Genetic analysis of heat tolerance for production and health traits in US Holstein cows

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Summary

Heat stress reduces milk production, depresses fertility and increases the incidence of health disorders in dairy cows. Our first goal was to estimate genetic components of milk yield (MY) and somatic cell score (SCS) across lactations considering heat stress. Our second goal was to reveal genes responsible for thermotolerance. Data included 254k MY and 356k SCR test-day records of 20k Holstein cows. Multi-trait repeatability test-day models with random regressions on THI values were used to estimate variance components. The models included herd-test-date and DIM classes as fixed effects, and generic and heat tolerance additive and permanent environmental as random effects. Genetic variances for MY under-heat stress increased 3.9 and 6.5% between consecutive parities, suggesting that cows become more sensitive as they age. Heritability estimates for MY at THI 78 were between 0.17 to 0.32. Genetic correlations between general merit and heat tolerance ranged from -0.30 and -0.55, indicating production and thermotolerance are antagonistic. For SCS, heritability estimates for SCS at THI 78 were between 0.10 and 0.16. For this trait, genetic correlations between general merit and thermotolerance were always positive, ranged from +0.10 to +0.43. Whole-genome scans were performed using ssGBLUP. For MY, as expected, the region on BTA14 that harbors *DGAT1* was associated with general merit in all three parities. One region on BTA15 was associated with thermotolerance across lactations; this region harbors *PEX16, MAPK8IP1,* and *CREB3L1*, genes implicated in thermogenesis and cellular response to heat stress. For SCS, regions on BTA6 and BTA29 were implicated in general udder health in all parities. These regions harbor genes, such as *CXCL13*, *SCARB2*, and *FAT3*, that are involved in immune response. Notably, genes *DLX1* and *DLX2* which downregulate cytokine signalling pathway were associated with SCS thermotolerance in all lactations. Overall, this study contributes to better understanding of the genetics underlying heat stress and point out novel opportunities for improving thermotolerance in dairy cattle.

Keywords: heat stress, variance components, repeatability test-day model, ssGBLUP

Introduction

Heat stress negatively impacts the performance and health of farm animals. In dairy cows, heat stress decreases milk yield, reduces milk quality and depresses fertility. Economic losses attributable to heat stress are estimated to be between $897 and $1500 million per year for the US dairy industry (St-Pierre *et al.,* 2003). Different approaches including cooling, shading and nutrition are commonly used to mitigate the effects of heat stress. However, these practices increase production costs and in general, they cannot eliminate heat stress completely. One of the complementary strategies of reducing the effects of heat stress is the selection of heat

tolerant animal. Several studies have reported the existence of additive genetic variability for heat stress in dairy cattle (Ravagnolo *et al.,* 2000, Aguilar *et al.,* 2009). Note that selective breeding of dairy cattle for thermotolerance is permanent, cumulative, and hence, the most cost-effective approach for mitigating the effects of heat stress in dairy cattle.

The first objective of the study was to estimate the variance components for milk yield (MY) and somatic cell score (SCS) across lactations using multi trait repeatability test-day model considering heat stress. Test day records of MY and SCS were merged with daily THI value based on meteorological records from public weather station, and were jointly analysed using test-day models that include random regressions on a function of THI values. The second objective of the study was to identify and characterize genomic regions, and preferably individual genes, responsible for the variation in thermotolerance underlying production and udder health. For this, test-day records of MY and SCS, weather data, and genome-wide SNP markers were jointly analysed. This study will contribute to better understanding of the genetics underlying heat stress and subsequent use of marker-assisted selection in breeding program.

Material and methods

Phenotypic and genotypic data

Data comprised 254k MY and 356k SCR test-day records of 20k Holstein cows calved from 2006 through 2016 on two farms in the State of Florida, USA. For MY, lactation records were required to have at least 8 test days, and for SCS, lactation records were required to have at least 5 test day records and records should be in between 5 and 305 DIM. Genotype data for 60k SNP markers were available for 6,362 cows with test-day records and 1,592 sires in the pedigree. Hourly THI were calculated using meteorological records as described by Ravagnolo *et al.* (2000); mean daily THI 3 days prior the test-day was assigned to each test-day record as suggested by Bohmanova *et al.* (2007).

Statistical Model

The following multi-trait repeatability test day model was used to estimate the variance components for heat tolerance, considering multiple lactations as different traits.

$$y_{klmn} = HTD_{kl} + DIM_m + a_n + p_n + v_n[\phi(THI)] + q_n[\phi(THI)] + e_{klmn}$$

Where y_{klmn} is the test day record of MY or SCS in herd-test-date k within parity l, DIM class m, effects p_n, v_n, p_n and q_n are the generic and heat tolerance additive and permanent environmental effects of cow n, and e_{klmn} is the residual. Threshold of heat stress was set to 68 and thus Φ (THI) was equal to max (0, THI-68).

