Genetic parameters for novel mastitis traits defined by combining test-day somatic cell score and differential somatic cell count in the first lactation of Japanese Holsteins

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ABSTRACT

In this study, we aimed to improve current udder health genetic evaluations by addressing the limitations of monthly sampled somatic cell score (SCS) for distinguishing cows with robust innate immunity from those susceptible to chronic infections. The objectives were to (1) establish novel somatic cell traits by integrating SCS and the differential somatic cell count (DSCC), which represents the combined proportion of polymorphonuclear leukocytes and lymphocytes in somatic cells and (2) estimate genetic parameters for the new traits, including their daily heritability and genetic correlations with milk production traits and SCS, using a random regression test-day model (RRTDM). We derived 3 traits, termed ML_SCS_DSCC, SCS_4_DSCC_65_binary, and ML_SCS_DSCC_binary, by using milk loss (ML) estimates at corresponding SCS and DSCC levels, thresholds established in previous studies, and a threshold established from milk loss estimates, respectively. Data consisted of test-day records collected during January 2021 through March 2022 from 265 herds in Hokkaido, Japan. From these records, we extracted records between 7 to 305 d in milk (DIM) in the first lactation to fit the RRTDM. The model included the random effect of herd-test-day, the fixed effect of year-month, fixed lactation curves nested with calving age groups, and random regressions with Legendre polynomials of order 3 for additive genetic and permanent environmental effects. The analysis was performed using Gibbs sampling with Gibbsf90+ software. The averages (ranges) of the daily heritability estimates over lactation were 0.086 (0.075–0.095) for SCS, 0.104 (0.073–0.127) for ML_SCS_DSCC, 0.137 (0.014–0.297) for SCS_4_DSCC_65_binary, and 0.138 (0.115–0.185) for ML_SCS_DSCC_binary; the heritability curve for SCS_4_DSCC_65_binary was erratic. Genetic correlations within the trait decreased as the DIM interval widened, especially for those integrating DSCC, indicating that these traits should be analyzed using RRTDM rather than repeatability models. The averages (ranges) of genetic correlations with milk yield over lactation were 0.01 (−0.22 to 0.28) for SCS, −0.05 (−0.40 to 0.13) for ML_SCS_DSCC, −0.08 (−0.17 to 0.09) for SCS_4_DSCC_65_binary, and −0.08 (−0.22 to 0.27) for ML_SCS_DSCC_binary. Compared with SCS, the newly defined traits exhibited slightly stronger negative genetic correlations with milk yield. Especially in late lactation stages, the genetic correlation between ML_SCS_DSCC and milk yield was significantly below zero, with a posterior median of −0.40. Furthermore, the new traits showed positive correlations with SCS, having estimates varying from 0.68 to 0.85 for ML_SCS_DSCC, 0.14 to 0.47 for SCS_4_DSCC_65_binary, and 0.61 to 0.66 for ML_SCS_DSCC_binary, depending on DIM. Considering that ML_SCS_DSCC and ML_SCS_DSCC_binary have relatively high heritability (compared with SCS) and favorable genetic correlations with milk production traits and SCS, their incorporation into breeding programs appears promising. Nevertheless, their genetic relationships with (sub)clinical mastitis require further investigation.
Key words: mastitis, somatic cell score, differential somatic cell count, immune-associated trait

INTRODUCTION

Because of its high prevalence and detrimental effects on economics and animal welfare, mastitis is a major concern in the dairy industry (Hogeveen et al., 2019). In Hokkaido, Japan, mastitis caused an estimated annual loss of $60 million from 2015 to 2018, and the situation has worsened because of bovine leukemia virus (Nakada et al., 2023). In the same area, approximately 20% of

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

culled dairy cows were culled involuntarily due to mastitis (Hokkaido Dairy Milk Recording and Testing Association, 2022). Mastitis is primarily managed through antibiotics, but outcomes are often not as expected, prompting concerns regarding antimicrobial resistance (Ruegg, 2017). Effective strategies to control mastitis without antibiotics are needed urgently (Sharma et al., 2018). In this context, genetic selection is considered one of the most efficient approaches to tackling mastitis (Martin et al., 2018).

Genetic selection offers several advantages, particularly because genetic merit can be permanently accumulated in the population (Martin et al., 2018). The success of a genetic selection program relies heavily on precise phenotyping and the heritability of the targeted trait; however, establishing a successful selection program becomes challenging when dealing with complex disease traits such as mastitis. The poor heritability of clinical mastitis (**CM**; 0.01–0.03; Carlén et al., 2004; Yamaguchi et al., 2019;) makes a national recording system (Heringstad et al., 2000) or constant reporting of producer-recorded health data (Koeck et al., 2012; Pritchard et al., 2013) necessary to collect the large amounts of data required for reliable estimations of breeding values. The recording and reporting process can be laborious and costly, and the inconstancy in trait definition (i.e., the criteria for defining an outbreak of CM) between producers, veterinarians, and nations can introduce bias into the results (Koeck et al., 2012; Pritchard et al., 2013). In countries without consistent and reliable records of CM, the SCS (the logarithmic transformation of SCC) has been used as a proxy for genetic selection on udder health (Ali and Shook, 1980; Cole and VanRaden, 2018).

The SCS increases as leukocytes, particularly PMN, are recruited from the blood into the mammary gland to phagocytize invading organisms. This immune response is crucial to prevent further infection by pathogens. Because the magnitude of the SCS is closely linked to the extent of inflammation in the mammary gland, cows with the lowest SCS averages during lactation are deemed to have the highest resistance to IMI and mastitis (Shook and Schutz, 1994). In addition, SCS has moderate heritability (0.07–0.17; Carlén et al., 2004; Yamazaki et al., 2013) and a favorable, positive genetic correlation with CM (0.59–0.88; Carlén et al., 2004; Yamaguchi et al., 2019), implying that indirect selection on SCS can be as effective as direct selection based on CM records (Martin et al., 2018). Furthermore, SCS records are available through routine milk testing in DHI programs at low cost, thereby providing information on IMI and CM at monthly intervals. These characteristics make SCS an objective, cost-effective, and convenient alternative to CM records for genetic selection (Shook and Schutz, 1994).

The genetic evaluation of SCS has been widely implemented since the 1990s and remains a pivotal measure for improving resistance to mastitis, but the approach has limitations (Shook and Schutz, 1994; Heringstad et al., 2000). Although selection for low SCS aims to identify animals without IMI, it might inadvertently favor animals with less robust innate immune responses to IMI. Consequently, long-term selection for low SCS can potentially compromise leukocyte recruitment (Kehrli and Shuster, 1994; Schukken et al., 1997) and thus general immunity against infectious diseases. Despite debates regarding this theory (Rainard et al., 2018), the current consensus is that a better tool is required to differentiate between cows with robust innate immunity and those predisposed to chronic infections (Shook and Schutz, 1994; Heringstad et al., 2000).

In 2017, a new tool known as the differential somatic cell count (**DSCC**) was described to determine the proportion of PMNs and lymphocytes in somatic cells during monthly DHI testing (Damm et al., 2017). Given that lymphocytes typically constitute a small proportion of milk cells, fluctuations in DSCC primarily reflect changes in PMN levels (Damm et al., 2017). Our previous research revealed that high levels of SCS do not necessarily negatively affect milk production, as long as DSCC is also at a high level (Huang et al., 2023). This finding suggests that cows with elevated SCS and DSCC may be in the early stages of infection, when the infection is well controlled by abundant PMNs. In contrast, when the SCS is high and the DSCC is low, indicating chronic infection, the productivity of cows becomes significantly impaired (Schwarz et al., 2020; Huang et al., 2023). Previous studies have demonstrated that the ability of cows to recruit PMNs in response to IMI is heritable (Paape et al., 2002). When encountering IMI, cows with rapid recruitment of PMNs have an increased likelihood of spontaneous cure and complete recovery of milk production (Shuster et al., 1996; Burton and Erskine, 2003; Mehrzad et al., 2004). Because of variations in PMN proportions (i.e., DSCC level), productivity and the ability to counter IMI differ markedly among cows with the same SCS, thus presenting an opportunity to define a new genetic trait that combines information from SCS and DSCC to improve resistance to mastitis and minimize the associated milk loss.

