



## Bayesian inference of the inbreeding load variance for fertility traits in Brown Swiss cattle

Maria Martinez-Castillero,<sup>1</sup>  Luis Varona,<sup>2</sup> Sara Pegolo,<sup>1\*</sup>  Attilio Rossoni,<sup>3</sup>  and Alessio Cecchinato<sup>1</sup> 

<sup>1</sup>Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020, Legnaro PD, Italy

<sup>2</sup>Unidad de Genética Cuantitativa y Mejora Animal, Instituto Agroalimentario de Aragón (IA2), Universidad de Zaragoza, Calle de Miguel Servet, 177, 50013, Zaragoza, Zaragoza, Spain

<sup>3</sup>Associazione Nazionale Allevatori di Razza Bruna, Loc. Ferlina, 204, 37012, Bussolengo VR, Italy

### ABSTRACT

Our study investigated the inbreeding load for fertility traits in the Italian Brown Swiss dairy cattle breed. Fertility traits included continuous traits (i.e., interval from calving to first service, days open, and calving interval) and categorical traits (i.e., calving rate at first insemination and nonreturn date at d 56). We included only records of the first 3 parities of cows that calved between 2010 and 2018. We traced up the pedigree of the cows with records as far as possible, ending up with a total of 73,246 animals. The final data set consisted of 59,864 records from 34,921 cows. We analyzed all models using a Bayesian approach that included a covariate with total inbreeding in addition to systematic, permanent environment, additive genetic, and inbreeding load effects. We then evaluated the trends in heritabilities and ratios of the inbreeding load using a continuum of partial inbreeding coefficients from 0.001 to 0.100 as reference. Posterior estimates of heritabilities tended to decrease across the continuum, whereas ratios of the inbreeding load tended to increase, more noticeably in categorical traits (calving rate at first insemination and nonreturn date at d 56). From the results obtained, we confirmed the presence of heterogeneity in inbreeding depression. We then predicted the inbreeding load effects, which had a low reliability of prediction, explained by having only 513 ancestors generating inbreeding. However, reliability of prediction was high enough for some of the individuals, obtaining a favorable prediction of inbreeding load for a relevant percentage, which improved the phenotypic performance of their inbred descendants. These results make it feasible to implement breeding and management strategies that select ancestors with a favorable inbreeding load prediction. In addition, it opens the possibility to define a global

index for the expected consequences of the inbreeding generated by each individual.

**Key words:** inbreeding load heterogeneity, inbreeding depression, dairy cattle, fertility, animal breeding

### INTRODUCTION

Inbreeding is caused by the mating of related individuals (Falconer and Mackay, 1996). It increases the probability of recessive alleles in homozygosis and decreases the percentage of heterozygous loci, thereby losing the advantage of overdominance (Charlesworth and Willis, 2009). Its most common consequence is inbreeding depression, which reduces the phenotypic yield of fitness-related traits (Falconer and Mackay, 1996). In dairy cattle, inbreeding depression has been reported in traits related to production (Smith et al., 1998; Biffani et al., 2002; Croquet et al., 2007), survival (Thompson et al., 2000; Sewalem et al., 2006), and fertility (Adamec et al., 2006; González-Recio et al., 2007; Pryce et al., 2014).

Inbreeding depression is a genetic phenomenon. It depends on the genotype of the ancestors whose alleles produce identity by descent in their progeny (Hedrick and Garcia-Dorado, 2016), as confirmed by the heterogeneity of inbreeding depression among sire families (Mi et al., 1965; Miglior et al., 1994). Brewer et al. (1990) proposed that the founder individuals' alleles control the heterogeneity of inbreeding depression. Lacy et al. (1997) proposed the decomposition of inbreeding into individual founder effects. Later on, Caballero and Toro (2000) suggested a Mendelian decomposition of inbreeding that split inbreeding among founders and the Mendelian sampling of the nonfounders. This Mendelian decomposition was the basis on which Casellas (2018) suggested a linear model for predicting the inbreeding loads of the individuals generating inbreeding. Later, Varona et al. (2019) proposed a new parameterization for predicting inbreeding loads for the individuals generating inbreeding and those not gen-

Received December 24, 2020.

Accepted May 4, 2021.

\*Corresponding author: [sara.pegolo@unipd.it](mailto:sara.pegolo@unipd.it)

**Table 1.** Mean (SD in parentheses) of continuous traits and incidence (%) of categorical traits in addition to minimum, maximum, and percentage of censored records in Brown Swiss cows after editing

Trait	Mean	Incidence	Minimum	Maximum	% of censored records
Continuous					
Interval from calving to first service	91.8 (41.0)	—	20	259	20.3
Days open	127.8 (70.2)	—	20	394	24.2
Calving interval	419.5 (75.4)	—	277	761	—
Categorical					
Calving rate (%)	—	49.9	—	—	—
Nonreturn rate at d 56 (%)	—	33.1	—	—	—

erating it. The availability of prediction of inbreeding loads allows us to distinguish between favorable and unfavorable effects on a specific trait. This information could help develop optimal breeding strategies whereby individuals able to provide favorable inbreeding effects on a trait (e.g., decreasing the number of days open) are mated (Man et al., 2002). It could also serve as a tool for artificially purging individuals carrying deleterious alleles, ensuring that individuals with an unfavorable inbreeding load effect are not used as reproducers (Varona et al., 2019). However, this novel approach has been applied only to growth traits in beef cattle (Varona et al., 2019) and horses (Poyato-Bonilla et al., 2020). In dairy cattle, inbreeding depression is expected to be relevant for fertility traits, as most farms mate purebreds (Sørensen et al., 2008), population size is finite, and selection is intensive (Weigel, 2001). For these reasons, using the approach of Varona et al. (2019), we conducted a study with the objectives of (1) estimating the genetic parameters and inbreeding load variances using a continuum of partial inbreeding coefficient as a reference and (2) predicting the inbreeding load effects on fertility traits in the Brown Swiss dairy cattle breed.

