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Characterization of a novel heat tolerance trait and subsequent haplotype block-based analysis to identify associated regions in Dutch Holstein cattle

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ABSTRACT

Heat stress is a major environmental challenge affecting dairy cattle, leading to behavioral changes, production losses, and welfare concerns. As heat stress events intensify and become more frequent due to climate change, identifying animals that are able to maintain high production levels during heat stress events, commonly referred to as heat tolerance, is crucial for sustainable dairy production. In this study, we develop a pipeline to quantify the population-wise impact of heat stress on a dairy cattle population and subsequently define individual-based heat tolerance traits. Data from 677,318 Dutch Holstein cows were analyzed, including 15.6 million mid-infrared spectra and 762 million records from automated milking systems. An iterative approach using kernel regression was employed to estimate the population-wise effects of heat stress. Results indicate that fat and protein percentages decrease approximately linearly with increasing temperature-humidity index (THI), with an absolute reduction of 0.3% as THI increases from 30 to 70. In contrast, milk yield remains stable until a THI of 60, after which production losses increase quadratically, reaching a loss of 5.0% at a THI of 75. We subsequently define the heat tolerance phenotype of an animal as the slope from a linear regression model of the residuals of the population-wise models against THI for milk yield, concentration of fat, protein, lactose, and specific fatty acids. Compared with reaction-norm models, individual records per cow are combined into one joint record before model fitting, thus reducing computing times and allowing more flexibility in the design of the model. Heritabilities for heat tolerance traits ranged from 0.05 to 0.12, and genetic variances indicate substantial potential for breeding as an improvement of the population by 1 genetic standard deviation would already offset 69% of the losses in fat percentage, 65% in protein percentage, and 11% in milk yield. Heat tolerance based on milk yield showed favorable correlations with most commercial traits, whereas heat tolerance based on fat and protein percentage showed negative correlations with health and resilience. A GWAS using both SNPs and haplotype blocks from the software HaploBlocker identified potential QTL across the genome, with particularly strong signals on BTA5, 14, and 20. These findings support the long-term potential of genetic improvement through breeding for heat tolerance but highlight the need for complementary management strategies to mitigate heat stress impacts in the short term.

Key words: heat tolerance, heat stress, haplotype block, GWAS

INTRODUCTION

Heat stress is an important environmental challenge that affects dairy cattle and has profound physiological impacts resulting in behavioral changes, productive losses, and animal welfare issues (Marino and Allen, 2017; Herbut et al., 2021). As global temperatures continue to rise due to climate change (Cheng et al., 2022), the frequency and intensity of heat stress events are projected to increase, exacerbating these impacts (Pörtner et al., 2022). This, in turn, causes economic losses, not only in dairy cattle production, but also in other livestock species worldwide (St-Pierre et al., 2003; Ross et al., 2015; McManus et al., 2020).

Various measures to combat heat stress have been developed across different domains of the animal production sectors. This includes adapting nutrition strategies to optimize diets and enhance energy availability to reduce metabolic heat production (Ríus, 2019), as well as modifying housing and management systems to implement cooling strategies (Johnson, 1987; D'Emilio et al., 2017), such as the use of fans, sprinklers, or shade structures.

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

In this manuscript, we will focus on the potential of genetic improvement through breeding to improve heat stress adaptation. Genetic differences in heat stress adaptation across breeds are well established (Copley et al., 2024) and are used in crossbreeding when introducing genetic material to low-production environments while maintaining local adaptation (Jordan, 2003; Michael et al., 2021). However, relatively little attention in applied breeding is given to within-population differences. Australia is the only country that explicitly includes heat tolerance in its selection index (Nguyen et al., 2016), whereas the Netherlands incorporates a general resilience trait in the dairy cattle breeding goal (Poppe et al., 2022). Although genetic improvement has less of a short-term impact, it remains an important consideration for a long-term sustainable breeding goal (Ravagnolo and Misztal, 2000; Garner et al., 2016).

Heat stress in this context is defined as an environmentally challenging condition. The most common way to quantify this condition is the use of weather data such as the daily peak temperature or humidity (National Research Council, 1971). To quantify the challenge level of an animal, potential indicator traits such as increased rectal temperature and respiratory rate can be used (Li et al., 2020; Yan et al., 2021). For the purpose of breeding, it is required to define a trait to describe how well the animal is responding to heat stress, aiming either for heat tolerance or heat resilience (Misztal et al., 2024). In essence, heat tolerance describes the ability of an animal to maintain its production levels during heat stress, whereas heat resilience describes the ability of an animal to quickly return to its production level after heat stress.

As the large-scale recording of rectal temperature and respiratory rate is impractical in the commercial setting due to high cost, more and more attention is given to the use of routinely collected traits such as milk yield to measure the impact of heat stress across lactations, weather conditions, and production environments to model genotype by environment interactions (**GxE**; Kipp et al., 2021; Vinet et al., 2023). Currently, 2 main approaches are used in animal breeding to model GxE interactions, and more specifically, heat stress adaptation. First, multitrait models are applied, where traits recorded in different environments are modeled as being genetically correlated traits (Falconer, 1952). Practical analysis suggests measurable benefits in this approach when genetic correlations fall below 0.8 (Copley, 2024). Second, reaction-norm models are employed to estimate interaction effects between traits and environmental conditions (Calus and Veerkamp, 2003; Kolmodin et al., 2002). Weather conditions have been shown to strongly affect fertility traits (Ojo et al., 2025), but correlations between milk yield traits in different weather conditions are usually high, failing to separate heat tolerance from overall production levels (Aguilar et al., 2010).

On a technical level, a reaction-norm model is a linear mixed model (Henderson, 1975) with this or a similar structure (Su et al., 2006):

$$y = \mathbf{X}b + \mathbf{Z}_u u_0 + \mathbf{H}_u u_h + \mathbf{Z}_a a_0 + \mathbf{H}_a a_h + e,$$

where **X**, **Z**, and **H** are incidence matrices for the fixed effects (b), breeding values for the intercept (a_0) and slope (a_h) of the reaction to environmental conditions, and nongenetic components u_0 and u_h that are modeled as random effects. Here, intercept and slope components are usually modeled as correlated in a multivariate normal distribution with the respective correlations σ_{u_0,u_h} and σ_{a_0,a_h}. The interested reader is referred to Carabano et al. (2017) for an extended overview of reaction-norm models.

The aim of this study is to develop a pipeline for the estimation of the population-wise and individual-based impact of heat stress on milk traits in dairy cattle. A further aim for this pipeline is to be both computationally efficient and able to include nonlinearity and interaction between parameters. Based on this, we are defining novel heat tolerance traits and critically assessing the potential of genetic improvement through breeding by estimating variance components, identifying associated regions by use of a GWAS, and conducting a haplotype block-based analysis.

MATERIALS AND METHODS

The pipeline developed in this study is a combination of various individual steps that are building on each other, starting with an estimation of the population-wise effects of heat stress, to subsequently define individual-based phenotypes, and lastly perform quantitative analysis on the phenotypes. In the following paragraphs, these steps are described in detail with a schematic overview of the pipeline provided in Figure 1. All analyses were conducted using R (R Core Team, 2017) with exemplary code for the individual steps provided in Supplemental Files S1–S4 (see Notes).

Materials

Data from the Dutch dairy cattle breeding company CRV (Arnhem, the Netherlands) were used, including 677,318 animals from 1,478 farms spread across the Netherlands and covering the period from 2013 to 2021. Of these animals, 348,133 were genotyped using different genotyping arrays, primarily using the Illumina

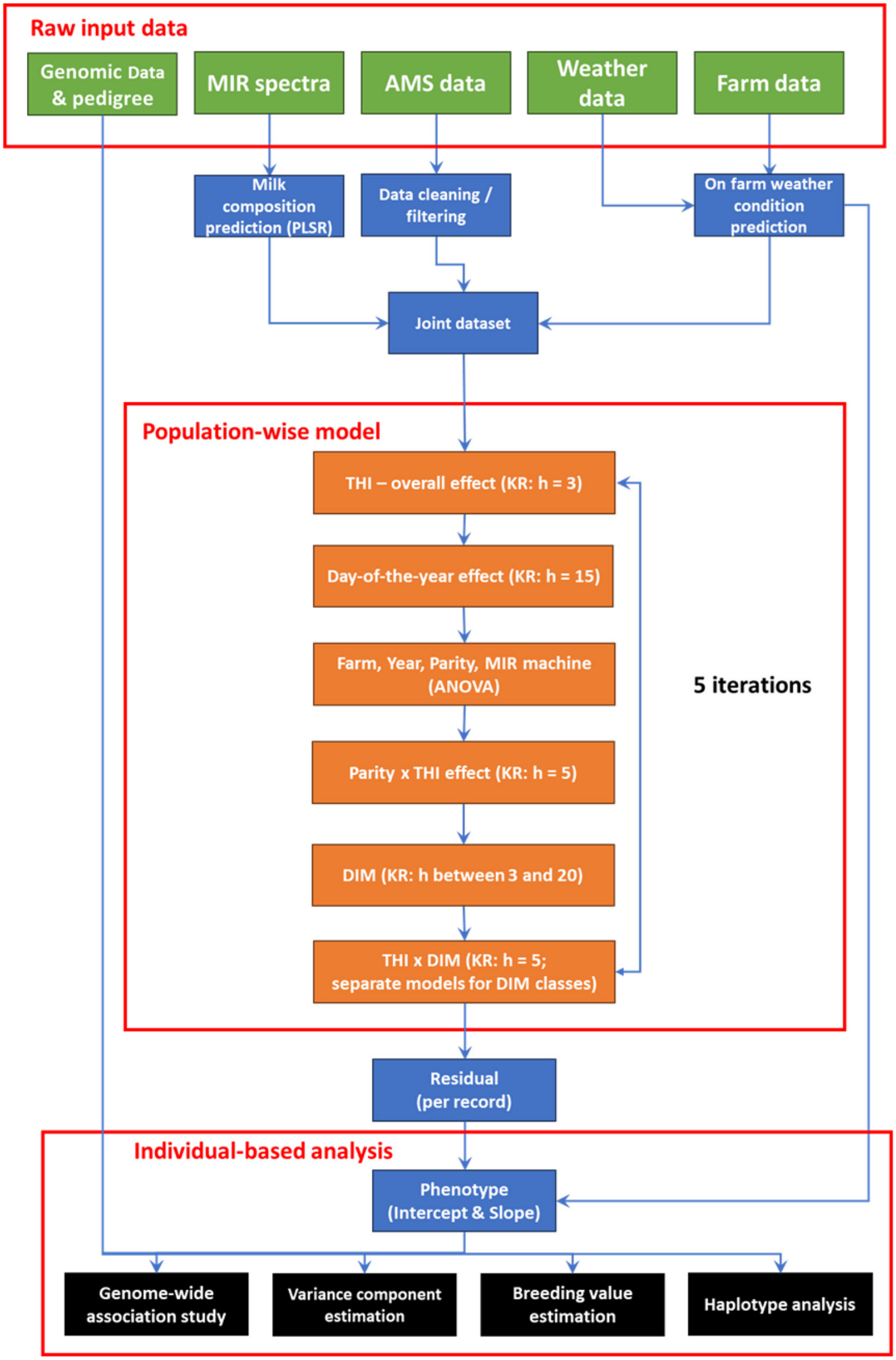

Figure 1. Schematic overview of the processing pipeline for the estimation of population-wise effects of heat stress and subsequent individual-based analysis. PLSR = partial least squares regression.