As the first step to determine whether integrating SCS and DSCC improves current genetic evaluations of mastitis resistance based solely on SCS, we aimed to (1) define novel udder health traits by combining test-day records of SCS and DSCC and (2) use random regression test-day models (**RRTDM**) to estimate the genetic parameters for these traits, including their variance components, heritability, and genetic correlations with milk

production traits and SCS, during the first lactation of Japanese Holsteins.

MATERIALS AND METHODS

Data

The phenotypic data used in this study were test-day records collected in the Tokachi Subprefecture of Japan from January 2021 to March 2022 and provided by the Tokachi Federation of Agricultural Cooperative (Hokkaido, Japan). Because the data were retrieved from pre-existing databases, Animal Care and Use Committee approval was not required. The dataset has been used and described in detail by Huang et al. (2023). The SCS was calculated as $\log_2(\text{SCC}/100,000) + 3$ (Wiggans and Shook, 1987). We extracted test-day records from the first lactation, covering 7 to 305 DIM. We excluded cows with unknown parents and those that had calved outside the age range of 21 to 32 mo (Nishiura et al., 2015). Each contemporary group (i.e., herd-test-day; **htd**) was required to have at least 3 animals to be enrolled (Oliveira et al., 2019). This stipulation resulted in a dataset consisting of 124,255 test-day records from 36,706 cows in 265 herds.

The pedigree data were provided by the Holstein Cattle Association of Japan, Hokkaido Branch. We included cows with phenotypic records and their ancestors until the fifth generation, resulting in a dataset of 164,427 animals for genetic analysis.

Trait Definition

Previous studies on the genetic analysis of DSCC suggested its potential use in breeding programs. The genetic correlation between SCS and DSCC differed from unity, with point estimates ranging from 0.60 to 0.66 (Bobbo et al., 2019; Pegolo et al., 2021; Ablondi et al., 2023). In addition, DSCC may have higher heritability than SCS (Bobbo et al., 2019; Ablondi et al., 2023). Furthermore, we argue that it is essential to consider the interaction between DSCC and SCS. The meaning of a DSCC value varies depending on the SCS value, and the 2 parameters have interactive effects on milk production and udder health (Huang et al., 2023). Therefore, we defined 3 traits derived from the combination of SCS and DSCC, as described in the following sections.

SCS_4_DSCC_65_binary. This trait corresponds to udder health group D defined by Schwarz et al. (2020) as cows with an SCC above 200,000 cells/mL (i.e., SCS >4) and a DSCC below 65%; this is believed to be an indication of chronic mastitis. Cows in this group experienced a significant reduction in productivity (Schwarz et al., 2020) and were at increased risk of being culled

(Schwarz et al., 2021), making it desirable to exclude these animals from breeding programs. For this binary trait, a test-day record with an SCS higher than 4 and a DSCC lower than 65% was assigned a value of 1; otherwise, it was assigned a value of 0 (Figure 1).
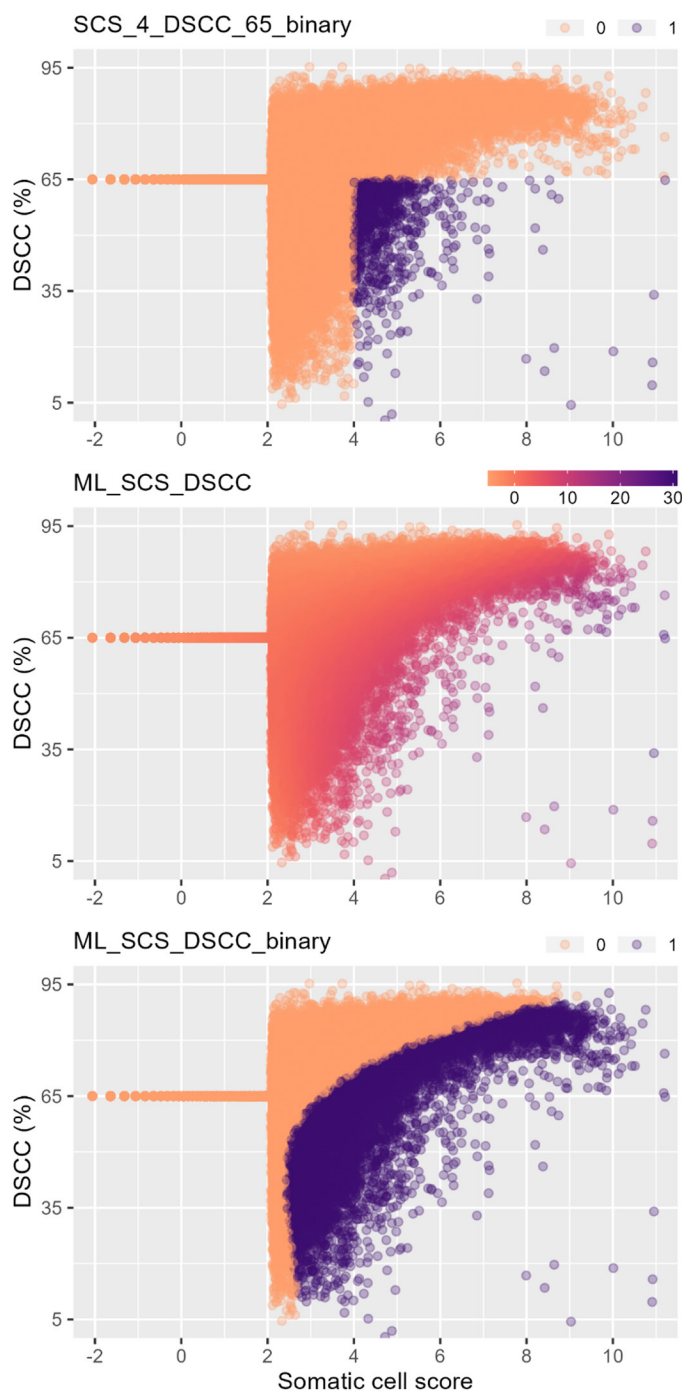

Figure 1. Distributions of our new traits as functions of SCC and differential SCC (DSCC).

ML_SCS_DSCC (%). The trait was derived from milk loss (**ML**; %) estimated at various levels of SCS and DSCC, according to the method described by Huang et al. (2023) but with modifications to avoid selection bias. In brief, we fitted a generalized additive model (**GAM**) using the "bam" function within the "mgcv" package (Wood, 2017) in R 4.2.2 (R Core Team, 2022) to estimate the interactive effects of SCS and DSCC on milk yield, where the effects of confounding factors, such as DIM, season, cow, and herd, were precluded. To include all records from all cows in the analysis, we divided the phenotypic data into 5 subsets by randomly selecting one-fifth of the cows from each herd and fitting a GAM to each subset of data. Using the fitted models, we estimated milk loss as a percentage change of milk yield relative to that found at an SCS of 2 and a DSCC of 65%. This computation results in estimates similar to that in Table 4 of Huang et al. (2023), but here we reversed the sign to facilitate the comparison with SCS (i.e., the lower, the better). Specifically, a negative value for this trait indicates that the cow is expected to produce more milk than she can produce with an SCS of 2 and a DSCC of 65%, whereas a positive value indicates she would produce less milk at the corresponding level. Because of the high correlations between the estimates derived from the 5 models (Pearson correlation coefficient >0.99 for all pairs of comparison), we averaged the estimates to derive the values of ML_SCS_DSCC (Figure 1).