## MATERIALS AND METHODS

### Data Set

The data for the study consisted of records of the fertility traits of Brown Swiss cattle collected by the Breeders Federation of Alto Adige/Südtirol (Associazione Provinciale delle Organizzazioni Zootecniche Altoatesine/Vereinigung der Südtiroler Tierzuchtverbände, Bolzano/Bozen, Italy) in northeastern Italy. Fertility traits included continuous traits [interval from calving to first service (**ICF**), days open (**DO**), and calving interval (**CInt**)] and categorical traits [calving rate at first insemination (**CR**) and nonreturn rate at d 56 (**NR56**)]. The ICF and DO records were considered censored if the cow's pregnancy was not confirmed. For DO and ICF, we discarded records covering periods of

less than 20 d. For CR, 1 referred to a cow being pregnant, and for NR56, 1 referred to a cow with no second insemination registered and an observation interval of >56 d. A detailed description of the data set is given in Martinez-Castillero et al. (2020). A total of 49,184 cows were available, with 234,877 phenotypic records of all fertility traits before data editing. We included only records regarding the first 3 parities of cows that calved between 2010 and 2018. The original pedigree had 2,793,159 animals, and we extracted the subpedigree, including all animals with records and all their known ancestors, ending up with a total of 73,246 animals. After editing, 21,921 to 34,921 cows with phenotypic information remained, with between 29,860 and 59,864 phenotypic records. The average ( $\pm$ standard deviation) phenotypes of the continuous fertility traits were 91.8 d ( $\pm 41.0$ ) for ICF, 127.8 d ( $\pm 70.2$ ) for DO, and 419.5 d ( $\pm 75.4$ ) for CInt, with an incidence of censored records of 20.3% for ICF and 24.2% for DO. The incidence of categorical traits was 49.9% for CR and 33.1% for NR56 (see Table 1).

### Statistical Analyses

**Genetic Models.** The phenotypic data from the fertility traits were analyzed with a model based on Varona et al. (2019):

$$\mathbf{y} = \mathbf{fc} + \mathbf{Xb} + \mathbf{W}_1\mathbf{h} + \mathbf{W}_2\mathbf{p} + \mathbf{Za} + \mathbf{Ki} + \mathbf{e},$$

where  $\mathbf{y}$  is the vector of phenotypic records of the continuous traits (ICF, DO, and CInt) or the liabilities of the categorical traits (CR and NR56);  $\mathbf{b}$  is the vector of systematic effects: parity and year-season of calving (a combination of the year and the season of calving in 12 categories of 5 d each; season 1 = calvings from April to September, season 2 = October to March);  $\mathbf{h}$ ,  $\mathbf{p}$ ,  $\mathbf{a}$ ,  $\mathbf{i}$ , and  $\mathbf{e}$  are the vectors of random effects of herd, permanent environment, animal additive genetic, inbreeding load, and residuals, respectively;  $\mathbf{f}$  is the vector of total inbreeding of the recorded individual;  $c$  is the covariate

with total inbreeding;  $\mathbf{a}$  and  $\mathbf{i}$  are genetic effects:  $\mathbf{a}$  (the additive genetic effect) is expressed in all phenotypes, whereas  $\mathbf{i}$  (the inbreeding load) is expressed in the phenotypes of the inbred descendants (Varona et al., 2019); and  $\mathbf{X}$ ,  $\mathbf{W}_1$ ,  $\mathbf{W}_2$ ,  $\mathbf{Z}$ , and  $\mathbf{K}$  are the incidence matrices corresponding to the vectors of systematic, herd, permanent environment, additive genetic, and inbreeding load effects, respectively. Following transformation of the partial inbreeding matrix described by Caballero and Toro (2000) and further developed by Varona et al. (2019),  $\mathbf{K} = \mathbf{T}(\mathbf{I} - \mathbf{P})$ , where  $\mathbf{T}$  is a lower triangular matrix with each of the nonzero elements corresponding to the partial inbreeding coefficient ( $\mathbf{F}_p$ ) linking the phenotype of an inbred individual with the ancestor causing inbreeding. The  $\mathbf{F}_p$  were obtained by Mendelian decomposition of inbreeding, following the procedure of García-Cortés et al. (2010). For computational reasons, we then multiplied by 10 to obtain the inbreeding load variance for an  $\mathbf{F}_p$  from one ancestor of 0.10.  $\mathbf{P}$  is a projection matrix with 0 in the diagonal and 0.5 in the elements that link individuals with its sire and dam, and  $\mathbf{I}$  is the identity matrix. A detailed description of the procedure for calculating the  $\mathbf{F}_p$  and an R script is presented in Supplemental Files S1 and S2 (<https://figshare.com/s/fld2e7flf7a71355170c>).

Under a hierarchical Bayesian scheme, it was assumed that prior distributions for the herd, permanent environment, and residual effects had multivariate Gaussian distributions:

$$\mathbf{h} \sim N(\mathbf{0}, \mathbf{I}\sigma_h^2),$$

$$\mathbf{p} \sim N(\mathbf{0}, \mathbf{I}\sigma_p^2),$$

$$\mathbf{e} \sim N(\mathbf{0}, \mathbf{I}\sigma_e^2),$$

where  $\sigma_h^2$ ,  $\sigma_p^2$ , and  $\sigma_e^2$  are the herd, permanent environment, and residual variances, respectively. In addition, the prior distribution of the additive genetic and inbreeding load effects was

$$\begin{pmatrix} \mathbf{a} \\ \mathbf{i} \end{pmatrix} \sim N\left(\begin{pmatrix} \mathbf{0} \\ \mathbf{0} \end{pmatrix}, \mathbf{G} \otimes \mathbf{A}\right),$$

where  $\mathbf{A}$  is the numerator relationships matrix, and

$$\mathbf{G} = \begin{pmatrix} \sigma_a^2 & \sigma_{ai} \\ \sigma_{ai} & \sigma_i^2 \end{pmatrix},$$

where  $\sigma_a^2$ ,  $\sigma_i^2$ , and  $\sigma_{ai}$  are the additive genetic variance, the inbreeding load variance, and the covariance between the additive genetic and the inbreeding load effects, respectively. Finally, the prior distribution of the systematic effects ( $\mathbf{b}$ ) and the variance components ( $\sigma_h^2, \sigma_p^2, \sigma_e^2$ , and  $\mathbf{G}$ ) was uniform within appropriate bounds.