EuroG MD chip (EuroGenomics, The Hague, the Netherlands), and subsequently imputed to obtain a joint panel of 76,438 SNPs. Furthermore, 5,322,943 animals were included in the pedigree, tracing back up to 30 generations until 1910. Mid-infrared (**MIR**) spectra were generated through routine milk recording procedures (ICAR, 2023) by QLIP (Zutphen, the Netherlands) with spectra per cow being generated on a monthly basis, resulting in a total of 15,596,136 MIR spectra. Although no milk composition data were available for this dataset, estimates for fat percentage (**F%**), protein percentage (**P%**), lactate, and individual fatty acids were estimated using a smaller separate dataset including 1,740 MIR spectra with the respective information, using partial least squares regression (Soyeurt et al., 2006). Details on the estimation procedure are provided in Supplemental File S5 and Supplemental Figures S1 and S2 (see Notes). Furthermore, milk yield data were collected using automated milking systems (**AMS**) with 762,173,326 records from individual milkings across 934 farms.

For all subsequent analyses, the dataset was reduced to a panel of animals for which data from all data sources were simultaneously available in sufficient quantity (at least 5 MIR spectra per animal), resulting in a panel of 5,929,221 MIR spectra from 346,248 animals across 772 farms (Table 1). No animal procedures were conducted specifically for this study. All data were obtained retrospectively from routine recording as part of standard herd management practices. As such, no additional ethical approval was required.

Meteorological data from the Copernicus Climate Change Service (**C3S**; Copernicus Climate Change Service, 2024) were used, gridded at a 0.1-degree resolution for longitude and latitude, with hourly records on temperature, precipitation, wind, pressure, and humidity based on satellite observations, ground-based weather stations, and climate models. For reference, 0.1 × 0.1 degrees corresponds to an ~11 × 7 km grid in the Netherlands, providing a much finer grid than the use of weather stations from the Royal Dutch Meteorological Institute, as used in Ojo et al. (2025), to derive the temperature-humidity index (**THI**; National Research Council, 1971). Within C3S, hourly weather records are merged into a daily average that is subsequently used. Weather data on farm was approximated using the weather conditions of the closest data point based on the global positioning system coordinates of the farms (Figure 2). Because the Netherlands is very flat and the used grid is narrow, these values should be highly representative for each specific farm. It should be taken into account that for more hilly regions, a more sophisticated approach to combine data from multiple weather stations in the area (Gote et al., 2024) or weather recording on farm might be necessary. Of all records, 22.7% were in conditions above THI = 60, and only 1.03% were above THI = 70. For instance, in 2017, there were essentially no extreme heat waves with conditions above THI = 70. Whereas MIR spectra were approximately equally distributed across the years from 2013 to 2020, fewer records were available in the

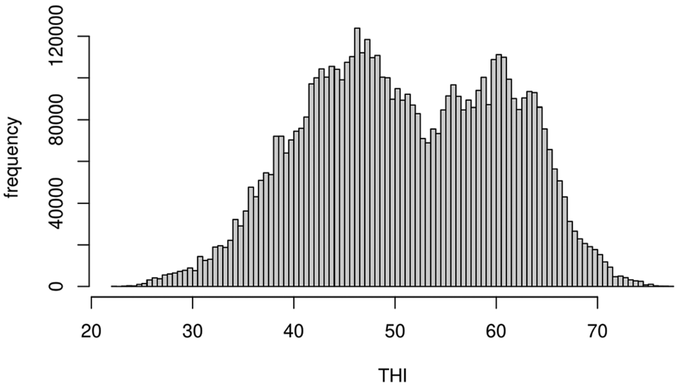

Figure 2. Distribution of weather conditions based on THI of the last 3 d (including the same day) for the used MIR records based on the Copernicus Climate Change Service (Copernicus Climate Change Service, 2024) and global positioning system coordinates of farms.

Table 1. Overview of the dataset used

Year	Number of spectra	Number of animals	Number of farms	Records (THI >60)	Records (THI >70)
2013	679,485	210,399	738	138,839	7,111
2014	700,179	220,893	749	145,835	4,204
2015	743,947	235,003	759	124,920	3,206
2016	817,012	255,491	762	216,088	10,514
2017	719,829	255,491	764	180,272	1,260
2018	717,618	245,471	765	230,861	11,114
2019	708,815	239,885	758	160,580	14,473
2020	571,811	248,317	752	111,043	8,588
2021	270,524	247,990	745	39,599	393

summer of 2021, resulting in just 15.9% of records being above THI = 60 in 2021.

Preprocessing of Data

Before conducting the main analyses, we performed preprocessing of data to ensure its consistency and reliability. This involved calculating THI values, filtering milk yield data, and applying statistical smoothing techniques.

The THI was calculated from the weather data using the average daily temperature (T, in °C) and the relative humidity (RH; National Research Council, 1971) using the following equation:

$$\mathrm{THI} = (1.8\cdot \mathrm{T} + 32) - [(0.55 - 0.0055\cdot \mathrm{RH})(1.8\cdot \mathrm{T} - 26)].$$

In all subsequent models, the average THI from the last 3 d (including the current day) was used in accordance with Mattalia et al. (2023). Herafter, THI will refer to the average THI from the last 3 d.

The AMS data were aggregated to calculate daily milk yield per animal. Basic data cleaning protocols, similar to those used at CRV for routine genetic evaluations, were employed to avoid double-counting of records, among other issues. Additionally, strict filters were applied to focus on normal lactations, only considering lactations with an overall length between 200 and 450 d and a daily milk yield greater than 0 and less than 60 L for at least 95% of the days in lactation. If milk yield changed by more than 10 L from one day to the next, records from one week before and after these cases were excluded, as no reliable health data were available to correct for such outliers. Additionally, the first 10 d and the last 5% of lactation days were excluded to avoid issues with the fit of the lactation curve.

Based on the remaining data, the lactation curve l for each individual cow was estimated using a kernel regression-based approach, using a Nadaraya–Watson estimator (**NW**; Nadaraya, 1964). The conceptual idea of kernel regression is to calculate a conditional expectation for a given time point t (in this case, number of DIM). For this, a weighted average of all records of a cow is computed in which records with a more similar number of DIM are weighted more strongly, as follows:

$$l\left(t\right) = \frac{\sum_{\mathrm{i}} y_{t_i} K\left(t - t_i\right)}{\sum_i K\left(t - t_i\right)},$$

where t_i and y_{t_i} correspond to the number of DIM and milk yield of a given record i, and K is a kernel function to control the weighting of each record. Weightings of records are derived from a Gaussian distribution, as follows:

$$K_{expected}(x) = \phi_{0,25}(x),$$

where $\phi_{\mu,\sigma}$ is the density of a Gaussian distribution with mean μ and standard deviation σ. In the context of kernel regression, σ is commonly referred to as the bandwidth of the kernel function, with a high bandwidth relative to the range of possible values leading to a smoother fit as weights are split more across records. For our purposes, x corresponds to the time-wise distance between a record t_i and the time point t for which we want to perform an estimation.

Subsequently, we want to compare the current performance of a cow against its expected performance based on its lactation curve. Even to determine the current performance, smoothing via kernel regression is applied to reduce random daily fluctuations. In comparison to the lactation curve, a much smaller bandwidth is used to put more emphasis on records on the same day and nearby days. Importantly, only past and present records are included, for instance, to avoid including records from a heat period immediately after:

$$K_{current}(x) = \phi_{0,3}(x)\cdot 1_{x>0}.$$

We express the current relative performance of an animal as the ratio between the current performance from kernel regression against the expected performance, corresponding to the ratio of l evaluated either with the $K_{expected}$ and $K_{current}$ kernel function. Instead of using the aggregated fat or protein yield in kilograms, we here consider the product of F% and P% on a test day with the current relative performance of milk yield to focus on the relative changes in production on an individual level, which will subsequently be referred to as relative yields of fat and protein (**RY_F** and **RY_P**). No smoothing was applied to F% and P% estimates because only monthly test day records were available from MIR.

Population-Wise Effects of Heat Stress

For the estimation of population-wise effects of heat stress, a combination of parametric and nonparametric modeling approaches was used that was fitted iteratively by using the residuals of the previous step as the response variable in the next step, with a schematic overview of all effects included given in Figure 1. Source code for the derivation of population-wise effects of heat stress on milk yield is given in Supplemental File S1 (see Notes).

First, the overall effect of THI was estimated using an NW estimator with a bandwidth of 3. Subsequently, a day of the year effect was estimated using an NW estimator with a bandwidth of 15. Next, fixed (class) effects for farm, year, parity, and machine (used to generate the MIR spectra) were estimated using a linear regression model. Parities 4 and higher were considered as a joint class. Next, THI by parity (THI × parity) effects were estimated using 4 separate NW estimators, in which only reports from parity 1, 2, 3, or ≥4, respectively, were used. Because fewer records were available compared with the overall THI effect, a bandwidth of 5 was used. Next, an effect for DIM was fitted again using an NW estimator. For DIM ≤350, a bandwidth of 3 was used, but because fewer observations were available later in lactation and only limited differences in this phase are expected, a bandwidth of 20 was used for DIM >350. Lastly, an interaction effect between THI and DIM (THI × DIM) was fitted by assigning observations to separate DIM classes, for which separate THI effects with a bandwidth of 5 were estimated: 30 or fewer days, 31 to 100 d, 101 to 200 d, 201 to 300 d, and 301 or more days.

This iterative procedure was repeated 5 times. Subsequently, THI × DIM and THI × parity effects were centered around zero by subtracting the weighted mean of the effects for the different classes of DIM and parity (based on frequency). The subtracted effect from centering was added to the overall THI effect.

The aforementioned bandwidths were primarily chosen based on visual inspection of the obtained fitted curves to avoid unstable or overfitted estimates in regions with limited records while still obtaining a good local fit. Larger (future) datasets may allow for the use of smaller bandwidths and reduce potential prediction biases, whereas smaller datasets might require increased bandwidth to reduce prediction variance (Hassanpour et al., 2023). Initially, models included an adaptive choice of bandwidth to use larger bandwidths in areas with fewer observations; however, this was later dropped to simplify models and stabilize prediction in areas with a lower number of observations (Brockmann et al., 1993). No multivariate NW estimators (Nadaraya, 1964) were used because this would have required an increase of bandwidths to obtain similar robustness as the number of observations in extreme THI values was limited.

Individual-Based Heat Tolerance

After estimating population-wise effects of heat stress, we defined heat tolerance traits by modeling residual variations at the individual level. The individual phenotype of an animal for our heat tolerance trait is defined based on its relative performance compared with the expected performance estimated using the previously derived population-wise model.

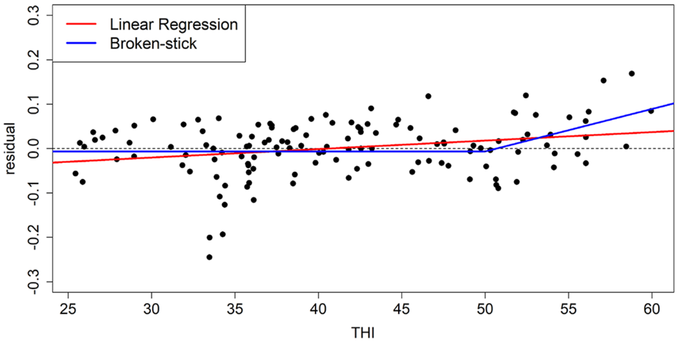

Figure 3. Example visualization of the calculation of the phenotype for heat tolerance based on milk yield. Dots represent individual observations for the same cow.