ML_SCS_DSCC_binary. This binary trait is a compromise between the SCS_4_DSCC_65_binary and ML_SCS_DSCC traits. The SCS_4_DSCC_65_binary trait is simple to derive. Nonetheless, genetic selection based on this trait is similar to using the independent culling level method for simultaneous selection of low SCS and high DSCC, in the sense that only cows with an SCS higher than 4 and DSCC lower than 65% would be excluded from breeding programs. Such a method can be inefficient and unable to maximize economic benefits. In contrast, ML_SCS_DSCC is computationally intensive to derive, requiring the understanding of GAM and a specific statistical package (i.e., "mgcv"), which potentially limits its use. To address this limitation, we determined a threshold ML_SCS_DSCC (the ML_SCS_DSCC_binary trait) according to the prevalence of subclinical mastitis in the study population. We estimated this prevalence as the proportion of observations with an SCS greater than 4 (Dohoo and Leslie, 1991), thus resulting in an estimate of 9.95%. Therefore, we coded a test-day record with a value of ML_SCS_DSCC below the 9.95% as 1; otherwise, we coded it as 0. The value of ML_SCS_DSCC_binary depends on the interaction between the values of SCS and DSCC (Figure 1). More specifically, even though a cow might have a relatively low SCS, she might still be coded as 1 if her DSCC was low or vice versa. The ML_SCS_DSCC-binary trait can conveniently be derived using a quintic function: SCS + 11.4 × DSCC − 57.8 × DSCC^2 + 150 × DSCC^3 − 174.9 × DSCC^4 + 62.8 × DSCC^5 > 3.5; if true, 1; else 0, in which the value of DSCC should be input as a proportion rather than a percentage (e.g., 0.65 for 65% of DSCC).

Statistical Models

Depending on the nature of the trait, we estimated the (co)variance components for SCS and the newly defined traits using a univariate linear or threshold RRTDM:

$$y_{ijklt} = htd_i + YM_j + \sum_{m=0}^{5} \phi(t)_{km} \beta_{km} + \sum_{m=0}^{3} \phi(t)_{lm} a_{lm} + \sum_{m=0}^{3} \phi(t)_{lm} pe_{lm} + e_{ijklt},$$

where y_{ijklt} was either the record of SCS or ML_SCS_DSCC, or the liability of SCS_4_DSCC_65_binary or ML_SCS_DSCC_binary of the lth animal on DIM t; htd_i was the random effect of the ith herd-test-day (4,637 levels); YM_j was the fixed effect of the jth year-month (15 levels), which was included to account for time trends in the phenotypes (Schaeffer, 2018); $\phi(t)_{km}$ was a matrix of Legendre polynomial order of 4 ($m = 0$–4) plus Wilmink's exponential function (exp$^{-0.05t}$; $m = 5$) at DIM t for fixed regressions (Schaeffer et al., 2000; Nishiura et al., 2015); β_{km} was the mth fixed regression coefficient of the kth group for the age of calving (6 levels; 21–32 mo, 2 mo per group); $\phi(t)_{km}$ was a matrix of Legendre polynomial order of 3 ($m = 0$–3) at DIM t for random regressions; and a_{lm} and pe_{lm} were the mth random regression coefficients of the lth animal's additive genetic effect (164,427 levels) and permanent environmental effect (**PE**; 164,427 levels), respectively. Lastly, e_{ijklt} was the random effect of residuals, which we assumed constant across the lactation. In matrix notation, the model can be written as follows:

$$\mathbf{y} = \mathbf{Vhtd} + \mathbf{Xb} + \mathbf{Za} + \mathbf{Wpe} + \mathbf{e},$$

where $\mathbf{y}$ was a vector of observations, $\mathbf{htd}$ was a vector of herd-test-day effects, $\mathbf{b}$ was a vector of fixed effects, $\mathbf{a}$ was a vector of additive genetic effects, $\mathbf{pe}$ was a vector of PE effects, $\mathbf{e}$ was a vector of residuals, and $\mathbf{V}$, $\mathbf{X}$, $\mathbf{Z}$, and $\mathbf{W}$ were the corresponding incidence matrices. The covariance structure of models was defined as

$$VAR\begin{bmatrix} htd \\ a \\ pe \\ e \end{bmatrix} = \begin{bmatrix} \mathbf{I}\sigma_h^2 & 0 & 0 & 0 \\ 0 & \mathbf{A} \otimes \mathbf{G} & 0 & 0 \\ 0 & 0 & \mathbf{I} \otimes \mathbf{P} & 0 \\ 0 & 0 & 0 & \mathbf{I}\sigma_r^2 \end{bmatrix},$$

where **I** was an identity matrix; σ_h^2 was the variance of the random *htd* effect; **A** was the numerator relationship matrix accounting for additive genetic relationships between animals; $\otimes$ was the Kronecker product; **G** and **P** were 4×4 covariance matrices of the random regression coefficients for additive genetic and PE effects, respectively; and σ_r^2 was the variance of the residuals.

We used a bivariate RRTDM to clarify the genetic relationship between SCS, the newly defined traits (i.e., SCS_4_DSCC_65_binary, ML_SCS_DSCC, and ML_SCS_DSCC_binary), and milk production traits (i.e., the yields of milk, protein, fat, and lactose). The analysis was performed for each pair of somatic cell traits and milk production traits ($4 \times 4 = 16$ models) and for SCS and the newly defined traits ($1 \times 3 = 3$ models). Fixed and random effects were defined as in the univariate model, and the covariance structure was defined as follows:

$$VAR \begin{bmatrix} htd \\ a \\ pe \\ e \end{bmatrix} = \begin{bmatrix} \mathbf{I} \otimes \mathbf{H} & 0 & 0 & 0 \\ 0 & \mathbf{A} \otimes \mathbf{G} & 0 & 0 \\ 0 & 0 & \mathbf{I} \otimes \mathbf{P} & 0 \\ 0 & 0 & 0 & \mathbf{I} \otimes \mathbf{R} \end{bmatrix},$$

where **H** was a 2×2 covariance matrix for the random *htd* effects between traits; **G** and **P** were 8×8 covariance matrices of the random regression coefficients for additive genetic and PE effects, respectively, between traits; and **R** was a 2×2 covariance matrix for the residuals between traits. Other components were defined as in the univariate model.

Parameter Estimation

The daily additive genetic variance $\left[\hat{\sigma}_{g_i}^2(t) \right]$, PE variance $\left[\hat{\sigma}_{pe_i}^2(t) \right]$, and heritability $\left[\hat{h}_i^2(t) \right]$ of trait i at DIM t can be calculated as follows:

$$\hat{\sigma}_{g_i}^2(t) = \phi'(\mathbf{t}) \hat{\mathbf{G}}_\mathbf{i} \phi(\mathbf{t});$$

$$\hat{\sigma}_{pe_i}^2(t) = \phi'(\mathbf{t}) \hat{\mathbf{P}}_\mathbf{i} \phi(\mathbf{t}); \text{ and}$$

$$\hat{h}_i^2(t) = \frac{\hat{\sigma}_{g_i}^2(t)}{\hat{\sigma}_{h_i}^2 + \hat{\sigma}_{g_i}^2(t) + \hat{\sigma}_{pe_i}^2(t) + \hat{\sigma}_{r_i}^2},$$

where $\phi'(\mathbf{t}) = [\phi_0(t) \; \phi_1(t) \; \phi_2(t) \; \phi_3(t)]$ was a vector of third-order Legendre polynomials at DIM t; $\hat{\mathbf{G}}_\mathbf{i}$ and $\hat{\mathbf{P}}_\mathbf{i}$ were the estimated 4×4 covariance matrices of the random regression coefficients for additive genetic and PE effects, respectively, and $\hat{\sigma}_{h_i}^2$ and $\hat{\sigma}_{r_i}^2$ were the estimated htd and residual variances for trait i, respectively.