**Gibbs Sampler.** We analyzed all models using a standard Bayesian approach and estimated the marginal posterior distributions of unknown parameters by Gibbs sampler using software developed by Varona et al. (2019). The total number of iterations was 2,500,000, with a burn-in of 500,000 and without thinning. Analysis of the inbreeding coefficients ( $\mathbf{F}$ ) for the individuals in the pedigree was carried out in the INBUPGF90 program of the family of BLUPF90 software programs (Misztal et al., 2015). Convergence of the models was confirmed by visual inspection using R software (R Core Team, 2018), which was also used for the post-Gibbs analyses and for estimating the correlations between additive genetic and inbreeding load [ $r_{(a,i)}$ ], heritabilities ( $h^2$ ), and the ratios of the inbreeding load ( $i^2$ ), herd ( $he^2$ ), and permanent environment ( $pe^2$ ) variances, and the slope of  $c$  ( $\beta$ ). The  $r_{(a,i)}$  was defined as

$$r_{(a,i)} = \frac{\sigma_{ai}}{\sqrt{\sigma_a^2 \times \sigma_i^2}},$$

where  $\sigma_{ai}$  is the covariance between the additive genetic and the inbreeding load, and  $\sigma_a^2$  and  $\sigma_i^2$  are the additive genetic and inbreeding load variances, respectively. The  $i^2$  was defined as the proportion of phenotypic variation caused by variability in the inbreeding load variance. The calculation of  $i^2$  requires the assumption of a reference point defined by the inbreeding caused by one specific ancestor. We used a continuum from  $F = 0.001$  to  $F = 0.100$  in sequences of 0.001 to reflect the trend associated with  $i^2$  in different scenarios to an extreme ( $F = 0.100$ ) with highly inbred individuals. To estimate  $i^2$  across the continuum, we rescaled the estimates depending on the reference point of  $F$  by multiplying it by the square of the ratio between the 2 reference points (the original and the new one). Thus, the posterior estimate of the inbreeding load for ICF with a reference point of  $F = 0.025$  and based on  $F = 0.100$  was  $30.842 = 493.471 \times (0.025/0.100)^2$ . We then calculated the corresponding  $h^2$  that reflects the change in the magnitude due to  $i^2$ . The estimates of  $\beta$  were defined as the deviations from  $F$ . Furthermore, the reliability of the predictions of the  $j$ th inbreeding load was approximated as

**Table 2.** Number of animals, sires, dams, and generations from the edited pedigree,<sup>1</sup> number of partial inbreeding coefficients ( $F_p$ ), and number of ancestors generating inbreeding to more than 10, 100, 1,000, and 10,000 individuals

Item	Continuous traits <sup>2</sup>	Categorical traits <sup>3</sup>
Animals (no.)	61,062	73,246
Sires (no.)	2,585	2,859
Dams (no.)	39,772	47,045
Generations from pedigree (no.)	12	13
$F_p$ (no.; % of total)	178,252	268,525
>0.001	92,241 (51.74)	137,837 (51.33)
>0.01	5,891 (3.30)	7,518 (2.80)
>0.10	124 (0.07)	162 (0.06)
Maximum	0.125	0.125
Ancestors generating inbreeding (no.)	443	513
>10	153	188
>100	81	93
>1,000	27	29
>10,000	8	9

<sup>1</sup>The original pedigree was 2,793,159 animals, 116,329 sires, 1,255,039 dams, and 39 generations.

<sup>2</sup>Continuous traits, in days: interval from calving to first service, days open, and calving interval.

<sup>3</sup>Categorical traits, in %: calving rate and nonreturn rate at d 56.

$$r(i_j) = \sqrt{1 - \left[ \frac{\text{PSD}(i_j)^2}{\hat{\sigma}_i^2} \right]}$$

where  $\text{PSD}(i_j)$  is the posterior standard deviation of the  $j$ th inbreeding load, and  $\hat{\sigma}_i^2$  is the posterior mean estimate of the inbreeding load variance.

## RESULTS AND DISCUSSION

### Mendelian Decomposition of Inbreeding

The fraction of inbred animals from the original pedigree (2,793,159 individuals) was 59.6%, with an average  $F = 0.020$  ( $\pm 0.031$ ). After editing (73,246 individuals), the incidence of inbred animals was 94.6%, and  $F$  ranged from 0.031 (25th percentile) to 0.058 (75th percentile), 0.071 (90th percentile), and 0.080 (95th percentile), with an average of 0.045 ( $\pm 0.023$ ). The average  $F$  was twice as high in the edited pedigree as in the original pedigree. This difference can be explained by the loss of information in the edited pedigree, as the records were obtained from a group of farms. The majority of the genealogy is not connected with the phenotypic records.

Mendelian decomposition of inbreeding from the edited pedigree generated 268,525  $F_p$  and an average of 3.66 ancestors whose alleles can generate identity by descent. The average  $F_p$  was 0.0023, with a standard deviation of 0.003. As shown in Table 2, the distribution was asymmetric, with 7,518  $F_p$  greater than 0.01 (2.80% of the total) and only 162 greater than 0.1

(0.06% of the total). The maximum  $F_p$  was 0.125. These results indicate that most of the ancestors generating inbreeding are from several generations ago, whereas recent ancestors generated only a small percentage of inbreeding. Moreover, as shown in Table 2, these  $F_p$  were generated by only 513 ancestors. Of these, 188 individuals generated inbreeding to more than 10 individuals, 93 to more than 100, 29 to more than 1,000, and 9 to more than 10,000. As the 513 ancestors are genetically linked to all individuals in the pedigree, the  $i$  of the remaining individuals can be predicted based on the assumption of variation in the additive genetic effect of the  $i$  and the genetic relationships between the individuals captured by the  $\mathbf{A}$  matrix.