To do this, the finally obtained residuals of the animal from the population-wise models are regressed against THI in a linear regression model, with the obtained slope of the regression model being the phenotype for the heat tolerance trait. The THI values were adjusted by 50 so that the intercept of the regression model represents the performance of an animal at THI = 50, which should represent its performance at thermoneutral conditions with the average THI of 51.1 across all samples and 47.8% of all records being generated at THI >50. An exemplary visualization showcasing the fitting procedure for a single cow is given in Figure 3. Thereby, for each of 17 milk components considered (F%, P%, milk yield [**MY**], lactose, and individual fatty acids) a separate heat tolerance trait is defined. Note that intercept and slope are explicitly not the result of a random regression model in which population-wise effects and intercept and slope are jointly estimated as in a reaction-norm model (Su et al., 2006).

To avoid a strong impact from records in cold weather conditions and to more reliably estimate the performance in thermoneutral conditions, a second version of each trait was considered in which observations below a THI threshold were set to that value, subsequently referred to as a broken-stick model. In the following, we used a threshold of THI = 50 to ensure a sufficient number of records above the threshold while still obtaining a reliable estimate of the performance in thermoneutral conditions. Only cows with a minimum of 5 records (or 50 in the case of milk yield) were given a phenotype in order to exclude cows for which no reliable estimation of the regression coefficients was possible.

The source code for the derivation of both heat tolerance phenotypes for milk yield is given in Supplemental File S2 (see Notes).

Variance Component Estimation

To assess the potential of using the previously defined heat tolerance traits for breeding, variance components and their resulting heritabilities were estimated using univariate linear mixed models using ASRemlR (Butler et al., 2009), including a farm effect and the performance in thermoneutral conditions (intercept) as covariates. For the random effect, a pedigree-based relationship was computed. In the pedigree, all phenotyped animals and ancestors from at most 3 generations prior were included. To increase model robustness, phenotypes with an absolute value above the 99% quantile were set to the 99% quantile with the appropriate sign to reduce the impact of extreme phenotypes. Genetic correlations between heat tolerance traits were subsequently estimated pairwise in bivariate models based on pedigree using ASRemlR (Butler et al., 2009). The source code used for all analyses is available in Supplemental File S3 (see Notes). Genetic correlations to other important breeding goal traits were estimated based on multiple across-country evaluation correlations (Schaeffer, 1994) following the approach described in Poppe et al. (2022).

Genome-Wide Association Study

In addition to the 76,438 SNPs included in this study, haplotype blocks derived using the software HaploBlocker (Pook et al., 2019) were considered in a GWAS. In short, a haplotype block in HaploBlocker is a specific sequence of alleles and only those individuals that carry this sequence in one of its 2 haplotypes (phased genotype) is carrying the given haplotype block. This effectively screens the population for cases of group-wise identity by descent (Donnelly, 1983), allowing haplotype blocks to overlap with potentially unique start and end points of each haplotype block, leading to longer block structures than identified with conventional haplotype block detection approaches (Barrett et al., 2005).

For this, genotypes were first phased using Beagle v5.4 (Browning et al., 2021; ne = 1,000; Pook et al., 2020), and subsequently, haplotype blocks were derived in HaploBlocker using window_size = 10 and min_majorblock = 1,000. Btau 4.0 (Bovine Genome Sequencing and Analysis Consortium et al., 2009) was used as the reference genome for both phasing and haplotype block calculation.

Genome-wide association studies were conducted for the 17 newly defined heat tolerance traits based on F%, P%, MY, lactose, and the specific fatty acids were performed using the R package statgenGWAS (van Rossum et al., 2020), including a fixed effect for both farm and performance in thermoneutral conditions (intercept). To reduce computing time, the data were split into 6 subsets, and p-values from the subsets were combined using the weighted Z-score method as suggested by (Willer et al., 2010).

To aid results from the GWAS, phenotypic differences between carriers and noncarriers of specific haplotype blocks were estimated using a linear regression of the observed phenotypes against haplotype block count (0, 1, 2). We here specifically avoid the direct estimation of the effect of a haplotype block as the sum of the effects of the included alleles, as this could introduce potential biases in estimation caused by linkage disequilibrium between SNPs and higher impact of shrinkage on rare variants.

The source code of the conducted GWAS study and the derivation of haplotype blocks is given in Supplemental File S4 (see Notes). Furthermore, the plot_block() function in the HaploBlocker R package (https://github.com/tpook92/HaploBlocker) was extended to visualize haplotype blocks based on their estimated effects.

RESULTS

Population-Wise Models

Results from the population-wise models indicate an essentially linear decrease in both F% (Figure 4A) and P% (Figure 5A) with increasing THI. The effect of THI is ~20% smaller for first-parity cows with a loss of 0.29% and 0.20% for F% and P% from THI = 30 to THI = 70, respectively, compared with later parities with average losses of 0.35% and 0.25%. Note here that overall concentration levels for first parity cows are slightly lower. Furthermore, the day of the year effect of days in June and July for both F% and P% is negative, resulting in further reduction of F% and P% (−0.1% and −0.06%, respectively) with substantially increased P% in autumn (up to +0.08%), whereas F% from autumn until the end of winter is quite constant at +0.03% (Figures 4C and 5C). The effect of DIM on F% and P% is substantial, with expected F% and P% around d 50 of lactation being almost 1% lower than for the very beginning and end of lactation (Figures 4D and 5D). In comparison, the absolute size of the THI × DIM effect is relatively low. Nonetheless, models indicate a lower impact of heat conditions on P% in early lactation because the positive effect is complementary to the overall negative effect of THI on P%, with the combination of both effects corresponding to the overall THI effect. For example, a cow is expected to have 0.061% reduced P% at THI = 60 (Figure 5A), whereas a cow in the first 30 d of lactation is expected to have 0.043% higher P% at THI = 60 (Figure 5B). Therefore, the expected overall THI effect of THI = 60 for a cow is a reduction of 0.017% (0.043 − 0.061; excluding the day of the year effect).

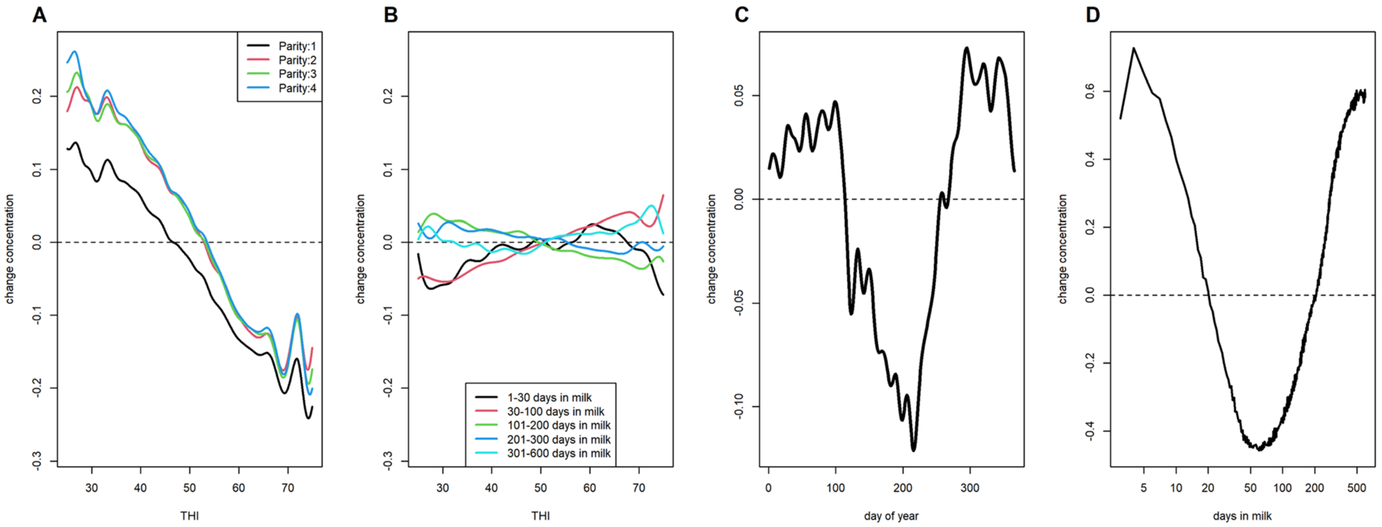

Figure 4. Estimated effects of THI × parity (A), THI × DIM (B), day of the year (C), and DIM (D) on fat percentage in milk.

Regarding individual fatty acids, a strong increase in concentration for C-18:1 *cis*-9 was observed in early lactation (<30 d, Figure 6D), as well as in first parity cows overall. Furthermore, the estimated THI × DIM effect suggests an even stronger increase under heat conditions (THI >65) for cows within the first 100 d of lactation (Figure 6B). The interested reader is referred to Supplemental Figures S3–S14 (see Notes) with results on lactose and the individual fatty acids.

Results reported in this study were obtained using a joint model including data from all parities, however, fitting separate models for data from each parity resulted in overall similar estimates for all effects (Supplemental Figures S15 and S16 [see Notes]). Noticeably, effect estimates for THI × DIM for the individual parities showed high variance in the fit, indicating the increased power obtained by the use of the joint dataset (Supplemental Figure S17, see Notes).

Models for milk yield suggest that yield is increasing up to THI 55 (Figure 7A). Therefore, partially compensating for reduced F% and P% at moderately increasing THI. However, as THI increases, milk yield drops by 0.08% (THI = 60), 0.44% (THI = 65), 1.7% (THI = 70), and 4.8% (THI = 75), approximately corresponding to a quadratic increase in losses. Hence, even amplifying losses for RY_F and RY_P, with animals in early lactation (<30

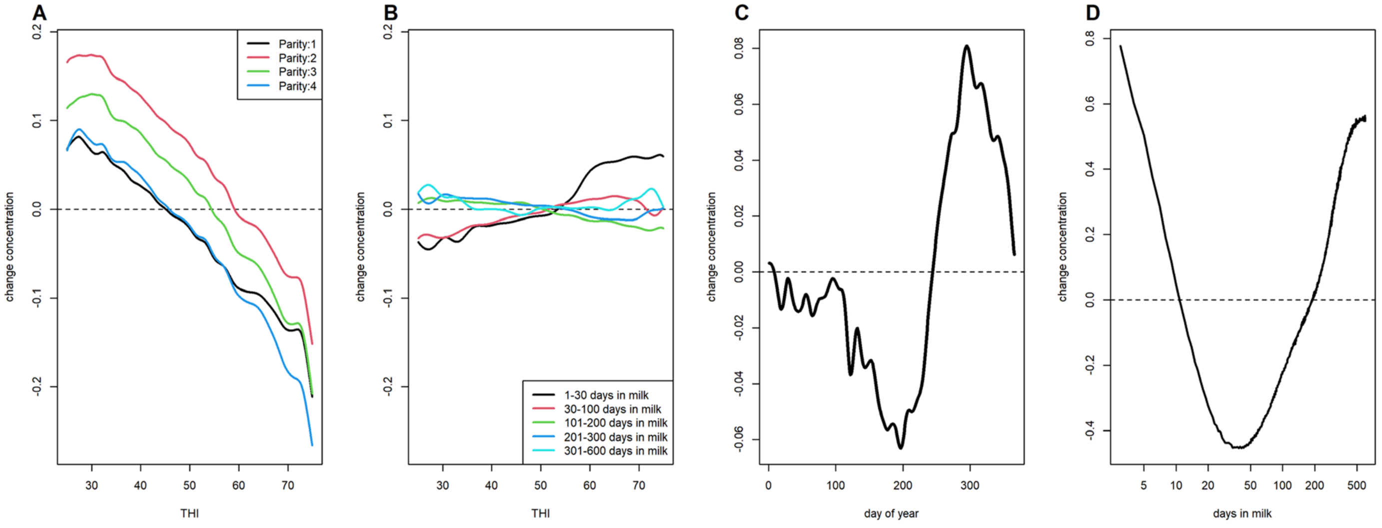

Figure 5. Estimated effects of THI × parity (A), THI × DIM (B), day of the year (C), and DIM (D) on protein percentage in milk.