Statistical Analysis

Variance components for MY and SCS using multi-trait repeatability test-day models were estimated in a Bayesian framework using GIBBS2F90, a program that implements joint sampling of correlated effects and traits. A total of 500,000 iterations were run with the first 100,000 discarded as burn-in and storing every 100th sample. Convergence was determined by graphical inspection of the posterior chain. The whole-genome association mapping was performed using single-step genomic BLUP methodology (ssGBLUP).

Results and discussion

Genetic dissection of heat stress for MY and SCS

Variance components of general production level (intercept) and animal ability to respond to heat stress (slope) for MY and SCS using multiple lactations (1-3) were estimated. Similarly, heritability and genetic correlation between generic and heat stress additive effects at heat stress level ϕ (THI) = 78 (i.e. 10 units above THI threshold of 68) for each trait and lactation were also estimated. Genetic variances for MY under-heat stress increased 3.9 and 6.5% between consecutive parities, suggesting multiparous cows are more affected by heat stress than primiparous cows. Genetic correlations between generic and heat tolerance effects for MY in all three lactations were negative, ranged from -0.30 to -0.55 which is in agreement with the findings of Aguilar *et al.* (2009) using also first three parities in US Holsteins. Heritability estimates for MY at THI 78 were between 0.17 to 0.32, which is comparable with the heritability estimates reported by Ravagnolo *et al.* (2000).

General additive genetic variances and heat tolerances additive genetic for SCS also increased with parity which concords with the findings of Hammami *et al*. (2015). Heritability estimates for SCS at THI 78 across parities ranged from 0.10 to 0.15 which is in the range of the heritability estimates by Santana *et al.* (2017) in Brazilian Holsteins. Genetic correlations between generic and heat tolerance effects for SCS in all three lactations were positive and ranged from +0.10 to +0.43.

Table 1. General (σ^2_a) and heat tolerance ($100\sigma^2_v$) additive variances, genetic correlation ($r^G_{(a, v)}$) and estimates of heritability $h^2_{f(10)}$ at THI of 78 (10 unit above THI threshold of 68).

	MY			SCS		
Parameters	Parity1	Parity2	Parity3	Parity1	Parity2	Parity3
σ^2_a	9.26	10.03	10.55	0.27	0.28	0.38
$100\sigma^2_v$	0.94	1.56	1.62	0.042	0.049	0.091
$10_{a\cdot v}$	-1.21	-1.17	-2.31	0.026	0.011	0.081
$h^2_{f(10)}$	0.32	0.24	0.17	0.11	0.10	0.15
$r^G_{(a,v)}$	-0.41	-0.30	-0.55	0.24	0.10	0.43

[1] σ^2_a = general additive genetic variance; $100\sigma^2_v$ = heat tolerance additive variance at 78 (10 units above a THI threshold of 68); $10_{a\cdot v}$ = additive genetic covariance between general and heat tolerance effect; $r^G_{(a, v)}$ = genetic correlation between general and heat tolerance effect

Whole-genome mapping for thermotolerance genes affecting MY and SCC

Single-step genomic BLUP methodology was utilized to perform GWAS of heat tolerance for MY and SCS. The association analysis identified several regions in the bovine genome strongly associated with general production level and animal ability to respond to heat stress. Figure 1 displays Manhattan plots for MY across the 3 lactations under study; the left plots show the result for general merit (intercept) while the right plots show the results for thermotolerance (slope). The results are presented in terms of the proportion of the genetic variance explained by 2.0 Mb SNP windows across the entire bovine genome. For MY, as expected, the region on BTA14 (14:1379063-3371507) that harbors *DGAT1* was associated with general merit in all three parities. The analysis also identified several regions associated with thermotolerance. Indeed, one region on BTA15 (15: 75595790-77595636) was strongly associated with heat susceptibility across the 3 parities. This region harbors *PEX16, MAPK8IP1,* and *CREB3L1*, genes implicated in thermogenesis and cellular response to heat stress.

Figure 2 displays Manhattan plots for SCS across lactations; the left plots show the result of general merit and the right plots show the results of SCS thermotolerance. One relevant region affecting general SCS merit was identified on BTA29 (29:2294521- 4291391) across all three parities. Interestingly, this region harbors *FAT3*, a calcium ion binding gene that impairs immunity, reduces smooth muscle function in teat sphincter, and hence increases counts of SCS and risk of mastitis (Mulligan *et al.,* 2006). Another genomic region affecting SCS was identified on BTA6 (6:92648735-94627787) across all three parities. This region harbors genes *CXCL13* and *SCARB2* which are important in mediating host response against bacterial invasions during infection. One region on BTA2 (2:24325003-26323407) was strongly associated with SCS thermotolerance. This region harbors two candidate genes, *DLX1* and *DLX2* which downregulate cytokine signaling pathway and prevent inflammation.