### Genetic Parameters

The posterior mean (and posterior standard deviation) estimates of variance components are presented in Table 3. The slope of the covariate with the total  $F$  was positive for continuous traits, ranging from 0.260 (−7.30; 7.67) in CInt to 8.43 (0.49; 16.45) in ICF, and negative for categorical traits ( $\sim 0.078$ ). The most important source of phenotypic variation in all fertility traits was the residual variance, ranging from 1,859.0 (1,793.2; 1,927.1) days squared in ICF to 6,485.5 (6,346.8; 6,627.5) days squared in DO for continuous traits, and was set to 1 for the categorical traits (CR and NR56). The inbreeding load variances were larger than the additive genetic variances, more noticeably in categorical traits [0.021 (0.013; 0.032) vs. 0.123 (0.025; 0.374) in CR and 0.019 (0.010; 0.030) vs. 0.146 (0.037; 0.382) in NR56], except for trait DO, where the estimate of genetic variance was larger [703.4 (546.4;

874.5) vs. 629.6 (159.0; 1,926.5)]. The posterior estimates of the genetic correlation between the additive and the inbreeding load effects were negative, ranging from  $-0.141$  ( $-0.751$ ;  $0.639$ ) in CInt to  $-0.281$  ( $-0.793$ ;  $0.445$ ) in ICF, and close to zero in DO  $-0.029$  ( $-0.775$ ;  $0.734$ ), whereas the HPD95 intervals included zero in all traits. In their study, Varona et al. (2019) obtained a negative correlation for weaning weight in the Pirenaica breed and a correlation close to zero in the Rubia Gallega breed and suggested that the different results could be due to the differences in the depths of the pedigrees available for each breed. In our final data set, the pedigree depth was smaller than that of the original pedigree (13 vs. 39 generations). Only a small number of ancestors generated inbreeding (513 individuals), which could explain the need for more information to estimate the correlations more accurately.

The relative magnitudes of the variance components are expressed by the heritabilities ( $h^2$ ) and the ratios of the inbreeding load ( $i^2$ ). These estimates, which depend on the amount of inbreeding generated by specific ancestors, are presented in Figure 1 under a continuum of different scenarios with different assigned  $F_p$ . The scenarios represent theoretical populations where all individuals are assigned  $F_p = 0.001$  to  $0.100$ . Posterior estimates of  $i^2$  tended to increase when the  $F_p$  assigned increased up to  $0.10$ , increasing up to around  $\sim 0.12$  in all traits except for DO, which increased around half as much ( $\sim 0.06$ ). Conversely, posterior estimates of  $h^2$  tended to decrease when the  $F_p$  assigned increased up to  $0.10$ , although not as much as  $i^2$  increased. In continuous traits, the decrease of  $h^2$  was more pronounced

compared with categorical traits. In continuous traits,  $h^2$  decreased in 1, 0.6, and 0.5% in traits ICF, DO, and CInt, respectively, whereas in categorical traits it decreased in only 0.2%. The changes in the estimates of  $h^2$  are due to the additional variance component, the  $i^2$ , contributing to the phenotypic variation, more pronounced as the reference point increased and in categorical traits.

The results obtained for  $i^2$  should be interpreted as the variation for one unit explained by the heterogeneity of the inbreeding depression effects in a theoretical population where each of the individuals has an  $F_p$  generated by a single, specific ancestor. Alternatively, it can be understood as the additional randomness in an inbred individual's phenotypic performance with a given probability of identity by descent from a single ancestor. However, large  $F_p$  are infrequent in commercial farms that use breeding strategies that try to avoid recent inbreeding, as in the pedigree we analyzed. The estimates of the  $i^2$  indicate that the heterogeneity of inbreeding depression had an almost negligible effect on our phenotypes. Nonetheless, we were able to confirm the presence of heterogeneity of inbreeding depression suggested in previous studies (Mi et al., 1965; Miglior et al., 1994; Fowler and Whitlock, 1999), although its effects should be noticeable only in the phenotypic variation in individuals with a large amount of inbreeding. However, the  $i^2$  we obtained were higher than those obtained by Varona et al. (2019) in beef cattle weaning weight, confirming the stronger influence of inbreeding depression on fitness-related traits such as fertility (DeRose and Roff, 1999; Casellas, 2018). The inbreed-

**Table 3.** Posterior mean estimates (HPD95<sup>1</sup> in parentheses) of variance components of fertility traits in Brown Swiss cows<sup>2</sup>

Item <sup>3</sup>	ICF	DO	CInt	CR	NR56
$\beta_{(F=0.10)}$	4.54 (−1.84; 10.79)	8.43 (0.49; 16.45)	0.26 (−7.30; 7.67)	−0.068 (−0.169; 0.039)	−0.087 (−0.191; 0.022)
$\sigma_a^2$	244.9 (177.4; 322.9)	703.4 (546.4; 874.5)	309.6 (229.3; 402.7)	0.021 (0.013; 0.032)	0.019 (0.010; 0.030)
$\sigma_{i(F=0.10)}^2$	493.5 (149.4; 1213.1)	629.6 (159.0; 1926.5)	770.1 (148.3; 2303.3)	0.123 (0.025; 0.374)	0.146 (0.037; 0.382)
$\sigma_{ia(F=0.10)}$	−103.9 (−369.3; 130.1)	−27.5 (−657.7; 529.4)	−72.8 (−448.3; 315.6)	−0.007 (−0.039; 0.027)	−0.013 (−0.005; 0.027)
$r_{(a,i)}$	−0.281 (−0.793; 0.445)	−0.029 (−0.775; 0.734)	−0.141 (−0.751; 0.639)	−0.157 (−0.706; 0.533)	−0.238 (−0.735; 0.565)
$\sigma_h^2$	646.6 (584.1; 713.8)	1,046.3 (942.6; 1,157.7)	554.3 (495.9; 617.0)	0.085 (0.074; 0.095)	0.098 (0.087; 0.111)
$\sigma_{pe}^2$	447.6 (360.9; 532.9)	1,278.9 (1,099.6; 1,457.3)	539.0 (438.1; 638.6)	0.054 (0.033; 0.076)	0.042 (0.019; 0.066)
$\sigma_e^2$	1,859.0 (1,793.2; 1,927.1)	6,485.5 (6,346.8; 6,627.5)	4,343.6 (4,255.5; 4,432.7)	1	1

<sup>1</sup>HPD95 = lower and upper bounds of the 95% highest posterior density region.

<sup>2</sup>ICF = interval from calving to first service; DO = days open; CInt = calving interval; CR = calving rate; NR56 = nonreturn rate at d 56.