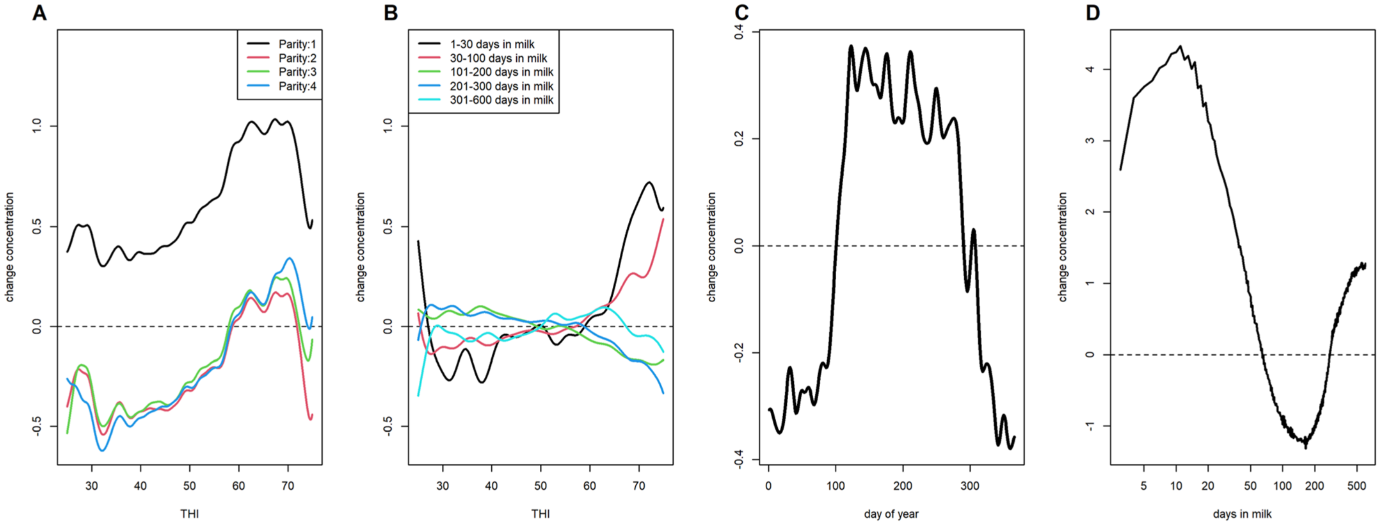

Figure 6. Estimated effects of THI × parity (A), THI × DIM (B), day of the year (C), and DIM (D) on C-18:1 *cis*-9 concentration in milk.

d) being slightly less affected. The effect of heat stress is amplified by animal age, with first-lactation cows having milk yield reduced by 3.0% at THI = 75, compared with increasing losses in the second (4.9%), third (5.4%), and later parities (5.8%). Furthermore, animals in the first 30 (and to some extent 100) DIM were less affected by heat stress conditions (Figure 7B). Results of the kernel regression to estimate population-wise effects indicate systematic biases in the fitting of the lactation curves based on DIM with an estimated effect of 0.01 at ~DIM 30, which should, however, be corrected by the inclusion of exactly this effect (Supplemental Figure S18, see Notes). Similarly, slightly lower milk yield was observed in spring and fall compared with summer and winter.

Obtained models when scaling solid components by the animal's relative milk yield resulted in very similar models to the concentration-based models up to THI = 60 and subsequent stronger losses in both RY_F and RY_P in line with the cumulative effects seen in F% or P% and milk yield depending on THI levels (Supplemental Figures S19 and S20, see Notes).

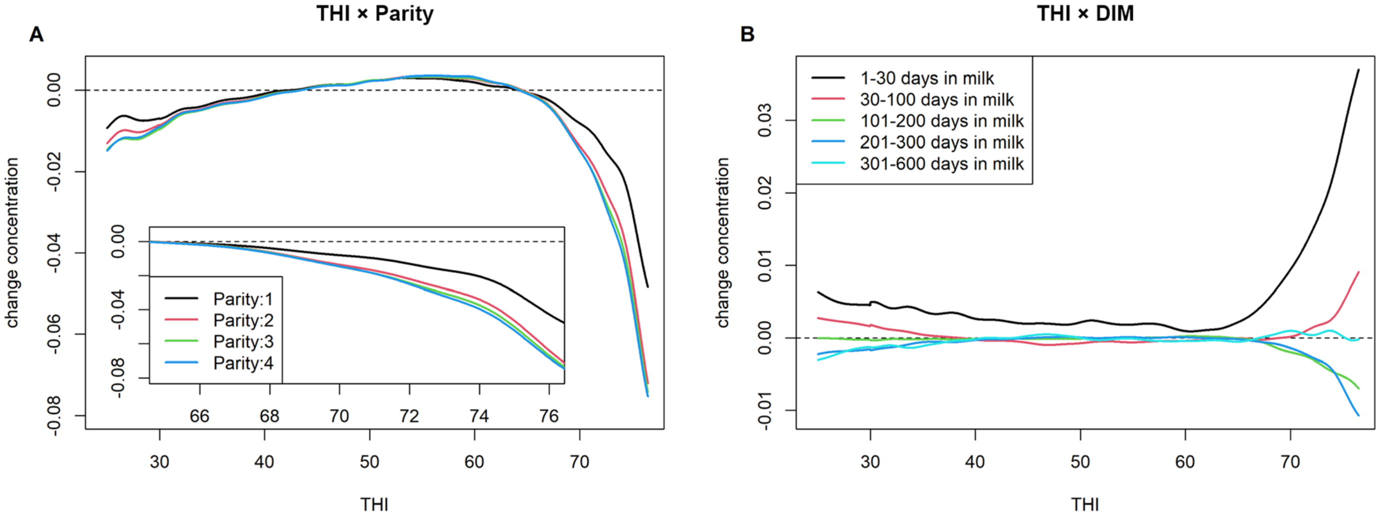

Figure 7. Estimated effects of THI × parity (A) and THI × DIM (B) on milk yield in percent relative to expectation based on the lactation curve.

Individual Phenotypes

Based on our newly proposed trait definition for heat tolerance traits, phenotypes for 273,135 out of 346,248 cows were derived (of which 56,505 are genotyped). When using the broken-stick model, the number of phenotyped cows was reduced to 271,634 because some cows had no observation associated with a THI higher than 50. In contrast, requiring a higher number of records heavily reduced the share of phenotyped animals (e.g., requiring 10 or 15 records would result in just 213,227 or 160,140 cows with phenotypes, respectively). The requirement of a higher number of records particularly affects young animals.

Estimated heritabilities for the heat tolerance trait for F% (**H_F%**) and P% (**H_P%**) were 0.046 and 0.116 (Table 2). Estimated genetic variances imply that a cow that is 1 genetic standard deviation (**gSD**) superior in H_F% is expected to have 0.086% higher F% at THI = 70 than an average cow with the same performance at THI = 50. The heritability for heat tolerance for milk yield (**H_MY**) was 0.095 with an expected 0.2% higher milk yield for a 1-gSD-superior cow at THI = 70.

Use of the broken-stick model resulted in similar heritabilities to those from the linear models (0.044 for H_F%, 0.107 for H_P%, 0.111 for H_MY). However, genetic variances substantially increased with 0.136% higher F%, 0.094% higher P%, and 0.42% higher milk yield for a 1-gSD superior cow at THI = 70 (Table 2). When comparing these numbers to the expected losses from the population-wise model, these improvements would already compensate for 69%, 65%, and 10% of the respective production losses at THI = 70 compared with THI = 50 (Table 2).

Requiring a higher number of records before assigning a phenotype to the animal did increase the heritability with, for instance, a minimum of 15 records (instead of 5) resulting in a heritability of 0.070 (instead of 0.046) for H_F%; however, this was only caused by a reduction of residual variance, as genetic variance stayed the same, or in case of H_MY, even slightly reduced (Supplemental Table S1, see Notes).

Estimated heritabilities for the intercepts were generally high for all milk composition traits considered, with values of 0.76 and 0.77 for H_F% and H_P%, respectively (Supplemental Table S2, see Notes). Note that phenotypes are not derived from a single milk sampling, and residual variances are reduced by correction for environmental effects. Thus, what is here reported as a heritability from a comparability perspective is more in line with repeated sampling/repeatability. No heritability was estimated for the intercept of H_MY because the intercept, by design of the trait, is close to zero for all individuals.

Based on estimated variance components, traits obtained from the broken-stick model showed the most promise, and therefore all subsequent analyses on genetic correlation and GWAS primarily focus on this trait definition. Note that correlations between the heat stress phenotypes derived from the broken-stick model and linear model on average were 0.91, resulting in very similar GWAS peaks and correlations to commercial traits.

The correlations between heat tolerance traits (Figure 8A) were similar to correlations between the different milk components themselves (Figure 8B; e.g., with F%

Table 2. Estimated variance components for different heat tolerance traits and their genetic variation compared with population-wise effects

Trait	Heritability (SE) in fully linear model	Heritability (SE) in linear model with threshold THI = 50)	Genetic variation[1] fully linear model/threshold (THI = 50)	Population-wise losses between THI 50 and THI 70
F%	0.046 (0.006)	0.042 (0.006)	0.086/0.136	0.196
P%	0.116 (0.009)	0.107 (0.009)	0.059/0.094	0.144
lact	0.059 (0.007)	0.059 (0.007)	0.025/0.042	−0.018
MY	0.095 (0.012)	0.111 (0.012)	0.0010/0.0042	0.042
C-4:0	0.068 (0.007)	0.059 (0.007)	0.051/0.079	−0.022
C-6:0	0.123 (0.009)	0.106 (0.008)	0.047/0.073	0.017
C-8:0	0.136 (0.009)	0.124 (0.009)	0.044/0.069	0.04
C-10:0	0.105 (0.008)	0.096 (0.008)	0.118/0.189	0.16
C-12:0	0.085 (0.008)	0.075 (0.007)	0.143/0.225	0.266
C-14:0	0.059 (0.007)	0.052 (0.007)	0.186/0.294	0.302
C-16:0	0.110 (0.008)	0.105 (0.008)	0.632/1.015	0.220
C-18:0	0.122 (0.009)	0.120 (0.009)	0.327/0.533	−0.529
C-18:1 *cis*-9	0.070 (0.008)	0.066 (0.007)	0.442/0.729	−0.536
C-18:1 *cis*-11	0.092 (0.007)	0.096 (0.007)	0.018/0.031	−0.001
C-18:2 *cis*-9,12	0.051 (0.006)	0.043 (0.006)	0.036/0.055	−0.052
C-18:3 *cis*-9,12,15	0.131 (0.009)	0.121 (0.009)	0.024/0.038	−0.048
CLA *cis*-9,*trans*-11	0.097 (0.008)	0.084 (0.008)	0.023/0.036	−0.015

[1] The genetic variation is expressed as the genetic standard deviation × 20 to express the differences between a ”standard” cow and 1 gSD superior cow in THI = 50 and THI = 70 conditions.