The genetic correlation $\left(r_{g_{i(t1),j(t2)}} \right)$ between trait i at DIM t1 and trait j at DIM t2 was estimated as follows:

$$r_{g_{i(t1),j(t2)}} = \frac{\phi'(\mathbf{t1}) \hat{\mathbf{G}}_\mathbf{ij} \phi(\mathbf{t2})}{\sqrt{\hat{\sigma}_{g_i}^2(t1) \times \hat{\sigma}_{g_j}^2(t2)}},$$

where $\hat{\mathbf{G}}_\mathbf{ij}$ was the estimated 4×4 additive genetic covariance matrix of the random regression coefficients between traits i and j, and $\hat{\sigma}_{g_i}^2(t1)$ and $\hat{\sigma}_{g_j}^2(t2)$ were the estimated additive genetic variance of trait i at DIM t1 and that of trait j at DIM t2, respectively. Other components were as defined previously. Note that traits i and j can be the same trait, such that in this case, $\hat{\mathbf{G}}_\mathbf{ij}$ equals $\hat{\mathbf{G}}_\mathbf{i}$.

We estimated variance components, heritability, and genetic correlation within a trait (between different DIM) using the univariate RRTDM and computed the genetic correlation between the traits using the bivariate RRTDM. These parameters were estimated through a Bayesian approach via Gibbs sampling, performed by the Gibbsf90+ module of the BLUPF90 software (Lourenco et al., 2022). We assumed a flat prior for all effects (i.e., the default in Gibbsf90+) and ran a single chain of 500,000 iterations for each analysis. After discarding 200,000 samples as a burn-in, we stored every 10th sample for thinning, resulting in a total of 30,000 post-Gibbs samples for parameter estimation. For each sampled iteration, we calculated the variance components, heritability, and genetic correlation to generate the posterior distribution (n = 30,000) of each parameter at the covered DIM (i.e., 7 to 305). We reported the median and the 95% highest probability density intervals to characterize the posterior distributions by using the R package "bayestestR" (Makowski et al., 2019).

RESULTS AND DISCUSSION

Descriptive Statistics and Variations Across Lactation

Table 1 shows descriptive statistics for somatic cell and milk production traits, as well as Pearson correlation coefficients between linear traits. The mean of SCS was 1.85, which is considerably lower than that reported earlier in Japan (>2.10; Hagiya et al., 2014; Nishiura et al., 2015). This difference can be attributed to an improvement in the genetic and management aspects of mastitis resistance within the studied population or to potential variations in udder health across different regions of Japan. The mean ML_SCS_DSCC was −0.37%, which indicated that, on average, the study population would produce 0.37% more milk than cows with an SCS

Table 1. Descriptive statistics and correlations between somatic cell traits and milk production traits[1]

Trait	Mean (SD)	MY (kg/d)	FY (kg/d)	PY (kg/d)	LY (kg/d)	SCS
				Pearson correlation coefficient		
SCS	1.85 (1.64)	−0.16	−0.08	−0.11	−0.19	—
ML_SCS_DSCC (%)	−0.37 (1.89)	−0.24	−0.12	−0.17	−0.27	0.74
	n test-day records (%)			Mean (SD)		
SCS_4_DSCC_65_binary						
Code 0	123,315 (99.25%)	30.4 (6.40)	1.21 (0.26)	1.02 (0.20)	1.39 (0.30)	1.83 (1.63)
Code 1	940 (0.75%)	22.7 (8.39)	1.01 (0.39)	0.80 (0.29)	0.99 (0.38)	4.71 (0.85)
ML_SCS_DSCC_binary						
Code 0	111,888 (90.05%)	30.7 (6.24)	1.22 (0.25)	1.02 (0.20)	1.40 (0.29)	1.57 (1.38)
Code 1	12,367 (9.95%)	26.7 (7.22)	1.14 (0.31)	0.93 (0.24)	1.19 (0.33)	4.33 (1.72)
Overall	124,255	30.3 (6.46)	1.21 (0.26)	1.01 (0.20)	1.38 (0.30)	1.85 (1.64)

[1]MY = milk yield; FY = fat yield; PY = protein yield; LY = lactose yield.

of 2 and a DSCC of 65% if only the effect of mastitis were considered. About 0.75% (n = 940) of test-day records from the first lactation were coded 1 for the SCS_4_DSCC_65_binary trait. This estimate was lower than previous reports of 1.5% (Bobbo et al., 2020) and 1.2% (Schwarz et al., 2020) in the primiparous cows and was attributable to the general better udder health in the studied animals. The frequency of a positive value for ML_SCS_DSCC_binary was 9.95% (n = 12,367). This was much higher than that for the SCS_4_DSCC_65_binary trait. Note that the estimated genetic parameters of binary traits are frequency dependent, particularly when estimated by a linear multivariate model (Carlén et al., 2009). This caveat was one of our reasons for using a threshold RRTDM for parameter estimation.

Pearson correlation coefficients between SCS and milk production traits varied from −0.08 to −0.19, depending on the trait (Table 1). The absolute values of the correlations were higher than those reported earlier (Miglior et al., 2007; Costa et al., 2019a), possibly because those studies included records from cows with more lactations. However, the overall rank of correlation strength was identical among studies, with lactose yield having the strongest negative correlation with SCS, followed by milk yield, and with fat yield having the weakest negative correlation. Compared with SCS, ML_SCS_DSCC revealed stronger negative correlations with milk production traits, with correlation estimates ranging from −0.12 to −0.27. By integrating information from DSCC in addition to SCS, ML_SCS_DSCC appears to have captured a more nuanced relationship between udder health and milk production. Moreover, we found a fairly strong correlation between SCS and ML_SCS_DSCC (r = 0.74).

Table 1 summarizes the yields of milk and milk components by groups in the binary traits. Milk production differed substantially between cows coded as 0 and 1 for SCS_4_DSCC_65_binary, such that the latter produced 7.7 kg less milk per day on average than the former. In comparison, the difference in milk production between

the 2 groups divided according to ML_SCS_DSCC_binary was smaller (i.e., 30.7 − 26.7 = 4.0 kg). Nevertheless, the within-group variation, as indicated by the standard deviation, was lower for groups divided by ML_SCS_DSCC_binary than was achieved by using SCS_4_DSCC_65_binary, implying a more distinct separation between the groups, with an effect of reducing the heterogeneity within each group.

We plotted the averages of SCS and ML_SCS_DSCC, as well as the proportion of positive values in the binary traits across lactation (Figure 2). The curves for SCS, ML_SCS_DSCC, and ML_SCS_DSCC_binary exhibited similar patterns, forming an inverted lactation curve with a nadir around 60 to 70 DIM. The day-to-day variations in SCS seemed to be more pronounced than those in ML_SCS_DSCC. Dohoo and Meek (1982) suggested that day-to-day fluctuation in SCS can be attributed to transient IMI, which causes a sudden influx of PMNs and prompt elimination of the causative pathogen. In a comparable scenario, we hypothesize that ML_SCS_DSCC would exhibit diminished fluctuations compared with SCS. Our previous study suggested that concurrent elevations in SCS and DSCC (i.e., proportions of PMNs) signified the onset of an IMI (Huang et al., 2023). During this stage, milk yield, and thus ML_SCS_DSCC, should scarcely be affected (Figure 1). Unlike with the other 3 traits, the nadir of SCS_4_DSCC_65_binary was ambiguous. The trait was in low frequency after the first few days of lactation, with a slight elevation in the last stage of lactation.