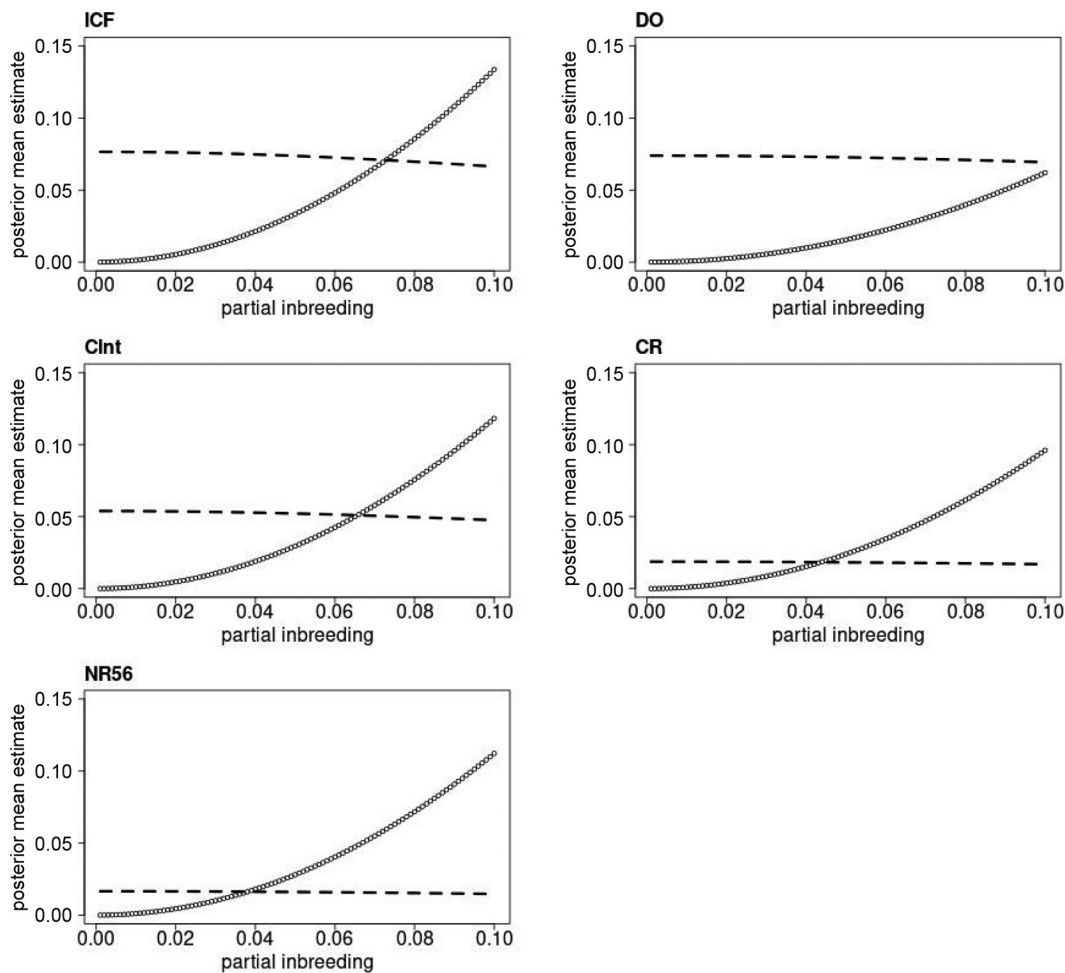
<sup>3</sup> $\beta_{(F=0.10)}$  = slope of the covariate with total inbreeding;  $\sigma_a^2$  = additive genetic variance;  $\sigma_{i(F=0.10)}^2$  = inbreeding load variance at a partial inbreeding coefficient of 0.10;  $\sigma_{ia(F=0.10)}$  = covariance between the additive genetic and inbreeding load effects at a partial inbreeding coefficient of 0.10;  $r_{(a,i)}$  = correlation between additive genetic and inbreeding load,  $r_{(a,i)} = \frac{\sigma_{ai}}{\sigma_a^2 \times \sigma_i^2}$ ;  $\sigma_h^2$  = herd variance;  $\sigma_{pe}^2$  = permanent environment variance;  $\sigma_e^2$  = residual variance.

ing depression we obtained could be due to the presence of recessive semideleterious alleles that negatively affect fertility, as seen in other studies (Adams et al., 2016; Hozé et al., 2020). Moreover, the distribution of these recessive alleles may be heterogeneous in individuals in the population and may determine the variability in individual inbreeding loads.

### Prediction of Inbreeding Loads

The procedure of Varona et al. (2019) provides predictions of the inbreeding loads ( $i$ ) for all of the individuals in the pedigree. However, in our study, the information available for predicting  $i$  of all individuals was associated with the genetic link to one of the 513 individuals generating  $F_p$ . The average reliability [ $r(i_j)$ ] was low, as shown in Table 4, ranging from 0.122 in CR to 0.179 in ICF. Given the low  $r(i_j)$ , most of the predictions of  $i$  were very close to zero. However,

a relevant fraction of the individuals had higher  $r(i_j)$  because they generate inbreeding in the phenotyped individuals or have a strong genetic link to them. Using as a threshold an  $r(i_j)$  of 0.3, the number of individuals with a greater value ranged from 3,901 in CR to 7,233 in DO. The distribution of the predicted  $i$  was variable and centered to zero, as shown in Figure 2. The slope with the covariate of the total  $F$  ( $\beta$ ) indicates the average effect of inbreeding, added for visualization purposes. That is, when  $i >$  or  $< \beta$ , the effect of inbreeding depression will be either positive or negative. The  $\beta$  obtained was positive for continuous traits, highest in DO (8.43), and close to zero in CInt (0.26), indicating a worsening in days in the phenotypic performance of the descendants due to inbreeding depression. In addition, it was negative for categorical traits ( $\sim 0.029$ ), indicating a worsening in percentage in the phenotypic performance of the descendants. However, a percentage of individuals had predictions of  $i$  with favorable



**Figure 1.** Trend of the posterior estimates of the inbreeding load variance ratio ( $i^2$ ; continuous line) and heritabilities ( $h^2$ ; dashed line) across a continuum of partial inbreeding coefficients.