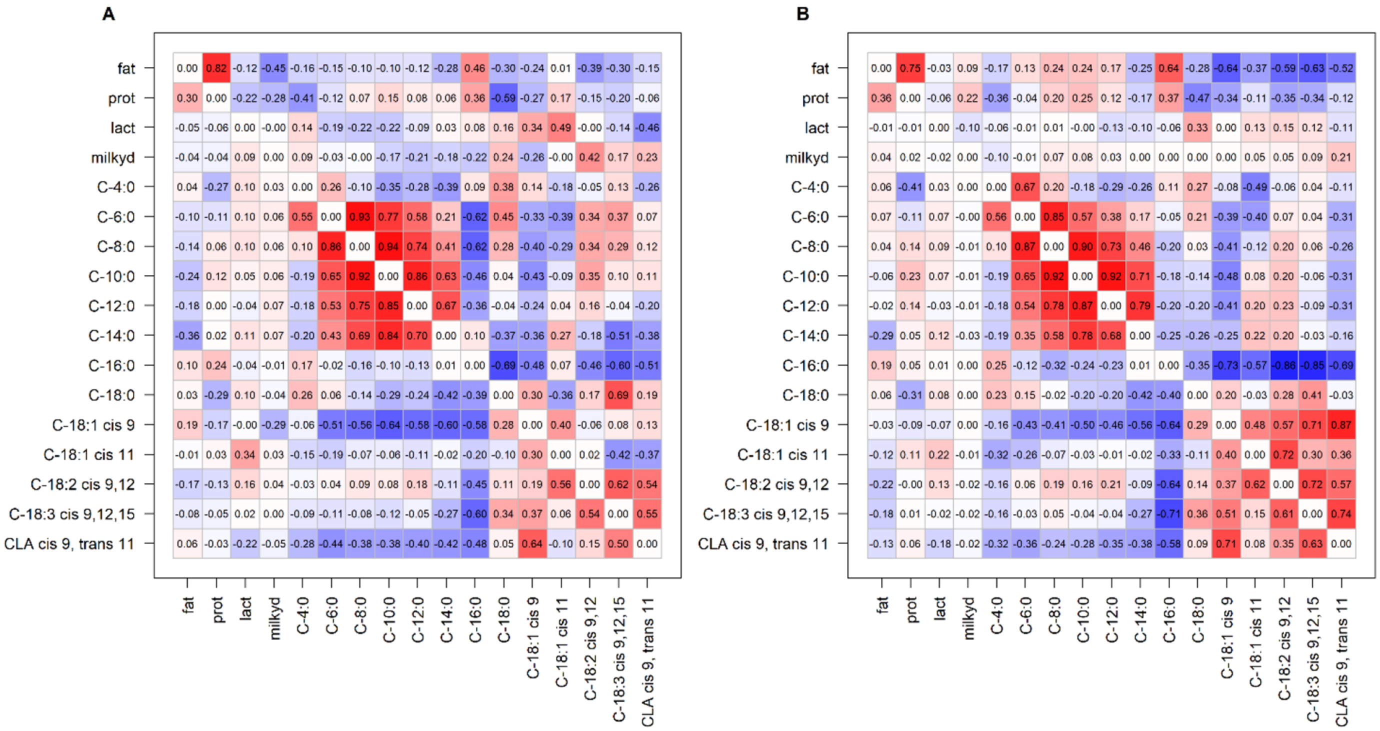

Figure 8. Correlations between heat tolerance traits based on milk yield and fat, protein, and fatty acid concentration based for the slope (A; heat tolerance) and the intercept (B;; performance at thermoneutral conditions). The slope is used for milk yield in both figures because by design, the intercept has a target value of 0 for all individuals. Genetic correlations are given above the diagonal and residual correlations below the diagonal.

and P% having a residual correlation of 0.33 and a genetic correlation of 0.82). Furthermore, high correlations among short-chain fatty acids (C-6 to C-14) and long-chain fatty acids (C18:1 *cis*-9 to CLA *cis*-9,*trans*-11) were observed, whereas correlations between the 2 groups were mostly negative. Correlations between H_MY and milk component-based traits were all around zero. Correlations between intercept and slope of the considered heat tolerance traits were mostly negative, with correlations of −0.48 and −0.36 for F% and P%, respectively, indicating that animals with lower overall production levels in absolute terms have a smaller relative drop in performance in heat conditions.

Correlations to commercial traits for H_MY were mostly beneficial, with an overall correlation of 0.29 to the total merit index (**NVI**; Table 3), with particularly favorable correlations to longevity (0.33). Conversely, correlations of H_F% and H_P% to the NVI were −0.14 and −0.09, with negative correlation to resilience (−0.22 / −0.19), claw health (−0.16 / −0.12), and subclinical ketosis (−0.12 / −0.19).

Genome-Wide Association Study

The use of HaploBlocker resulted in the identification of 154,318 haplotype blocks, effectively tripling the number of considered variants compared with when only using a panel of SNPs.

Results for GWAS studies showed significant effects in various regions of the genome, with the highest peak

Table 3. Estimated genetic correlations between selected commercial traits and newly developed heat tolerance traits based on MY, F%, and P%; a full list of traits and correlations to individual fatty acid based heat tolerance traits is given in Supplemental Figure S21 (see Notes)

Trait	H_F%	H_P%	H_MY
MY	+0.17	+0.19	+0.03
Fat yield	−0.24	−0.17	+0.04
Protein yield	−0.03	−0.04	+0.11
Longevity	−0.05	−0.01	+0.33
Fertility	−0.07	−0.07	+0.17
Udder health	−0.01	−0.01	+0.12
Subclinical mastitis	−0.04	−0.02	+0.11
Subclinical ketosis	−0.12	−0.19	+0.15
Resilience	−0.22	−0.19	+0.18
Stability	−0.17	−0.21	+0.27
Total merit index	−0.14	−0.09	+0.29

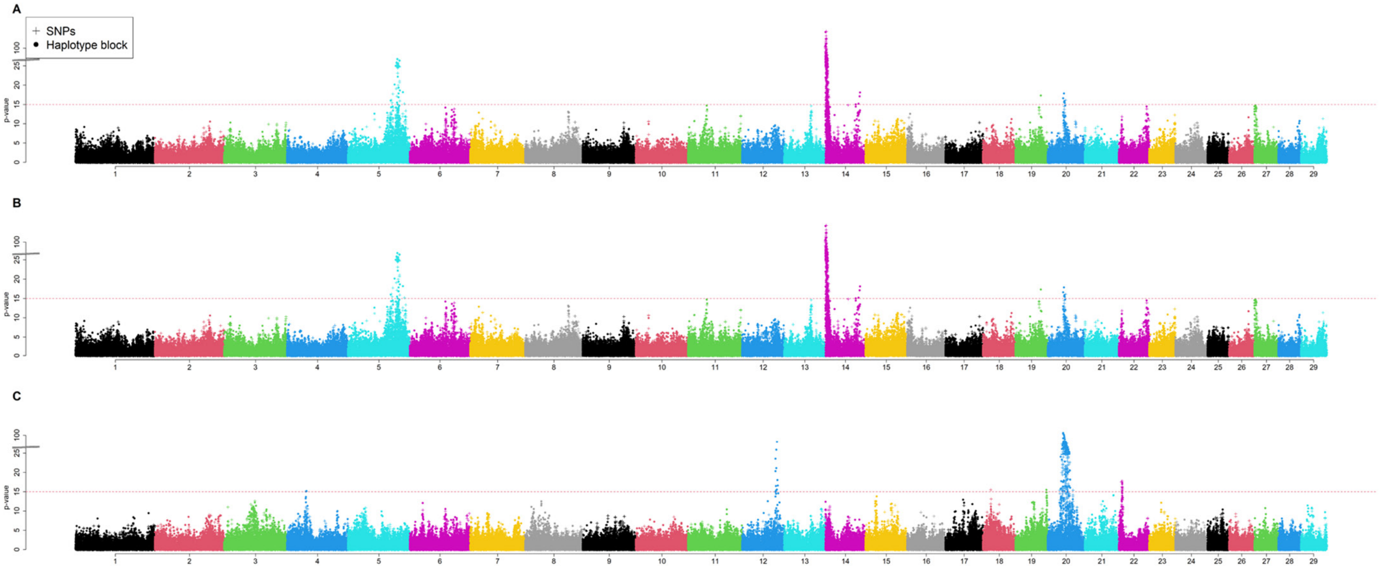

Figure 9. Manhattan plot based on a GWAS for the heat tolerance trait for H_F% (A), H_P% (B), and H_MY (C). The y-axes are log-scaled from a *P*-value of 10^{-30} onward, and a significance threshold of 10^{-15} is indicated by the red dashed line.

for most traits identified on chromosome 14 and peaks closely matching the location of DGAT1 (Figure 9). Note that all GWAS were also performed including DGAT1 as a fixed effect; however, with the exception of the DGAT1 region having no longer having any significant associations, the results remained basically the same. Across all traits, a series of SNPs showed highly significant hits ($P < 10^{-15}$) for multiple of the 17 considered traits, with the most frequent hits on BTA14 (0.1 Mb, 15 hits), BTA5 (11 hits, 102.0 Mb), BTA27 (10 hits, 38.9 Mb), and BTA 19 (9 hits, 52.1 Mb). An overview of hits per genome region is given in Figure 10, with a full list of the most frequently significant variants per chromosome given in Table 4. For example, the estimated effects of these peak SNPs and haplotype blocks for BTA14, BTA5, and BTA20 correspond to 0.77, 0.43, and 0.44 gSD of H_F% (Table 5), further indicating the potentially high impact of these variants. Furthermore, special attention was given to the peak on BTA20, as it showed a significant peak for H_F%, H_P%, and H_MY (Figure 11). The SNP with the highest peak on chromosome 20 for H_F% is at 36.4 Mb, with a frequency of 10.4% in the population. Contrarily, the highest peak haplotype block has a frequency of 7.0% and spans 10 SNPs from 33.2 Mb to 33.6 Mb. Note that all these SNPs have a frequency of, at minimum, 27.7% in the population, and only the combined sequence of allelic variants is present at a 7.0% frequency, which

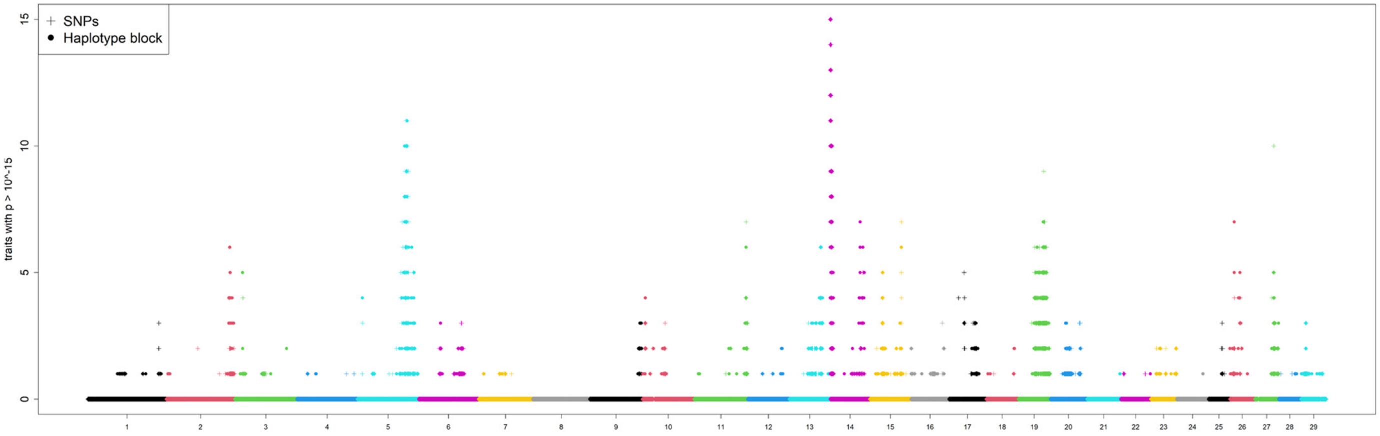

Figure 10. Number of heat tolerance traits each SNP or haplotype has a *P*-value $<10^{-15}$ for the 17 considered milk composition traits (using the slope estimated when setting records of THI <50 as THI = 50).