Variance Components

We estimated the variance components across the first lactation, including htd, additive genetic, PE, and residual variances for the 4 somatic cell traits (Figure 3A). For SCS, the daily additive genetic variances ranged from 0.18 (at 40 DIM) to 0.25 (at 203 DIM), with an average of 0.23. Although these estimates were lower than those
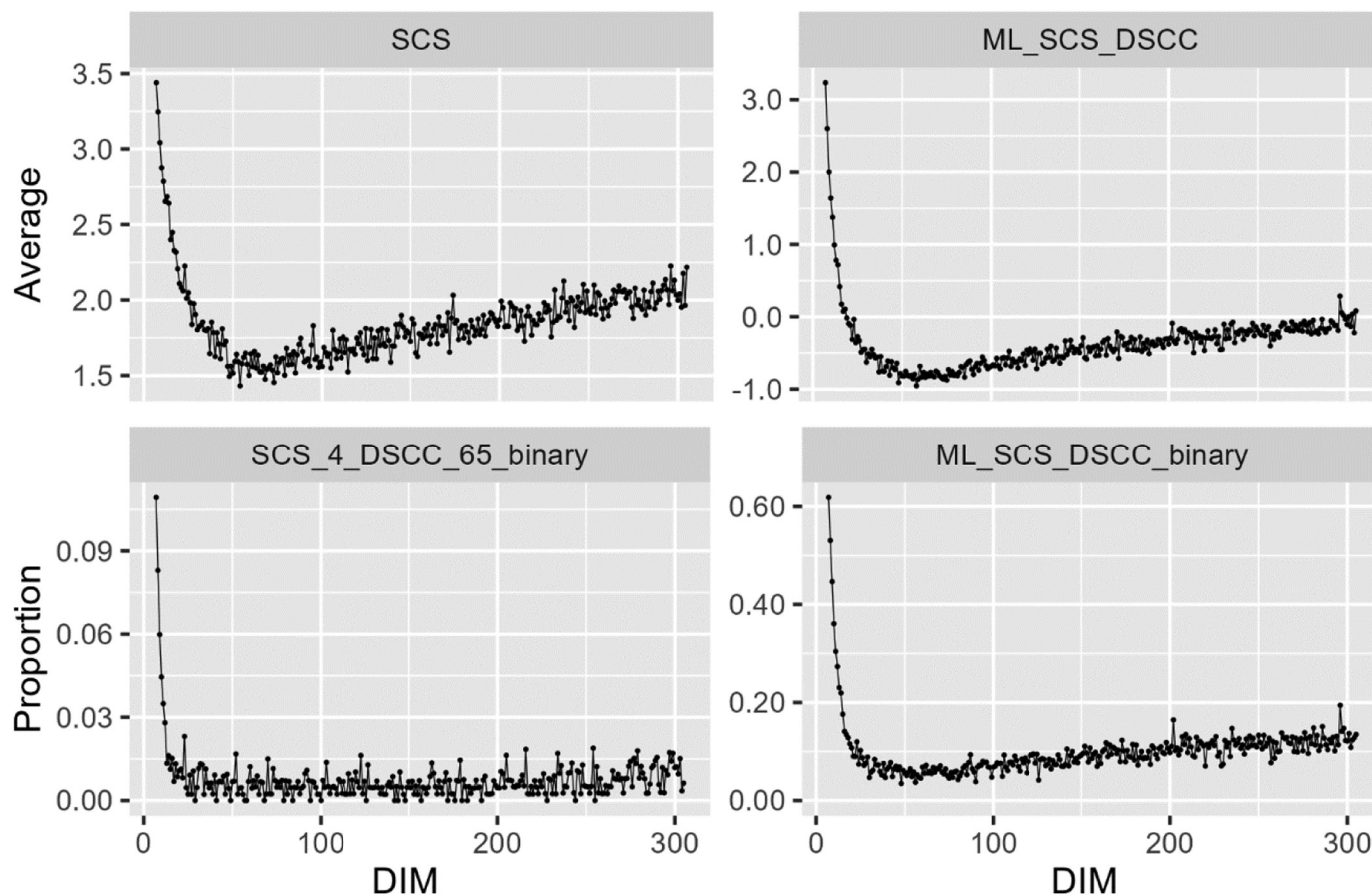
Figure 2. Average values of SCS and ML_SCS_DSCC and the proportions of positive values in SCS_4_DSCC_65_binary and ML_SCS_DSCC_binary across the first lactation.

reported earlier in the Japanese Holstein population (Sasaki et al., 2017), the patterns across lactation exhibited similarities between this and the previous study. The PE variance for SCS peaked at the initial stage of lactation (1.67 at 7 DIM). After reaching a nadir of 1.14 at 42 DIM, it rose slightly toward the end of the lactation, resulting in a lactation mean of 1.33. The observed trajectory for PE aligns with earlier findings (Haile-Mariam et al., 2001b; Mrode and Swanson, 2003). The htd variance of SCS (0.11) was notably lower than its residual variance (0.97), consistent with the observation by Rzewuska et al. (2011). This finding suggests that the difference in management practices between herds might not be the primary source of variations in SCS.

For ML_SCS_DSCC, the daily additive genetic variances ranged from 0.21 (at 55 DIM) to 0.54 (at 7 DIM), with an average of 0.35. The trend resembled that of SCS, albeit with more distinct fluctuations observed across different lactation stages. Regarding PE variances, the highest value (3.74) and lowest value (0.97) occurred at 7 and 67 DIM, respectively, resulting in a lactation mean of 1.37. The PE variances for ML_SCS_DSCC during early

lactation were nearly double those at the end of lactation. This contrast was more pronounced than that for SCS. Several factors have been proposed to influence the subpopulations of somatic cells and the recruitment of PMN during the periparturient period. These factors include periparturient hypocalcemia (Kehrli Jr. and Goff, 1989), blood cortisol level (Burton et al., 1995), heat stress (Alhussien and Dang, 2017), and imbalances in energy, glucose, fatty acids, selenium, and other (micro)nutrients (Ingvartsen and Moyes, 2013; Sordillo, 2016). The interaction of these factors, alongside variations in IMI risk across different environments, likely contributed to the environmental variances observed during early lactation. The htd and residual variances for ML_SCS_DSCC were estimated at 0.17 and 1.47, respectively.