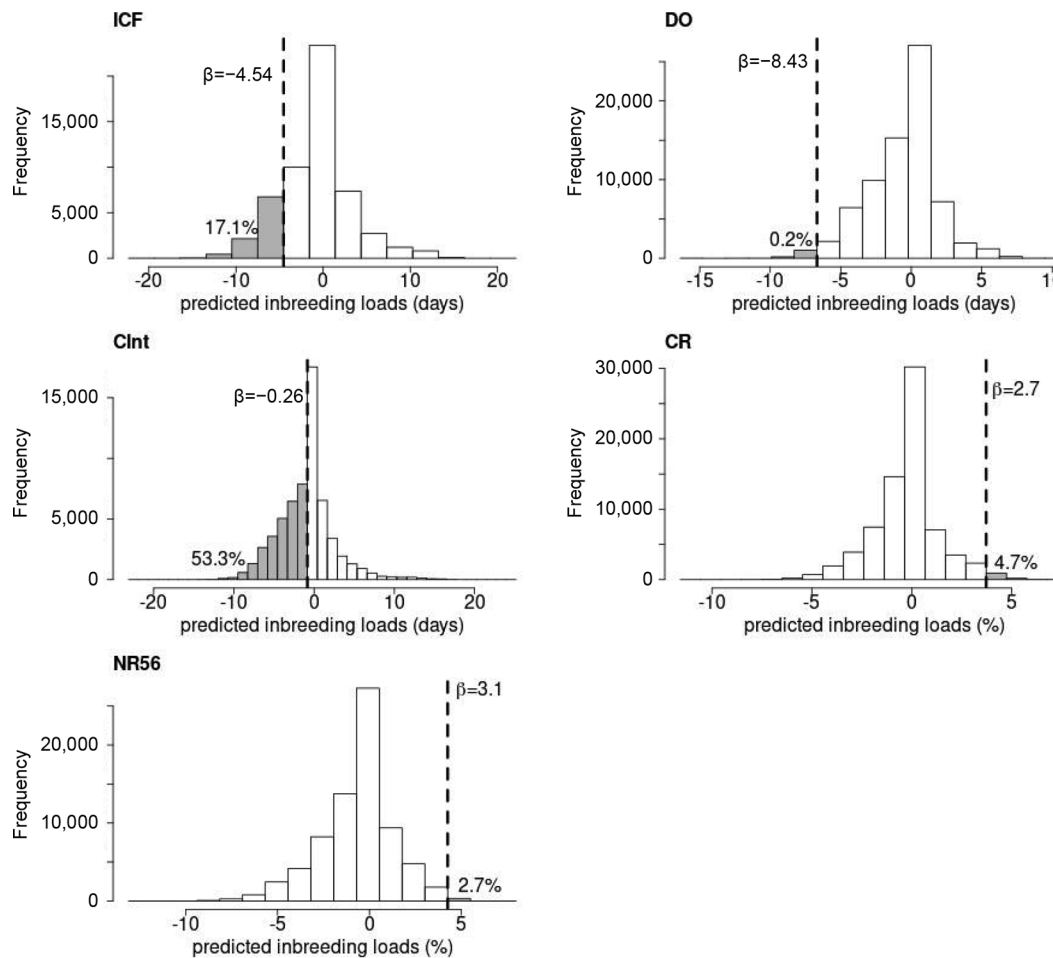
**Table 4.** Estimates of reliability (mean and SD) for each fertility trait, number of individuals with a reliability greater than 0.3, 0.4, 0.5, and 0.7, and maximum value of reliability in Brown Swiss cows

Trait <sup>1</sup>	Estimate of reliability		No. of individuals with reliability:				Maximum value of reliability
	Mean	SD	>0.3	>0.4	>0.5	>0.7	
ICF	0.179	0.109	6,856	629	29	3	0.820
DO	0.151	0.118	7,233	271	17	3	0.759
CInt	0.164	0.111	6,172	828	26	3	0.857
CR	0.122	0.099	3,901	65	13	3	0.770
NR56	0.132	0.105	4,222	74	14	4	0.779

<sup>1</sup>ICF = interval from calving to first service; DO = days open; CInt = calving interval; CR = calving rate; NR56 = nonreturn rate at d 56.

effects with respect to  $\beta$ , improving the phenotypic performance of their inbred descendants by reducing the number of days in continuous traits and increasing the incidence in categorical traits. These results were most noticeable for CInt (53.3%) and ICF (17.1%) and least noticeable for DO (0.2%). These variabilities can

also be seen in Table 5, which presents the predicted  $i$  for the 5 individuals with the highest  $r(i_i)$ , which correspond to old AI sires that generate inbreeding in a large number of individuals (370–15,272) and have a vast number of sons (53–1,930), grandsons (873–3,668), and great-grandsons (2,917–13,762). Availability of the



**Figure 2.** Predicted inbreeding load ( $i$ ) for continuous (ICF, DO, and CInt, in days) and categorical (CR and NR56, in %) fertility traits, the slope with the covariate of the total inbreeding coefficient ( $F$ ;  $\beta$ , dashed line), and the proportion of the predicted  $i$  with a positive effect with respect to the slope (gray area). ICF = interval from calving to first service; DO = days open; CInt = calving interval; CR = calving rate; NR56 = nonreturn rate at d 56.