Table 4. Overview of SNPs and haplotype blocks with the highest number of QTL hits ($P < 10^{-15}$) for the 17 heat tolerance traits considered

SNP ID	Chromosome	Location (Mb)	Number of associations ($P < 10^{-15}$)
Haplotype block	14	0.01–0.84	15
Haplotype block	5	102.05–102.57	11
ARS-BFGL-NGS-57448	27	38.88	10
BTA-45758	19	52.1	9
SNP_X14710_1740	11	107.17	7
Haplotype block	26	9.35–9.75	7
ARS-BFGL-NGS-39003	15	65.04	7
SNP_BES11_Contig346_1209	13	64.81	6
Haplotype block	2	130.49–131.78	6
MARC_33120_400	17	31.35	5
Haplotype block	3	16.22–16.85	5
Haplotype block	10	6.13–6.35	4
Haplotype block	20	31.69–34.04	3
Haplotype block	9	105.68–108.07	3
ARS-BFGL-NGS-100347	25	28.56	3
BTB-01958090	16	64.21	3
rs29024685	6	88.54	3
Hapmap40784-BTA-66134	29	10.41	3
AAFC03097715_86987	1	145.85	3
BTB-01381318	23	52.27	2
Haplotype block	18	57.99–60.29	2
Haplotype block	12	69.42–70.85	2
Haplotype block	7	47.1–58.16	1
BTA-64106	28	34.11	1
ARS-BFGL-NGS-38990	4	101.67	1
Haplotype block	22	61.06–61.33	1
Haplotype block	21	67.55–68.17	1
Haplotype block	24	53.88–54.18	1
—	8	—	0

essentially means that all carriers of the peak haplotype block also carry the peak SNP (Figure 12).

On a phenotypic level, carriers of the peak SNP have a higher heat tolerance in terms of H_F% (allele count 0: −0.0011; allele count 1: +0.0033; allele count 2: +0.0079; Figure 13). Similarly, animals carrying the peak haplotype also have higher heat tolerance (haplotype count 0: −0.0010; haplotype count 1: +0.0053; haplotype count 2: +0.0085). However, animals that carry the peak SNP and not the haplotype show a substantially lower change in heat tolerance (allele count 0: −0.0011; allele count 1: +0.0001; allele count 2: +0.0025 with haplotype count 0). When comparing haplotype blocks in chromosome 20 along the estimated effect for H_F% (Figure 14), the highest effects are estimated for blocks in strong linkage with the peak haplotype, with only minor differences between alternative haplotype blocks in the genome segment.

Computing Time

The entire pipeline for the estimation of population-wise effects took 41 min with a peak memory usage of 35 GB using a single core of an Intel Xeon Gold 2.1 GHz processor per trait. Both memory and computing times scale approximately linearly in the number of records included. The subsequent derivation of phenotypes required negligible computing resources, completing in 3.3 min with 1.6 GB peak memory for processing all traits combined. Note here that our pipeline mostly uses base R functions like *ksmooth*() and *lm*() and is by no means optimized for the computational efficiency of the concrete application at hand, thereby indicating that computing times for the presented pipeline should not be a major concern.

DISCUSSION

In this work, we are providing a comprehensive new pipeline for the estimation of both individual-based and population-wise effects of heat based on repeated measurements as an alternative to reaction-norm models. Our proposed approach allows more sophisticated models to include nonlinear effects or interactions between parameters, such as THI × DIM interactions. Although they were only applied to milk yield and milk composition traits in this study, the proposed population-wise models can be applied in the same way to other traits or species without restrictions, whereas the suggested concepts to define heat tolerance traits require the availability of longitudinal data.

Table 5. Overview of estimated effect of peak SNPs and haplotype on heat tolerance defined based on F%

SNP ID	Chromosome	Location (Mb)	Effect size (% of a gSD of H_F%)	Intercept (in F% compared with population average)	Frequency
Hapmap30383-BTC-005848	14	0.08	77	0.26	0.49
Haplotype block	5	98.95–101.08	43	0.14	0.11
Haplotype block	20	33.22–33.59	43	−0.14	0.09
Haplotype block	19	51.61–52.11	−12	−0.03	0.39
Haplotype block	27	2.17–3.59	−9	0.02	0.08
Haplotype block	11	38.1–38.56	−2	0.00	0.17
ARS-BFGL-NGS-75174	13	55.15	17	0.00	0.50
Haplotype block	22	56.52–61.81	−36	−0.01	0.04
Haplotype block	6	72.21–76.94	74	−0.04	0.01
Haplotype block	8	87.46–88.38	−19	0.00	0.19
Haplotype block	7	17.76–17.93	16	−0.02	0.03
Haplotype block	16	6.12–6.6	3	0.02	0.19
BTA-57027	23	51.95	−11	−0.01	0.36
Haplotype block	26	39.9–40.58	−5	−0.01	0.04
AAFC03014648_112378	29	44.72	−7	−0.01	0.28
Haplotype block	18	57.99–61.03	39	0.01	0.06
Haplotype block	15	65.56–66.27	15	−0.03	0.07
Haplotype block	28	43.17–43.85	37	0.01	0.08
Hapmap54726-rs29023280	10	25.98	14	0.00	0.24
Haplotype block	2	111.89–112.4	17	0.05	0.25
Haplotype block	3	12.63–14.03	10	0.03	0.12
ARS-BFGL-NGS-114711	17	69.28	17	−0.01	0.18
rs29013594	24	44.25	14	0.01	0.35
Hapmap57034-rs29025414	9	83.83	−8	0.01	0.43
Haplotype block	12	66.86–69.6	7	0.01	0.13
ARS-BFGL-NGS-57997	21	26.45	8	−0.02	0.12
Haplotype block	1	17.2–17.52	51	0.00	0.02
ARS-BFGL-NGS-57716	4	4.83	26	−0.01	0.25
Haplotype block	25	13.26–13.75	26	0.02	0.15

Population-Wise Models

Our results highlight the impact of weather conditions on dairy cattle indicated by a linear reduction of F% and P% across the entire spectrum of considered THI values, even in thermoneutral conditions. In contrast, milk yield itself was relatively stable until a THI of 60; however, with rising THI values, production losses increased quadratically. Animals in higher parities were more strongly affected by weather conditions, as also reported by Agui-

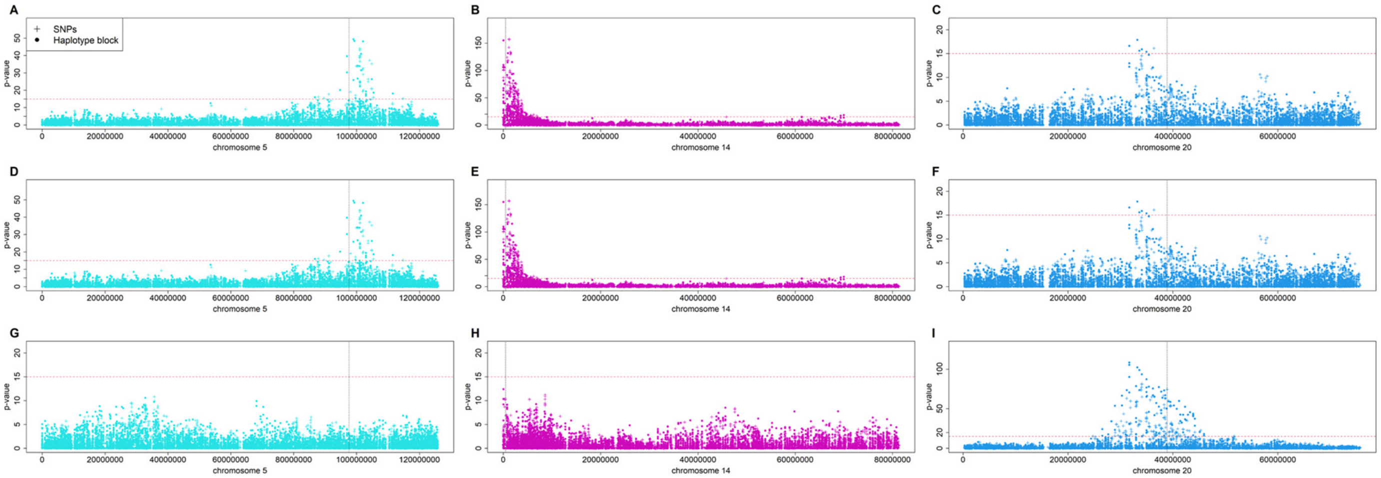

Figure 11. Manhattan plot based on a GWAS for the heat tolerance trait for H_F% (A–C), H_P% (D–F), and H_MY (G–I) on BTA5 (A, D, G), BTA14 (B, E, H), BTA20 (C, F, I). Location of CDKN1B and DUSP16 (Sigdel et al., 2019), DGAT1 (Prakapenka et al., 2024), and SLICK (Huson et al., 2014) are marked with vertical lines. A significance threshold of 10^{-15} is indicated by the red dashed line.