The liability of SCS_4_DSCC_65_binary showed the greatest additive genetic variance at 7 DIM (0.60), which diminished to nearly negligible levels (<0.02) between 50 and 100 DIM and peaked again at 232 DIM with a value of 0.49. The PE variance for this trait peaked at 7 DIM, with an estimate of 4.93. Subsequently, it declined sharply until 50 DIM and rose again toward the end of
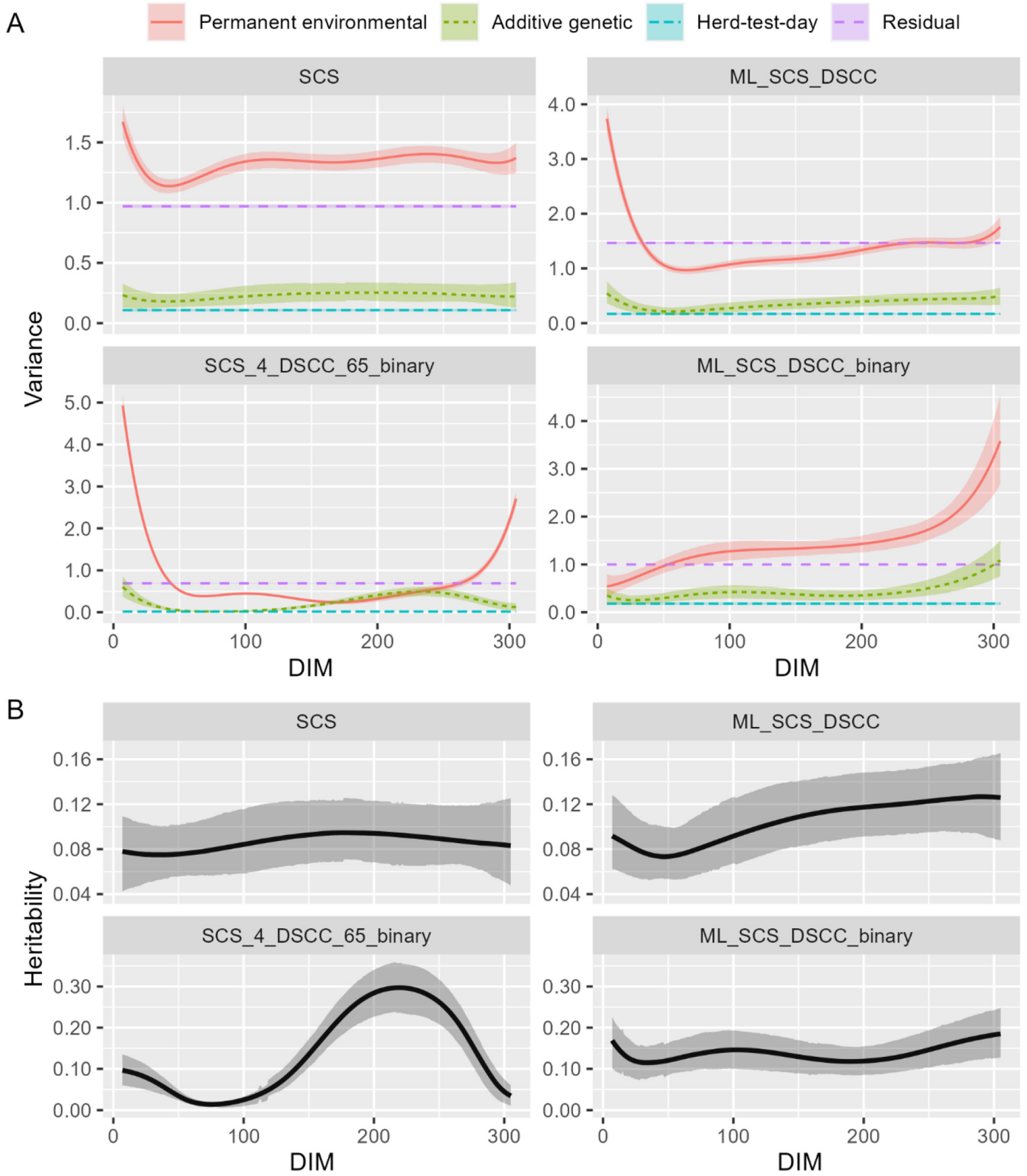
Figure 3. Estimates of variance components (A) and heritability (B) across the first lactation for SCS and our new somatic cell traits, which were defined by combining SCS and the differential SCC (DSCC). Estimates are expressed as the median (line) and the 95% highest posterior density interval (shading) of the posterior distribution for each parameter.

lactation. The htd and residual variances for the trait were estimated as 0.01 and 0.69, respectively. Notably, the residual variance estimate deviated from the expected value of one, the default residual variance for threshold models fitted in Gibbsf90+. This deviation indicated a potential convergence issue, likely related to the low frequency of positive values for the trait. In contrast, the liability of ML_SCS_DSCC_binary had the lowest genetic variance (0.25) at the beginning of lactation and the greatest (1.09) at the end of lactation. The PE variance followed a trend similar to that of the genetic variance, starting with the lowest value (0.54) and gradually increasing to a peak of 3.58 at 305 DIM. The htd and residual variance estimates for ML_SCS_DSCC_binary were 0.18 and 1, respectively. The residual variance estimate was as expected for a threshold model.

Heritability Estimates

We estimated heritability for somatic cell traits across the first lactation (Figure 3B). The lactation means of heritability were 0.086 for SCS, 0.104 for ML_SCS_DSCC, 0.137 for SCS_4_DSCC_65_binary, and 0.138 for ML_SCS_DSCC_binary. The trajectory of heritability estimates differed among traits also. The heritability curve for SCS was relatively flat, with lower estimates in the early lactation stages. As lactation progressed, it reached the nadir of 0.075 at 37 DIM and the peak of 0.095 at 176 DIM. These estimates fell within the range reported by earlier studies on Japanese Holsteins (Hagiya et al., 2014; Nishiura et al., 2015; Sasaki et al., 2017). In contrast, ML_SCS_DSCC had a higher estimate of heritability at 7 DIM than SCS on the same day (0.092 vs. 0.078). After reaching the nadir of 0.073 at 47 DIM, the estimated heritability of ML_SCS_DSCC gradually increased until the peak of 0.127 at 289 DIM.

The heritability estimates of ML_SCS_DSCC were almost always higher than those of SCS. A plausible explanation for the higher heritability estimates is that ML_SCS_DSCC accounts for the residual effects of CM by integrating information from the DSCC. Shook and Schutz (1994) proposed that SCS is limited in its inability to accurately identify CM caused by *Escherichia coli*. This environmental pathogen often triggers a dramatic but transient surge in SCS, a phenomenon that the current monthly DHI sampling may be inadequate to capture. Conversely, our observations revealed that, during the healing process of mastitis, a decrease in SCS coincided with a decrease in DSCC (Huang and Kusaba, 2022), implying that ML_SCS_DSCC might persist or even increase in the advanced stages of mastitis (Figure 1). This characteristic potentially enhanced its effectiveness in identifying CM events within the monthly sampling

framework. In a nutshell, the reduction in environmental variances, especially for those caused by sampling intervals, potentially contributed to the higher heritability estimates of ML_SCS_DSCC.

The heritability estimates for the liability of SCS_4_DSCC_65_binary exhibited an erratic pattern, reaching a nadir of 0.014 at 76 DIM and a peak of 0.297 at 289 DIM. Misztal (2006) argued that the reliability of genetic parameters determined by RRTDM depends heavily on data distribution, meaning that the estimates likely contain artifacts at time points where data are scarce. In the case of SCS_4_DSCC_65_binary, test-day records with positive values were scarce within 50 to 250 DIM (Figure 2). Further investigation is necessary to adjust the thresholds for SCS and DSCC to stabilize estimates. Alternatively, RRTDM can be performed using linear splines to enable more reliable estimates, as demonstrated in a longitudinal analysis of CM (Negussie et al., 2012). In contrast, the heritability curve for the liability of ML_SCS_DSCC_binary was more reasonable than that for SCS_4_DSCC_65_binary, falling between 0.115 at 33 DIM and 0.185 at 305 DIM. This is attributable to the relatively high frequency of 1 for the trait (Figure 2).

Genetic Correlations Within Somatic Cell Traits

We plotted the within-trait genetic correlation curves between the selected DIM and the remaining DIM, with 10, 155, and 275 DIM selected to represent early, middle, and late lactation, respectively (Figure 4). The within-trait genetic correlations for SCS were high between adjacent DIM but decreased as the DIM interval widened, especially for those between the initial and later stages of lactation. Nonetheless, the lowest genetic correlation for SCS, between 10 and 165 DIM, remained fairly high (0.56). This finding agrees with previous observations for SCS in the first lactation (Liu et al., 2000; Koivula et al., 2004; Mrode et al., 2012) and suggests that selection based on the lactation average of SCS can effectively reduce SCS at any stage of lactation.