**Table 5.** Details of the 5 individuals with the highest reliability in Brown Swiss cows

Item <sup>1</sup>	Individual ID				
	12783	12782	21519	12530	11170
Year of birth	2006	—	1995	—	—
ICF (d; reliability)	-22.32 (0.79)	8.07 (0.82)	22.12 (0.72)	1.18 (0.66)	7.41 (0.63)
DO (d; reliability)	-16.20 (0.72)	-1.46 (0.76)	10.81 (0.73)	4.88 (0.49)	0.17 (0.64)
CInt (d; reliability)	-13.12 (0.80)	2.46 (0.86)	12.77 (0.75)	-3.62 (0.68)	-12.86 (0.69)
CR (%)	0.027 (0.77)	-0.199 (0.76)	-0.013 (0.73)	-0.054 (0.67)	-0.204 (0.67)
NR56 (%)	-0.067 (0.78)	-0.286 (0.75)	0.106 (0.75)	-0.082 (0.70)	-0.087 (0.67)
Sons (no.)	251	403	1,930	385	53
Grandsons (no.)	3,548	3,583	3,688	3,290	873
Great-grandsons (no.)	13,762	8,636	2,917	8,772	7,167
F generated (no.)	15,272	2,552	370	3,873	11,170
F <sub>p</sub>	0.00614	0.0077	0.0201	0.0061	0.0027

<sup>1</sup>ICF = interval from calving to first service; DO = days open; CInt = calving interval; CR = calving rate; NR56 = nonreturn rate at d 56; F generated = number of individuals from which inbreeding is generated; F<sub>p</sub> = partial inbreeding coefficient.

predicted i opens new possibilities toward developing population breeding and management strategies instead of the general strategy of avoiding or limiting inbreeding (Meuwissen and Sonesson, 1998). Although it is highly recommended to avoid inbreeding generated by a common ancestor with an unfavorable prediction of inbreeding load (worsening the phenotype), those ancestors with a favorable inbreeding load prediction could be allowed—or even favored—in breeding strategies. Moreover, inbreeding cannot be completely eradicated in some populations due to their limited effective size. Therefore, an alternative strategy may include predicting the inbreeding loads in the selection index to generate a selection response that mimics and accelerates purging effects (Kristensen and Sørensen, 2005; Hedrick and Garcia-Dorado, 2016). However, implementing these approaches involves predicting inbreeding loads for all traits of interest, and these traits may be genetically correlated. In fact, the raw correlations among the predicted inbreeding loads for all individuals ranged up to 0.817 between CR and NR56, suggesting the possibility of the development of a multivariate approach, which could be of interest in future studies.

## CONCLUSIONS

This study confirms the presence of heterogeneity in the inbreeding depression effects, reflected in the variance in the inbreeding load effects. It also confirms the presence of a new source of phenotypic variation—the inbreeding load variance—when inbreeding is present. The results confirm the strong influence of inbreeding depression on fertility traits and the ability to predict the inbreeding loads of individuals. The study opens the possibility to develop alternative breeding strategies that include individuals with a favorable inbreeding load, which can improve the descendants' phenotype

(e.g., shortening DO or increasing CR). In light of this, the results could be used as a reference for creating a selection index in breeding strategies to obtain a response to selection by including traits of interest and predicting their inbreeding loads.

## ACKNOWLEDGMENTS

We thank the Italian Brown Swiss Cattle Breeders Association (ANARB, Verona, Italy) for providing pedigree information. The research was part of the project ECOLatte (funded under the National Rural Development Program—Sottomisura 10.2: Animal Biodiversity; Italy). The authors have not stated any conflicts of interest.

## REFERENCES

- Adamec, V., B. G. Cassell, E. P. Smith, and R. E. Pearson. 2006. Effects of inbreeding in the dam on dystocia and stillbirths in US Holsteins. *J. Dairy Sci.* 89:307–314. [https://doi.org/10.3168/jds.S0022-0302\(06\)72095-1](https://doi.org/10.3168/jds.S0022-0302(06)72095-1).
- Adams, H. A., T. S. Sonstegard, P. M. VanRaden, D. J. Null, C. P. Van Tassell, D. M. Larkin, and H. A. Lewin. 2016. Identification of a nonsense mutation in *APAF1* that is likely causal for a decrease in reproductive efficiency in Holstein dairy cattle. *J. Dairy Sci.* 99:6693–6701. <https://doi.org/10.3168/jds.2015-10517>.
- Biffani, S., A. B. Samoré, and F. Canavesi. 2002. Inbreeding depression for production, reproduction and functional traits in Italian Holstein cattle. 7th World Congress on Genetics Applied to Livestock Production. Communication No. 09-44.
- Brewer, B. A., R. C. Lacy, M. L. Foster, and G. Alaks. 1990. Inbreeding depression in insular and central populations of *Peromyscus* mice. *J. Hered.* 81:257–266. <https://doi.org/10.1093/oxfordjournals.jhered.a110988>.
- Caballero, A., and M. A. Toro. 2000. Interrelations between effective population size and other pedigree tools for the management of conserved populations. *Genet. Res.* 75:331–343. <https://doi.org/10.1017/s0016672399004449>.
- Casellas, J. 2018. On individual-specific prediction of hidden inbreeding depression load. *J. Anim. Breed. Genet.* 135:37–44. <https://doi.org/10.1111/jbg.12308>.