Conclusions

The results of this study reinforce the idea that there is a negative relationship between general production merit and thermotolerance, either for MY or SCS. Therefore, continued selection for general merit without consideration of heat tolerance will result in greater susceptibility to heat stress. In this study, we also performed GWAS with the purpose of understanding the genetic architecture of thermotolerance for MY and SCS. Our findings reveal that thermotolerance is a quantitative trait influenced by many regions of the genome. Several potential candidate genes with known roles in thermal regulation and immune response were identified in these genomic regions. Overall, the findings of this study contribute to better understanding of the genetics underlying heat stress and point out novel opportunities for improving thermotolerance in dairy cattle.

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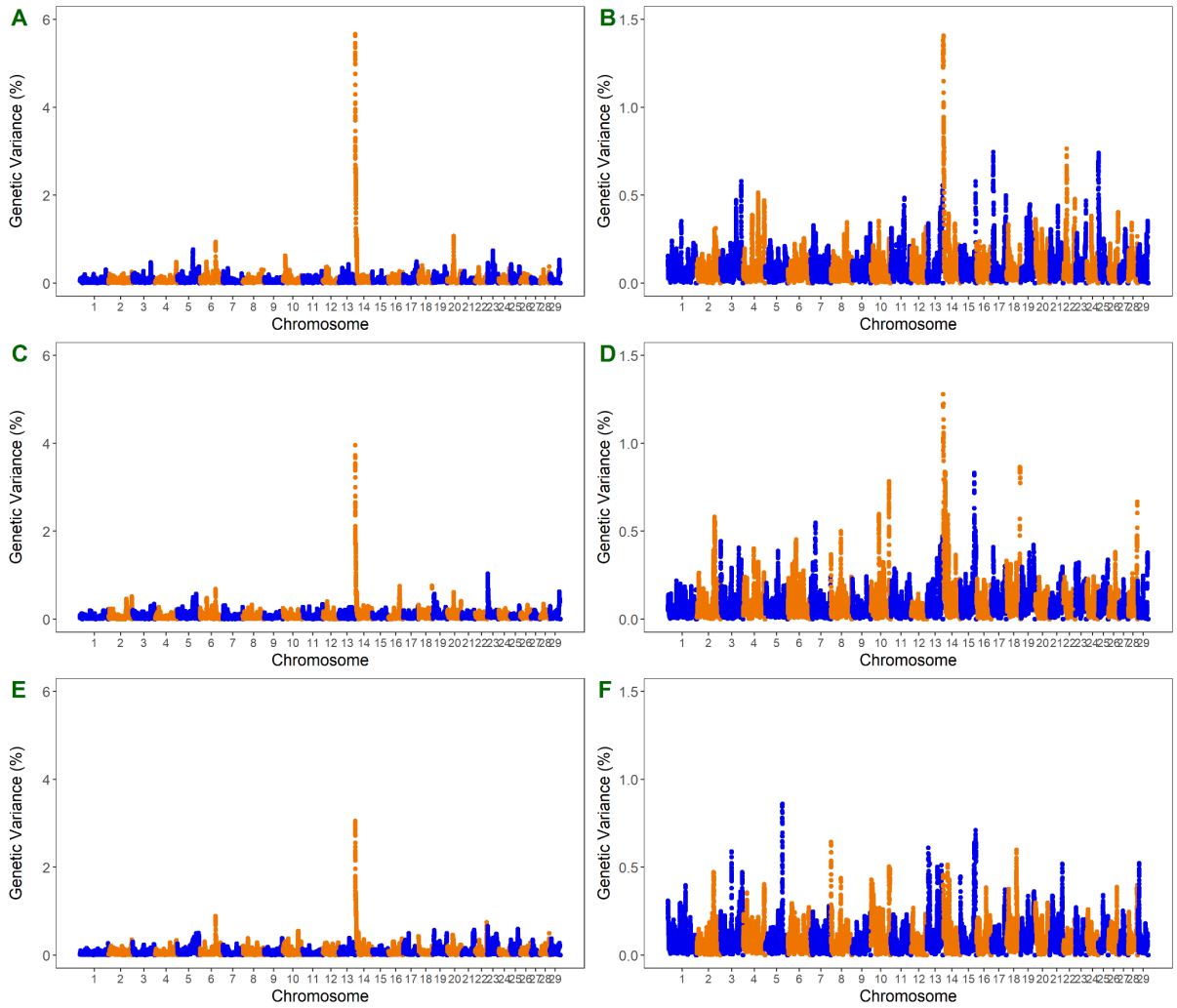

Figure 1. Manhattan plots showing the results of the genome-wide association mapping for Milk Yield: the left plots show the result of general merit and the right plots show the results of thermotolerance across lactations numbered vertically as lac1 lac2 and lac3.