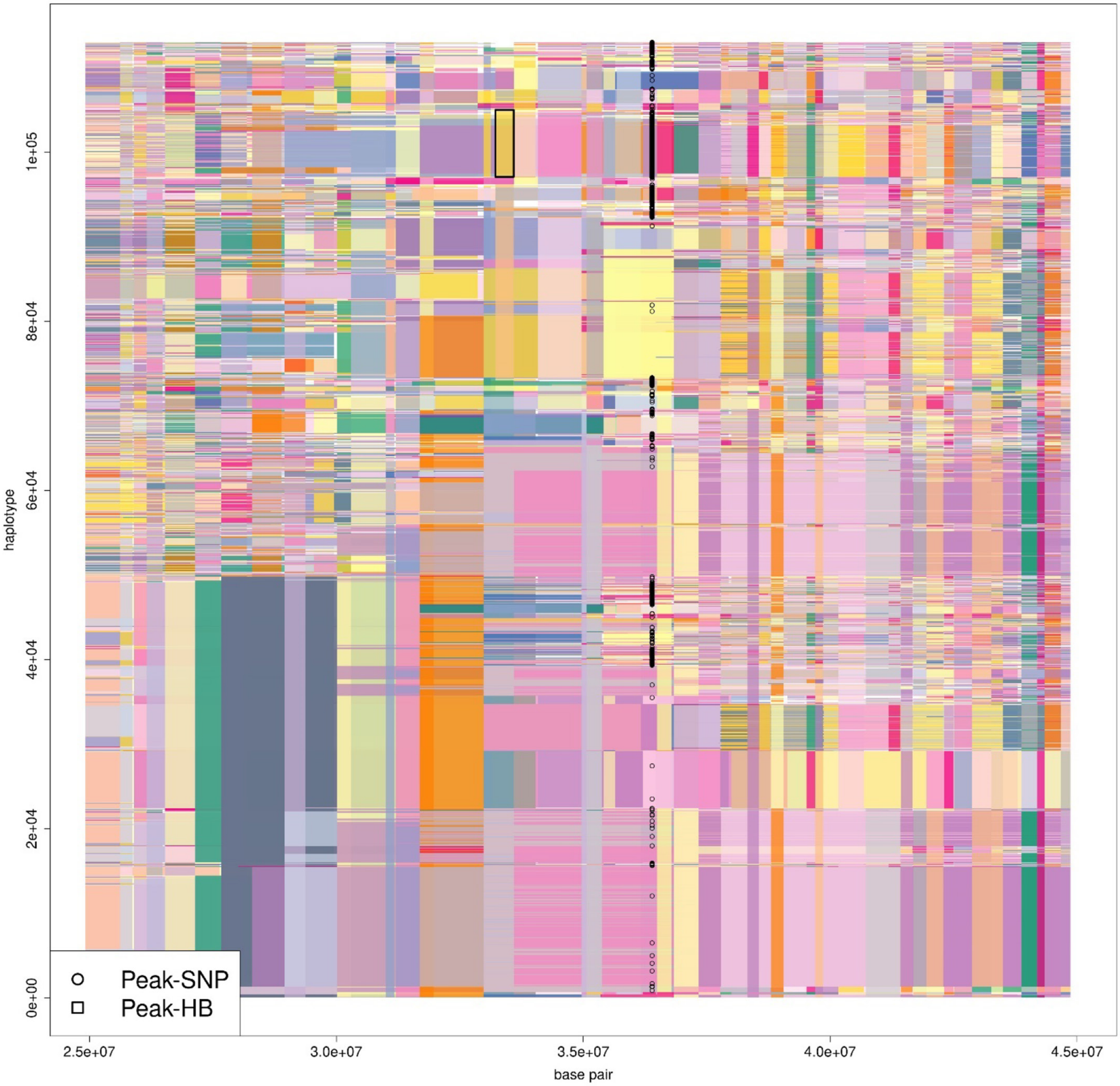

Figure 12. Haplotype-based visualization of individuals with a phenotype for H_F% and available genomic data. Haplotypes are sorted according to their similarity at 33.3 Mb according to Pook et al. (2019). Haplotypes with the beneficial variant at the peak SNP and haplotype block (HB) are indicated in black.

lar et al. (2010). In contrast to Aguilar et al. (2010), our results suggest a noticeable impact of heat for first-parity cows. To validate our results, the estimated effects of heat load were compared with estimates obtained with a reaction-norm model for the Dutch Holstein population within the European Union Rumigen project (Mattalia et al., 2023). When dropping interaction effects from the kernel regression and only considering cows from the first 2 parities (to match the analysis conducted within Rumigen), the obtained estimates of heat stress essentially perfectly match (not shown), further validating our approach.

Because only data from Dutch farms with a temperate maritime climate were considered in this study, effects

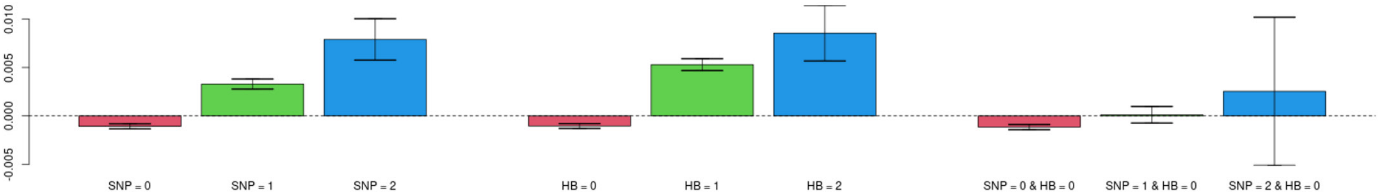

Figure 13. Average phenotypic level for H_F% depending on allelic variant on peak SNP and haplotype count at peak haplotype block (HB) on chromosome 20. The confidence band (95%) is calculated for the estimated mean phenotype.

could only be reliably estimated until THI = 75, where milk yield was reduced by 5%. Paired with a relative reduction of F% and P% of ~6% when THI increases from 50 to 75, resulting in a reduction of F-kg and P-kg of more than 10%, which is in line with estimates from McWhorter et al. (2023). In view of climate change (Cheng et al., 2022), the frequency and severity of such heat stress events is expected to increase and are common in other parts of the world today. Note that in our study, the most critical cases of potential heat stress in which an animal did not produce any milk were removed from the analysis, as they were very rare in our specific data and we had no health data to correct for other potential reasons of complete milk production losses. When extrapolating the trend of quadratic losses in milk yield to higher THI values, which should only be done with caution but highlights the potential effects of heat stress in regard to production traits, this would suggest losses of 15% to 20% at THI = 80 in milk production. Lastly, we expect farmers to already adapt feeding and husbandry practices in response to extreme weather conditions, leading to a potential underestimation of the effects of heat stress (Johnson, 1987; D'Emilio et al., 2017; Ríus, 2019). For example, consider that farmers are feeding more energy-dense food or feed additives during a heat wave to mitigate the effect of reduced feed intake, which should already compensate for some of the losses that would have occurred if management was not adapted.

Note that the interpretation of the individual effects of any population-wise models should be done with caution, as individual parameters can be highly interdependent, leading to multicollinearity (Alin, 2010). For example, the day of the year effect for days in summer showed the lowest expected F% and P%, potentially leading to a further underestimation of the effect of heat stress. We also considered models that include other, more detailed weather data, such as the number of hours in a day above a certain THI threshold, maximum THI and temperature, and air pressure (Misztal et al., 2024) as additional effects estimated via kernel regression. Whereas goodness-of-fit was basically not affected for thermoneutral conditions (THI between 35 and 65), the average absolute size of residual effects in extreme weather situations was reduced slightly, thereby indicating a potentially better fit, but also overfitting, as fitted curves obtained from the kernel regression showed more nonsystematic variation. For the reason of interpretability and to reduce overfitting, the models finally used in this study were kept relatively simple.

Although THI is not a perfect metric for determining the overall heat load, one of its core advantages is its simplicity, allowing for an interpretation of the estimated effects and subsequent fitting of a regression line. For more complex models, we propose to aggregate all effects associated with climatic conditions to derive an overall weather effect for each farm-by-day combination that should provide a more advanced rating of the severity of heat stress on a given day. However, because day of the year effects will also include other effects like the change in diet over the year, isolating the effect of heat stress will not be fully possible.

Breeding for Heat Tolerance

Regarding the definition of our newly suggested heat tolerance traits, our approach has similarities to the slope in a reaction-norm model (Su et al., 2006; Aguilar et al., 2010). However, in terms of its use in breeding, we are proposing to directly use the estimated slope as the trait in breeding, acting as a residual trait. Contrary to this, although the slope is an output from a reaction-norm model, the realized estimated breeding values at different THI values are usually output used from an reaction-norm model, as heritability for the slope in such models is a random effect and often not significantly different from zero (Copley, 2024), leading to high correlations of breeding values for production traits at different THI levels. Contrarily, our heat tolerance traits defined herein are residual traits of production traits and, if at all, showed a slightly negative correlation to overall production levels and thereby provide a tool to isolate the weather-dependent component of the trait.

As repeated records of a cow are combined into a single phenotypic record, our proposed approach results in reduced computational load compared with reaction-norm models, when applied in routine genetic evaluations.

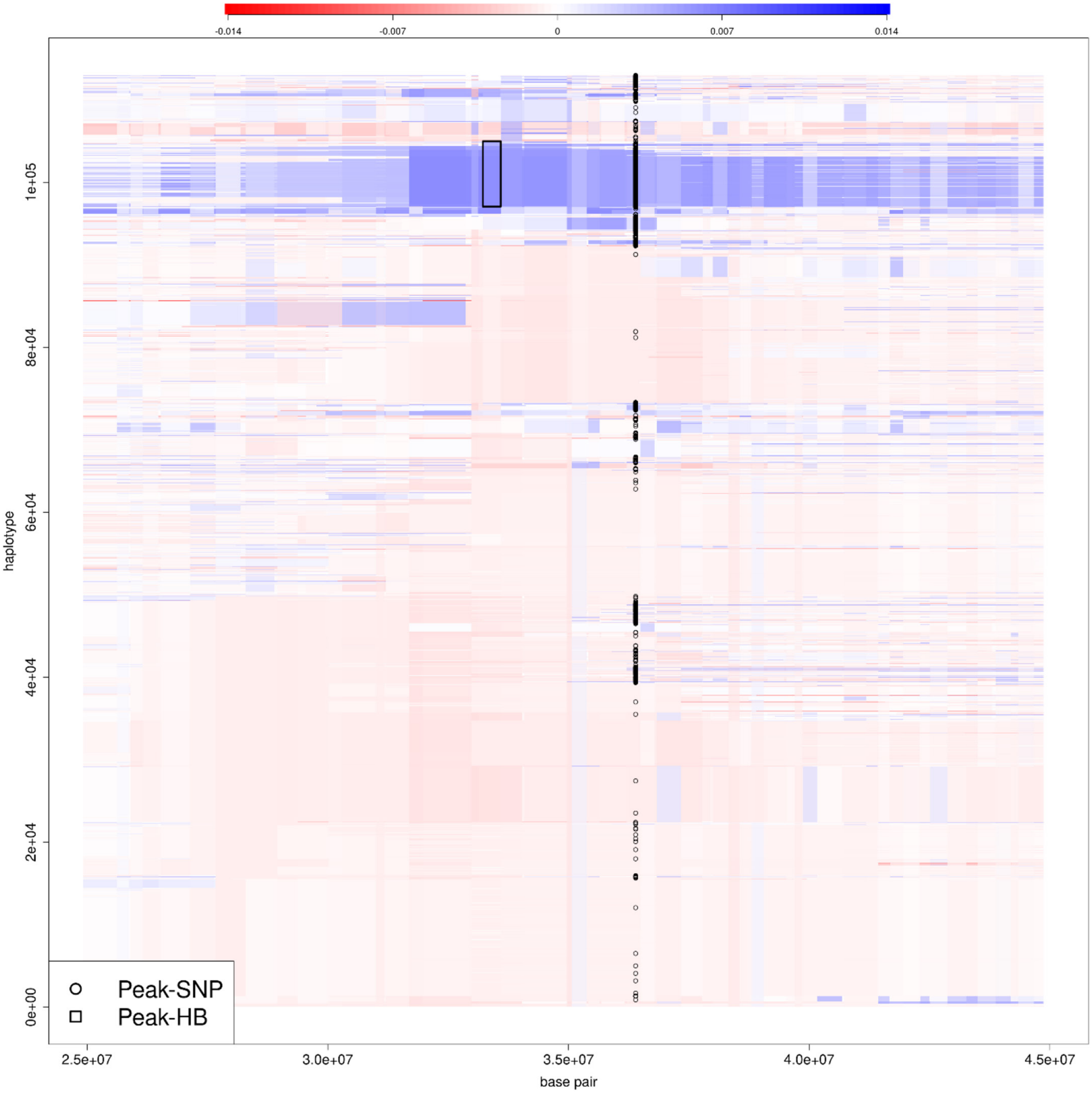

Figure 14. Haplotype based visualization of individuals with a phenotype for H_F% and available genomic data. Haplotypes are sorted according to their similarity at 33.3 Mb according to Pook et al. (2019). The color of blocks is chosen according to the estimated effect of each haplotype block (HB) based on phenotypic data with high heat tolerance indicated in blue and low heat tolerance indicated in red. Haplotypes with the beneficial variant at the peak SNP and haplotype block are indicated in black.