- Charlesworth, D., and J. H. Willis. 2009. The genetics of inbreeding depression. *Nat. Rev. Genet.* 10:783–796. <https://doi.org/10.1038/nrg2664>.
- Croquet, C., P. Mayeres, A. Gillon, H. Hammami, H. Soyeurt, S. Vanderick, and N. Gengler. 2007. Linear and curvilinear effects of inbreeding on production traits for walloon Holstein cows. *J. Dairy Sci.* 90:465–471. [https://doi.org/10.3168/jds.S0022-0302\(07\)72648-6](https://doi.org/10.3168/jds.S0022-0302(07)72648-6).
- DeRose, M. A., and D. Roff. 1999. A comparison of inbreeding depression in life-history and morphological traits in animals. *Evolution* 53:1288–1292.
- Falconer, D. S., and T. F. C. Mackay. 1996. *Introduction to Quantitative Genetics*. 4th ed. Addison Wesley Longman.
- Fowler, K., and M. C. Whitlock. 1999. The variance in inbreeding depression and the recovery of fitness in bottlenecked populations. *Proc. Biol. Sci.* 266:2061–2066. <https://doi.org/10.1098/rspb.1999.0887>.
- García-Cortés, L. A., J. C. Martínez-Ávila, and M. A. Toro. 2010. Fine decomposition of the inbreeding and the coancestry coefficients by using the tabular method. *Conserv. Genet.* 11:1945–1952. <https://doi.org/10.1007/s10592-010-0084-x>.
- González-Recio, O., E. López De Maturana, and J. P. Gutiérrez. 2007. Inbreeding depression on female fertility and calving ease in Spanish dairy cattle. *J. Dairy Sci.* 90:5744–5752. <https://doi.org/10.3168/jds.2007-0203>.
- Hedrick, P. W., and A. Garcia-Dorado. 2016. Understanding inbreeding depression, purging, and genetic rescue. *Trends Ecol. Evol.* 31:940–952. <https://doi.org/10.1016/j.tree.2016.09.005>.
- Hozé, C., C. Escoufflaire, M. Mesbah-Uddin, A. Barbat, M. Boussaha, M. C. Deloche, D. Boichard, S. Fritz, and A. Capitan. 2020. Short communication: A splice site mutation in *CENPU* is associated with recessive embryonic lethality in Holstein cattle. *J. Dairy Sci.* 103:607–612. <https://doi.org/10.3168/jds.2019-17056>.
- Kristensen, T. N., and A. C. Sørensen. 2005. Inbreeding—Lessons from animal breeding, evolutionary biology and conservation genetics. *Anim. Sci.* 80:121–133. <https://doi.org/10.1079/ASC41960121>.
- Lacy, R. C., G. Alaks, and A. Walsh. 1997. Hierarchical analysis of inbreeding depression in *Peromyscus polionotus*. *Evolution (N. Y.)* 51:1025. <https://doi.org/10.2307/2411182>.
- Man, W. Y. N., J. W. James, and F. W. Nicholas. 2002. Effect of inbreeding contribution from particular ancestors: A preliminary analysis of first lactation milk yields from Holstein Friesians in Australia. 7th World Congress on Genetics Applied to Livestock Production. Communication No. 23-01.
- Martinez-Castillero, M., H. Toledo-Alvarado, S. Pegolo, A. I. Vazquez, G. de los Campos, L. Varona, R. Finocchiaro, G. Bittante, and A. Cecchinato. 2020. Genetic parameters for fertility traits assessed in herds divergent in milk energy output in Holstein-Friesian, Brown Swiss, and Simmental cattle. *J. Dairy Sci.* 103:11545–11558. <https://doi.org/10.3168/jds.2020-18934>.
- Meuwissen, T. H. E., and A. K. Sonesson. 1998. Maximizing the response of selection with a predefined rate of inbreeding: Overlapping generations. *J. Anim. Sci.* 76:2575–2583.
- Mi, M. P., A. B. Chapman, and W. J. Tyler. 1965. Effects of mating system on production traits in dairy cattle. *J. Dairy Sci.* 48:77–84. [https://doi.org/10.3168/jds.s0022-0302\(65\)88164-4](https://doi.org/10.3168/jds.s0022-0302(65)88164-4).
- Miglior, F., E. B. Burnside, and W. D. Hohenboken. 1994. Heterogeneity among families of Holstein cattle in inbreeding depression for production traits. 5th World Congress on Genetics Applied to Livestock Production. Vol. 28, pages 479–482, Guelph, Ontario, Canada.
- Miszta, I. E., S. Tsuruta, D. Lourenco, I. Aguilar, A. Legarra, and Z. Vitezica. 2015. *Manual for BLUPF90 Family of Programs*. University of Georgia, Athens.
- Poyato-Bonilla, J., D. I. Perdomo-González, M. J. Sánchez-Guerrero, L. Varona, A. Molina, J. Casellas, and M. Valera. 2020. Genetic inbreeding depression load for morphological traits and defects in the Pura Raza Española horse. *Genet. Sel. Evol.* 52:62. <https://doi.org/10.1186/s12711-020-00582-2>.
- Pryce, J. E., M. Haile-Mariam, M. E. Goddard, and B. J. Hayes. 2014. Identification of genomic regions associated with inbreeding depression in Holstein and Jersey dairy cattle. *Genet. Sel. Evol.* 46:71. <https://doi.org/10.1186/s12711-014-0071-7>.
- R Core Team. 2018. R: The R Project for Statistical Computing. <https://www.r-project.org/>.
- Sewalem, A., G. J. Kistemaker, F. Miglior, and B. J. Van Doormaal. 2006. Analysis of inbreeding and its relationship with functional longevity in Canadian dairy cattle. *J. Dairy Sci.* 89:2210–2216. [https://doi.org/10.3168/jds.S0022-0302\(06\)72291-3](https://doi.org/10.3168/jds.S0022-0302(06)72291-3).
- Smith, L. A., B. G. Cassell, and R. E. Pearson. 1998. The effects of inbreeding on the lifetime performance of dairy cattle. *J. Dairy Sci.* 81:2729–2737. [https://doi.org/10.3168/jds.S0022-0302\(98\)75830-8](https://doi.org/10.3168/jds.S0022-0302(98)75830-8).
- Sørensen, M. K., E. Norberg, J. Pedersen, and L. G. Christensen. 2008. Invited review: Crossbreeding in dairy cattle: A Danish perspective. *J. Dairy Sci.* 91:4116–4128. <https://doi.org/10.3168/jds.2008-1273>.
- Thompson, J. R., R. W. Everett, and N. L. Hammerschmidt. 2000. Effects of inbreeding on production and survival in Holsteins. *J. Dairy Sci.* 83:1856–1864. [https://doi.org/10.3168/jds.S0022-0302\(00\)75057-0](https://doi.org/10.3168/jds.S0022-0302(00)75057-0).
- Varona, L., J. Altarriba, C. Moreno, M. Martínez-Castillero, and J. Casellas. 2019. A multivariate analysis with direct additive and inbreeding depression load effects. *Genet. Sel. Evol.* 51:78. <https://doi.org/10.1186/s12711-019-0521-3>.
- Weigel, K. A. 2001. Controlling inbreeding in modern breeding programs. *J. Dairy Sci.* 84:E177–E184. [https://doi.org/10.3168/jds.s0022-0302\(01\)70213-5](https://doi.org/10.3168/jds.s0022-0302(01)70213-5).

## ORCIDS

Maria Martinez-Castillero  <https://orcid.org/0000-0002-8531-648X>

Sara Pegolo  <https://orcid.org/0000-0001-6390-9826>

Attilio Rossoni  <https://orcid.org/0000-0001-5202-5785>

Alessio Cecchinato  <https://orcid.org/0000-0003-3518-720X>