The within-trait genetic correlations for ML_SCS_DSCC were lower than those for SCS, particularly between the initial and later stages of lactation. The estimate was as low as 0.10 between 10 and 305 DIM, suggesting that ML_SCS_DSCC in early lactation should be considered a distinct trait. Importantly, a test-day record with high SCS and low DSCC, indicating an increase in the presence of macrophages in milk, may represent distinct situations according to the lactation stage. This situation might be a physiological state in the periparturient period, where PMN recruitment is impaired because of the high level of cortisol around calving, leading to increased susceptibility to CM in this period (Burton
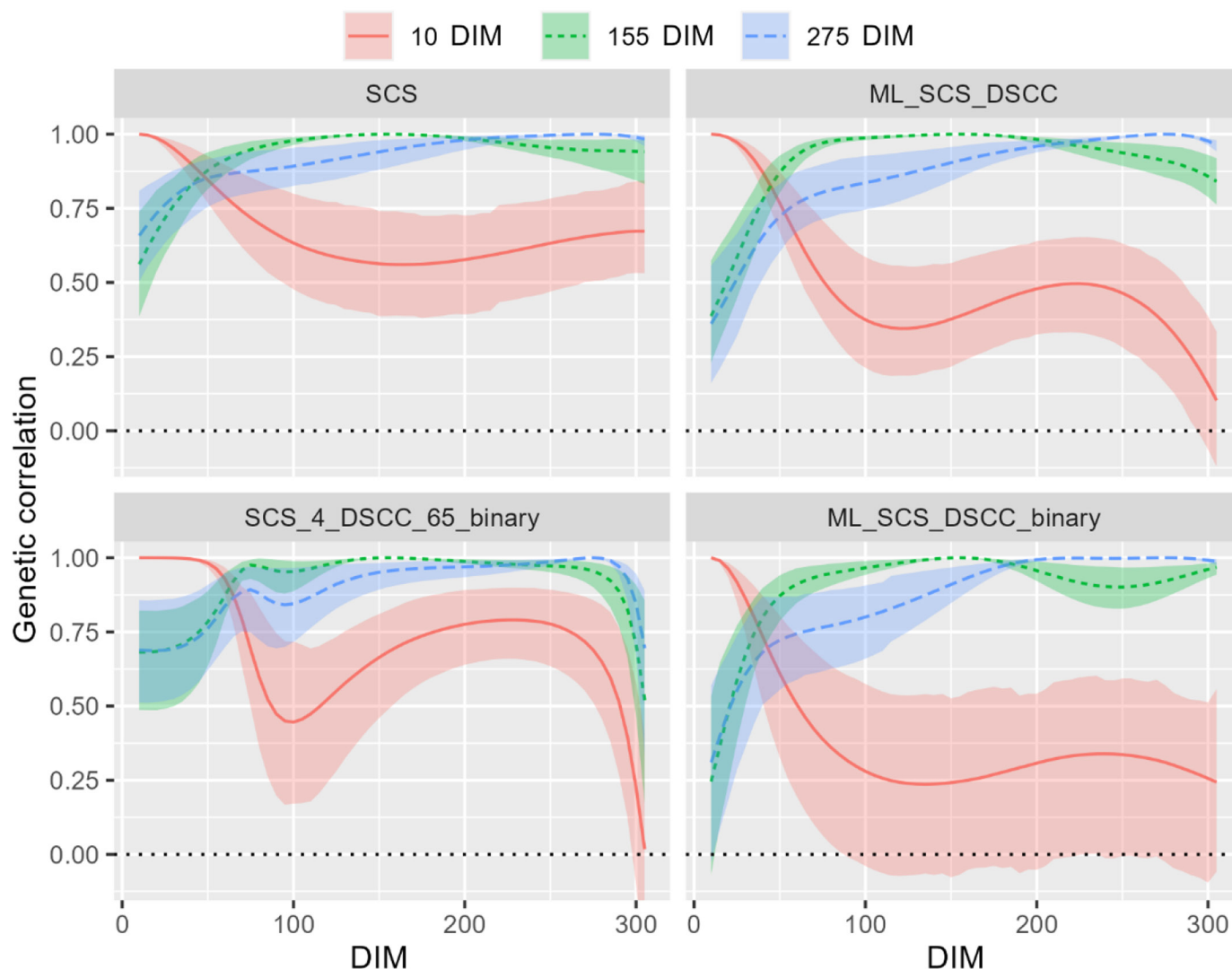
Figure 4. Estimates of genetic correlations between the given DIM and other time points during the first lactation for SCS and our new somatic cell traits. The new traits were defined by combining SCS and the differential SCC (DSCC). Estimates are expressed as the median (solid line) and the 95% highest posterior density interval (shading) of the posterior distribution.

and Erskine, 2003). Alternatively, the unique situation might be, as we mentioned earlier, the residual effect of CM during mid- and late lactation, which damaged the mammary glands and prompted the recruitment of macrophages for wound healing. The susceptibility of cows to CM and their ability to recover from CM are genetically different traits (Welderufael et al., 2017) that are controlled by distinct sets of genes (Welderufael et al., 2018). This genetic differentiation could explain the observed low genetic correlations between stages of lactation for ML_SCS_DSCC.

The within-trait genetic correlation curves for SCS_4_DSCC_65_binary were erratic, possibly reflecting the scarcity of positive records in specific time intervals (Figure 2). The within-trait genetic correlation curves

for ML_SCS_DSCC_binary resembled those for ML_SCS_DSCC but were accompanied by wider 95% highest probability density intervals.