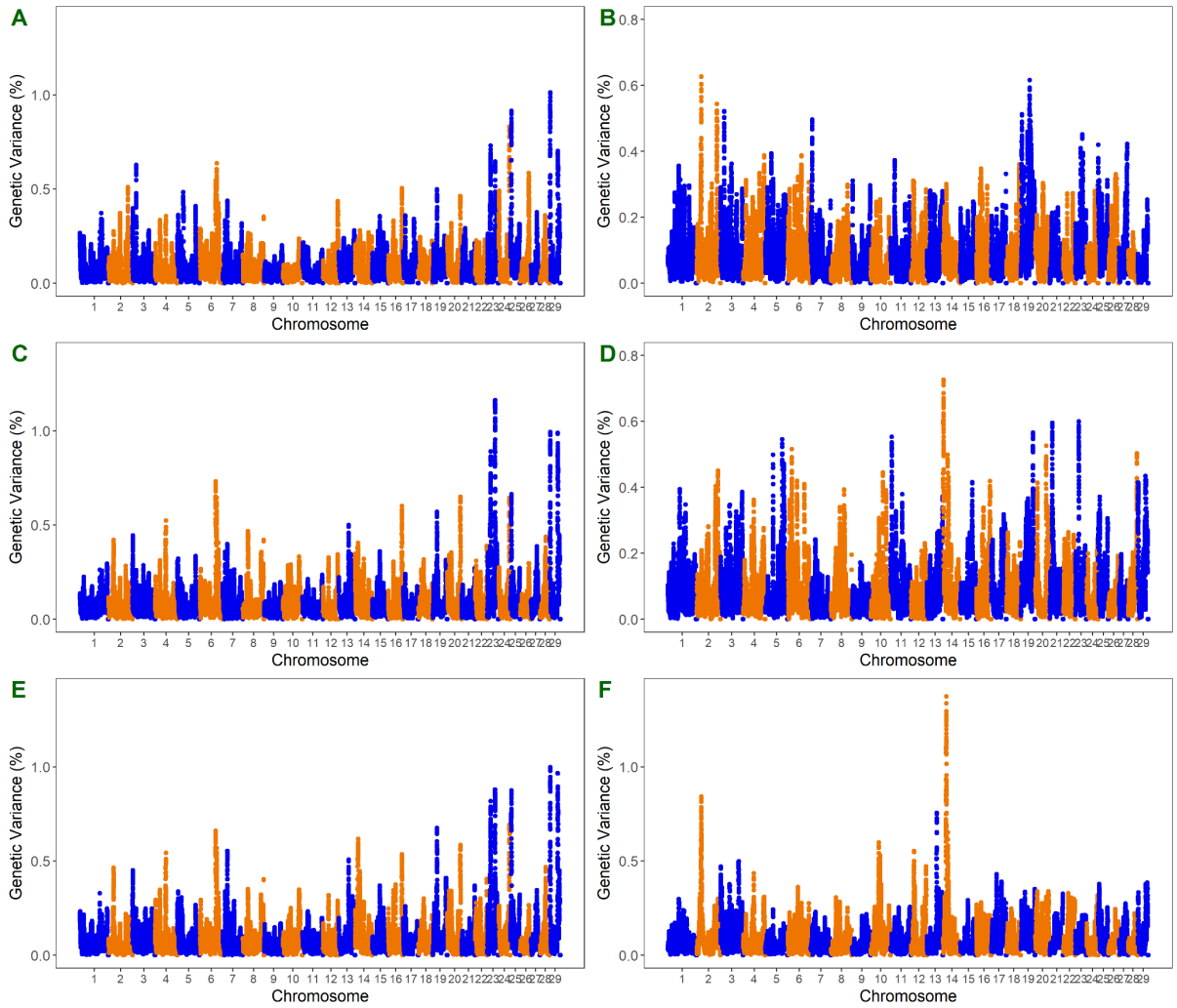

Figure 2. Manhattan plots showing the results of the genome-wide association mapping for Somatic Cell Score: the left plots show the result of general merit and the right plots show the results of thermotolerance across lactations numbered vertically as lac1, lac2 and lac3.