As shown above, the computation of precorrected phenotypes based on a population-wise model is relatively straightforward, even for large datasets. Furthermore, the overall flexibility to include additional effects is greatly increased.

It should still be noted that the here suggested heat tolerance traits can only be derived in case longitudinal data for the trait can be collected, to enable the deterministic fit of a linear regression model. Therefore, the proposed approach is highly suitable for traits like milk yield in dairy, daily gain in pigs, or egg production in poultry. On the contrary, for traits like conception rate, reaction-norm models should still be superior (Ojo et al., 2025), although we would argue that estimating a reliable breeding value for fertility traits is usually only possible for sires with a high number of offspring. Note that from a goodness-of-fit perspective, an iterative fitting procedure like ours is conceptually inferior compared with a joint or all-in-one model (Holland and Piepho, 2024), at least if both models are able to incorporate the same parameters and their effect structures. On the other hand, a downstream application like GWAS in our approach can be performed directly on phenotypes instead of estimated breeding values or deregressed proofs, thereby being conceptually better suited than application on values derived based on SNP effects (Ekine et al., 2014; Holland and Piepho, 2024).

A limitation of our approach is that the reliability of the phenotype of an animal is currently not accounted for in the prediction model. As such, cows with a higher number of observations will have more reliable phenotypes. This in turn also leads to higher heritability estimates when only including animals with a high number of individual records. Because genetic variance did not increase and fewer phenotypes for young animals are available, we do not expect higher prediction accuracies for young animals from this.

From a methodological perspective, this does not pose a conceptual issue but is an important consideration when interpreting the estimated heritability. As such, heritabilities of our phenotypes are more in line with repeatability estimates (National Research Council, 1988; Costa et al., 2019), and naturally much higher than heritability estimates based on a single record (Druet et al., 2005; Soyeurt et al., 2007). We explored integrating the R^2 values from the linear regression in our models as an indicator of the reliability of the individual phenotypes; however, values overall were low, with $R^2 < 0.01$ for 95% of all samples for H_MY (Supplemental Figure S19), as the variance of production levels is much higher than the isolated effect of weather conditions (Figure 3).

A potential solution for differences in the reliability of individual phenotypes could be to process each lactation of an animal as a separate phenotypic record. This should provide similar reliabilities between individual phenotypes and ensure that each phenotype is derived based on individual records from most seasons of the year, thereby, also avoiding potential biases introduced by partially completed lactations or frequent re-ranking of animals.

The low values for R^2 again highlight the need to use longitudinal data to improve the accuracy of prediction models, going even beyond the nowadays commonly used monthly test day recording scheme and instead considering weekly or ideally daily or per-milking recording. Although scaling of a reaction-norm model is linear in the number of records for many implementations solving mixed model equations, computing times with billions of records are expected to be critical, and our pipeline is able to summarize repeated records of the same animal efficiently, thereby resulting in subsequent prediction models that only include a single record per cow or least only one per lactation.

In terms of the overall potential for breeding, all our heat tolerance traits exhibited significant genetic variation. However, for practical breeding, it is probably necessary to combine individual heat tolerance traits into a joint trait. In this regard, H_F% and H_P% show a high correlation, making a combination of these 2 easily possible. Because genetic variances in both H_F% and H_P% are high relative to the population-wise effects of heat (69% and 65%, respectively), this indicates that with little weight in a selection index, reduced concentration due to higher THI can be compensated. Milk yield remained stable up to a THI of 60. However, at higher THI levels, production losses increased substantially. Despite substantial genetic variation in H_MY, a 1-gSD improvement would only offset 11% of the production losses. Nonetheless, because we found no negative correlations to milk, protein, or fat yield, paired with favorable correlations to other production traits (0.29 correlation to NVI), the use for breeding should come at limited risk.

Because there is currently no economic benefit in breeding for individual fatty acid concentration, there is no direct benefit of the use of the heat tolerance traits for individual fatty acids, however, these traits could still be beneficial for breeding purposes as indicator traits for heat stress with H_C-16:0 and H_C-18:0 showing significant correlation to resilience and H_C-18:3 *cis*-9,12,15 to longevity (Figure 9). Furthermore, association with other heat-related traits, such as rectal temperature, respiratory rate, or activity (Li et al., 2020; Yan et al., 2021), should be assessed to evaluate the potential to replace or complement costly recording schemes for such traits by the use of already available MIR data.

Haplotype Blocks for Association Studies

Results from the GWAS analysis confirmed a variety of peaks that were previously associated with heat stress adaptation, such as BTA5 (Sigdel et al., 2019), BTA14 (Prakapenka et al., 2024), and BTA19 (Bouwman et al., 2014), validating the suitability of our approach. Although BTA20 (Huson et al., 2014) is commonly associated with heat stress adaptation, this effect is usually associated with the SLICK gene, leading to a slicker fur.

However, because the SLICK variant originates from the Senepol breed (Mariasegaram et al., 2007) and is not present in Dutch Holstein, our results suggest an additional associated variant on BTA20 that is in close proximity to the SLICK gene and has a positive effect on the 3 primary considered heat tolerance traits H_F%, H_P%, and H_MY. Note that no fine mapping of the associated region to identify causal variants using sequencing and molecular analysis tools was performed within this study.

Overall, as the estimated effects for the respective peak variant in QTL regions on various chromosomes were high, relative to the overall genetic variation in the trait. To some extent, this will be caused by these variants having overestimated effects, as they were estimated to have the lowest *P*-value. Nonetheless, this also highlights the impact targeted breeding or introgression of a few key regions can have when breeding for heat tolerance. Because peak variants are typically present in low frequency, they nonetheless only explain a low share of the total variation.

The use of haplotype blocks (Pook et al., 2019) in a GWAS provides a powerful tool to increase the density of variants considered to add further impactful variation to a dataset, thereby increasing the power of the GWAS. This is particularly highlighted by the peak on chromosome 20, as the peak SNP of the GWAS showed practically no effect if the peak haplotype block was not present. Although no fine mapping was performed, this should provide clear evidence that the haplotype block in this case is better suited at identifying which individuals are carriers of the causal variant to subsequently help further narrow down the actually causal variant by allowing for the analysis of more aggregated variables of segments of the genome that frequency result in lower *P*-values and providing a visualization tool of local population structure to relate this directly to traits and their phenotypes. Nonetheless, for the identification of causal variants, molecular analysis is required; however, quantitative or statistical analysis by haplotype blocks can act as a powerful tool to assist such molecular analysis by identifying candidate regions and individuals (Gusev et al., 2016; McLaren et al., 2016).

Improving the current population to a level of suitability in more challenging climatic conditions from genetic variation within the Dutch Holstein population will be extremely challenging. At the same time, this also highlights the need to look into alternative strategies when aiming to improve the heat tolerance of the Dutch Holstein population. Exemplary strategies could include the use of crossbreeding to introduce genetic material from a locally adapted breed or the introgression of such diversity into the breeding population (Hoffmann, 2013; Galukande et al., 2013). Although practically not possible for production animals in Europe, the downsides of introgression could be bypassed by genome editing (Jinek et al., 2012), as already done in research with the introduction of the SLICK mutation into Holstein via the use of CRISPR-Cas9 (Cuellar et al., 2024). Before implementing such procedures, a functional understanding of the trait is needed, however, particularly to ensure that a variant is actually causal (Simianer et al., 2018), as we for example identified GWAS peaks on BTA20, in close proximity to the SLICK gene (Littlejohn et al., 2014), with the SLICK variant not even present in the Dutch Holstein population. Hence, there might be additional causal genes in the region that need to be accounted for. Furthermore, we observed lower overall production levels in cows carrying some of the QTL identified in this study, introducing a further potential source of confounding by identifying lower overall production level cows as particularly heat tolerant, as they have less to lose.

Between Heat Resilience and Tolerance

On a conceptual level, further consideration needs to be given to the fact that the heat tolerance traits defined herein primarily focus on which animals are able to maintain their production levels the best under heat conditions. However, reduction of production levels can sometimes be the only way for the animal to cope with the extreme stress situation to avoid more severe reactions, with the most extreme reaction being the likelihood of death (West, 2003), commonly referred to as the heat resilience of an animal (Misztal et al., 2024). In practical breeding, yield-based traits, as proposed in this work, should be complemented by the impact of heat on other traits (Ojo et al., 2025) and health records to capture both heat tolerance (ensuring stable production) and heat resilience (reducing the risk of morbidity and mortality) within the breeding objective of improving general heat stress adaptation (Poppe et al., 2022; Misztal et al., 2024; Ojo et al., 2025). Our results here indicate that cows that reduce their production levels by decreasing F% and P% tend to have worse resilience, whereas a smaller reduction of milk yield in heat conditions showed favorable correlations to health and resilience traits.

CONCLUSIONS

Our newly developed pipeline provides a computationally efficient tool to integrate sophisticated methodology to analyze the impact of heat stress on a population-wise level and subsequently define novel heat tolerance traits to be used for breeding based on traits with repeated measurements, such as milk production data. The heat tolerance traits defined in this study exhibit substantial genetic variance on a whole-genome level, a variety of associated regions with major effects, and, in the case of heat tolerance based on milk yield, beneficial correlations to key production traits, thereby making them suitable for use in breeding. Nonetheless, we want to emphasize that breeding efforts need to be complemented by other management approaches such as cooling, shading, and nutrition, as breeding primarily aims at long-term population improvement. To improve animal welfare and production in the short term, the integration of crossbreeding or intercrossing to a more adapted breed provides further ways to enhance tolerance and help animals cope with more extreme climatic conditions than are is expected in the temperate maritime climate of the Netherlands.

NOTES

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Nonstandard abbreviations used: AMS = automated milking system; C3S = Copernicus Climate Change Service; F% = fat percentage; gSD = genetic standard deviation; GxE = genotype by environment interaction; H_F% = heat tolerance trait for F%; H_MY = heat tolerance for milk yield; H_P% = heat tolerance trait for P%; HB = haplotype block; MIR = mid-infrared; MY = milk yield; NVI = total merit index; NW = Nadaraya–Watson; P% = protein percentage; PLSR = partial least squares regression; RY_F = relative yield of fat; RY_P = relative yield of protein; THI = temperature-humidity index.

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