Genetic Correlations Between Somatic Cell Traits and Milk Production Traits

We examined the genetic correlations between SCS, the newly defined traits, and milk production traits on the same DIM in the first lactation (Figure 5). The average genetic correlations between SCS and milk production traits throughout lactation were 0.01 with milk yield, 0.00 with protein yield, 0.10 with fat yield, and −0.04 with lactose yield. These estimates were close to zero and consistent with those estimated using a repeatability
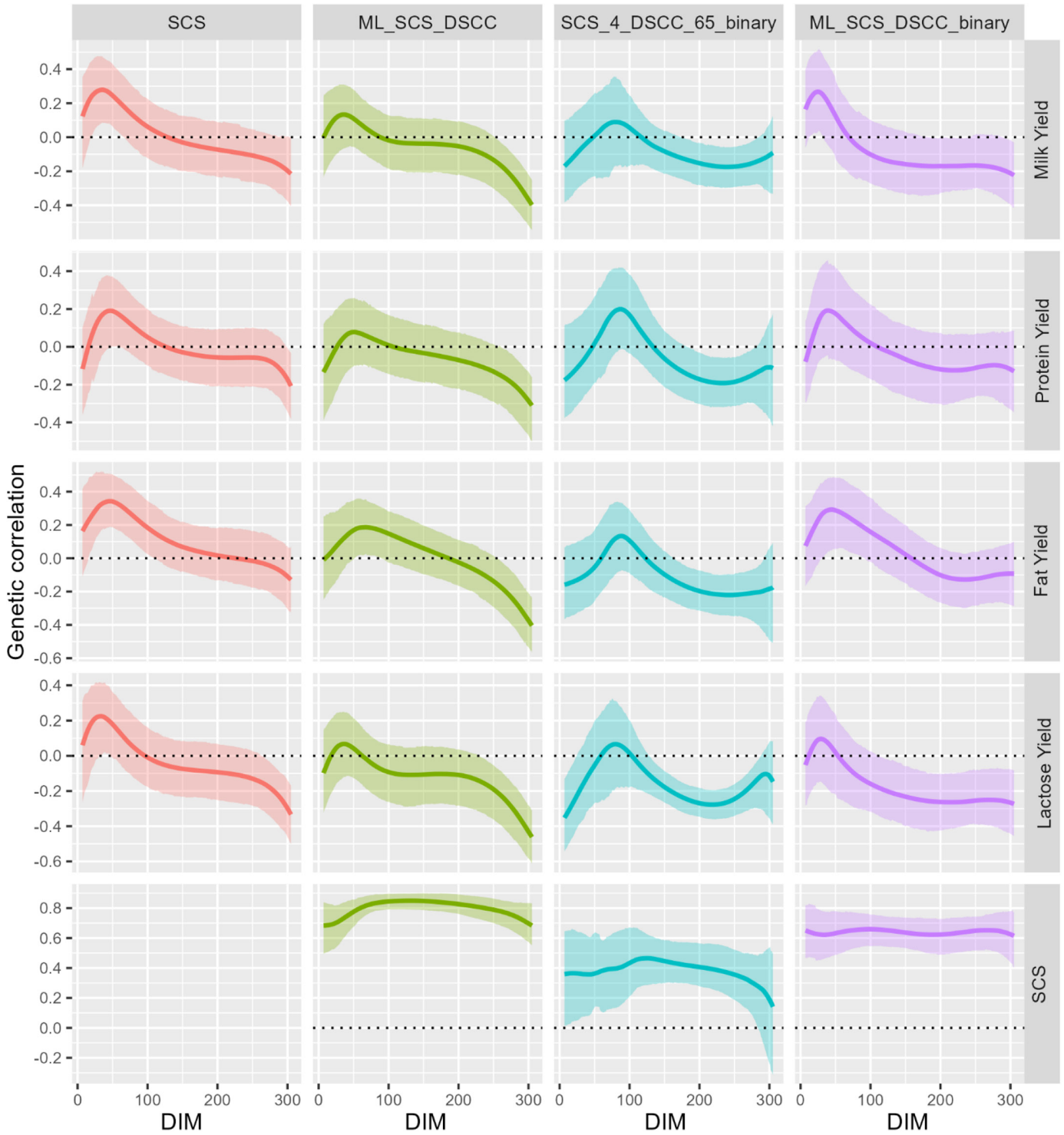
Figure 5. Genetic correlations among SCS, newly defined traits, and milk production traits on the same DIM across the first lactation. The new traits were defined by combining SCS and the differential SCC (DSCC). Estimates are expressed as the median (solid line) and the 95% highest posterior density interval (shading) of the posterior distribution.

model (Sneddon et al., 2015; Costa et al., 2019b). The trajectories of daily genetic correlations between SCS and yield traits were similar. For brevity, we focus here on the genetic correlation trajectory between SCS and milk yield. The daily genetic correlations reached a peak of 0.28 at 35 DIM and then gradually declined, reaching the lowest value of −0.22 at 305 DIM. This trend is consistent with previous findings (Haile-Mariam et al., 2001a; Yamazaki et al., 2013), where the genetic correlations changed from positive to negative as lactation progressed.

Compared with SCS, the average genetic correlations between ML_SCS_DSCC and production traits were slightly more negative: −0.05 for milk yield, −0.06 for protein yield, 0.00 for fat yield, and −0.12 for lactose yield. These negative correlations imply that selecting cows with low ML_SCS_DSCC would have a more favorable influence on milk production than would selection based on SCS. The daily genetic correlations between ML_SCS_DSCC and milk yield reached a peak of 0.13 at 36 DIM and then gradually declined until a nadir of −0.40 at 305 DIM. Notably, the negative genetic correlations between ML_SCS_DSCC and milk yield became significant at 250 DIM, suggesting that genetic selection based on ML_SCS_DSCC would have better impacts on milk production later during lactation. This effect could consequently enhance the longevity of dairy cows owing to the strong correlation between milk yield and survival rate in late lactation stages (Sasaki et al., 2017). Haile-Mariam et al. (2001a) proposed that the negative genetic correlations between SCS and milk yield in late lactation stages were likely related to chronic infection with major pathogens. Major pathogens such as *Staphylococcus aureus* and environmental *streptococci* induce long-term elevations in SCS, causing damage to the udder and substantial milk loss (Gonçalves et al., 2020). Therefore, cows with resistance to chronic infections caused by such pathogens are deemed to have lower SCS and greater milk yield during later lactation stages. This rationale holds for ML_SCS_DSCC, particularly considering that the trait can more effectively identify cows with impaired milk yield due to chronic infection than can SCS (Table 1).

The average genetic correlations between SCS_4_DSCC_65_binary and yield traits were −0.08 for milk yield, −0.06 for protein yield, −0.11 for fat yield, and −0.15 for lactose yield. The daily genetic correlations between milk yield and SCS_4_DSCC_65_binary were negative during the initial and later stages of lactation, reaching a peak of 0.09 at 79 DIM and a nadir of −0.17 at 240 DIM. We expected the negative genetic correlations across most lactation stages, given that milk production was compromised in cows with positive values of SCS_4_DSCC_65_binary (Table 1). In addition, most

of the average genetic correlations between ML_SCS_DSCC_binary and yield traits were negative: −0.08 for milk yield, −0.03 for protein yield, 0.04 for fat yield, and −0.17 for lactose yield. The daily genetic correlation between ML_SCS_DSCC_binary and milk yield was positive in the early lactation stage but became negative near 75 DIM and remained around −0.2 in the middle and late lactation stages.

Genetic Correlations Between SCS and the Newly Defined Traits

We also estimated the genetic correlations between SCS and the 3 newly defined traits on the same DIM in the first lactation (Figure 5). On average, ML_SCS_DSCC had the most favorable genetic correlation with SCS (0.80), followed by ML_SCS_DSCC_binary (0.64); SCS_4_DSCC_65_binary exhibited the lowest genetic correlation with SCS (0.38). The curve of genetic correlations between ML_SCS_DSCC and SCS was relatively flat, with the lowest values (both 0.68) at the beginning and end of lactation. The consistent positive correlations between the 2 traits suggest that selecting cows with low ML_SCS_DSCC likely reduces SCS, regardless of the lactation stage. Moreover, due to the higher heritability of ML_SCS_DSCC (Figure 3), selection on ML_SCS_DSCC would indirectly reduce SCS to a similar degree as direct selection on SCS.

CONCLUSIONS

ML_SCS_DSCC had higher heritability than SCS and favorable genetic correlations with both milk production traits and SCS. This indicates that genetic selection on ML_SCS_DSCC can efficiently reduce SCS levels while improving milk production simultaneously. Selection based on this trait retains cows with high SCS and high DSCC, indicative of effective leukocyte recruitment, thus alleviating concerns regarding possible negative consequences of selection for low SCS alone on cows' innate immunity. Alternatively, ML_SCS_DSCC_binary is easily derived using the proposed formula and provides reasonable estimates of genetic parameters comparable to those of ML_SCS_DSCC. Our results suggest that ML_SCS_DSCC or ML_SCS_DSCC_binary have the potential to improve current genetic evaluations of udder health, although their genetic relationships with IMI and CM require further investigation.

NOTES

Abbreviations used: CM = clinical mastitis; DSCC = differential somatic cell count; FY = fat yield; GAM = generalized additive model; htd = herd-test-day; LY = lactose yield; ML = milk loss; MY = milk yield; NARO = National Agriculture and Food Research Organization; PE = permanent environmental effect; PY = protein yield; RRTDM = random regression test-day model; WCGALP = World Congress on Genetics Applied to Livestock Production.

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