A limited number of Y chromosome lineages is present in North American Holsteins

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ABSTRACT

Holsteins are the most numerous dairy cattle breed in North America and the breed has undergone intensive selection for improving milk production and conformation. Theoretically, this intensive selection could lead to a reduction of the effective population size and reduced genetic diversity. The objective of this study was to investigate the effective population size of the Holstein Y chromosome and the effects of limited Y chromosome lineages on male reproduction and the future of the breed. Paternal pedigree information of 62,897 Holstein bulls born between 1950 and 2013 in North America and 220,872 bulls evaluated by multiple-trait across-country genetic evaluations of Interbull (Uppsala, Sweden) were collected and analyzed. The results indicated that the number of Y chromosome lineages in Holsteins has undergone a dramatic decrease during the past 50 years because of artificial selection and the application of artificial insemination (AI) technology. All current Holstein AI bulls in North America are the descendants of only 2 ancestors (Hulleman and Neptune H) born in 1880. These 2 ancestral Y-lineages are continued through 3 dominant pedigrees from the 1960s; namely, Pawnee Farm Arlinda Chief, Round Oak Rag Apple Elevation, and Penstate Ivanhoe Star, with a contribution of 48.78, 51.06, and 0.16% to the Holstein bull population in the 2010s, respectively. The Y-lineage of Penstate Ivanhoe Star is almost eliminated from the breed. The genetic variations in the 2 ancestral Y-lineages were evaluated among 257 bulls by determining the copy number variations (CNV) of 3 Y-linked gene families: PRAMEY, HSY, and ZNF280BY, which are spread along the majority (95%) of the bovine Y chromosome male-specific region (MSY). No significant difference was found between the 2 ancestral Y-lineages, although large CNV were observed within each lineage. This study suggests minimal genetic diversity on the Y chromosome in Holsteins and provides a starting point for investigating the effect of the extremely limited number of Y-lineages on male reproduction and other traits important for the future of the Holstein breed.

Key words: Y chromosome, copy number variation, paternal pedigree, Holstein, effective population size

INTRODUCTION

Holstein cattle, renowned for their milk production and recognized by their distinctive black (or red)-and-white markings, are the largest dairy cattle population in the world. In 2012, there were ~26 million Holstein cows registered by the World Holstein-Friesian Federation. Although the origin of Holsteins can be traced to the development of cattle in the north region of the Netherlands over the last 2 millennia (Lush et al., 1936), the history of Holsteins in North America is very short, only about 150 yr. The first group of Holsteins was brought to the United States in the 1850s (Lush et al., 1936). The breed quickly gained the attention of American cattle breeders and dairymen because of its reputation for high yields of quality milk. From then on, 8,800 Holsteins were imported to the United States before importation ceased in the early 1900s due to an outbreak of foot-and-mouth disease in mainland Europe (http://www.holsteinusa.com/holstein_breed/breedhistory.html). Obviously, the imported animals were sufficient for initiation of an American breeding program. To date, there are ~9.2 million milk cows in the United States and ~90% of these are Holstein, supplying ~86,000 million kg of milk (USDA, 2010). The average milk production per cow per year is 9,455 kg in the United States, which is significantly higher than that in other countries (WHFF, 2012).

The Holstein breeders in the United States established the first Holstein-Friesian herd book (HBB) association in 1871 (Lush et al., 1936), which is now the world’s largest Holstein association with more than 19 million registered Holsteins (http://www.holsteinusa.com/holstein_breed/breedhistory.html). In the late 1940s, modern AI was established [Perry, 1968; Wolf Foundation Prize in Agriculture in 1981 (http://www.wolffund.org.il/index.php?dir=site&page=winners&cs}
ing that CNV of several multicopy gene families, includ-

ing (2009, 2011, 2012; Yue et al., 2013, 2014) have revealed

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1995). In most farm-animal breeding programs, such

as many as X-linked genes (Tucker and Lundrigan,

during evolution, whereas male-limited transmission

provides an ideal genomic niche to enhance male fitness

because male-beneficial mutations on the Y chromo-

some are always transmitted through the sex (Rice,

1996; Bachtrog, 2013). One of the consequences of male-

limited transmission is the reduction of the effective Y

population size (Wilson Sayres et al., 2014). In an ideal

breeding population in which the female-to-male breeding

sex ratio is 1, there are only one-fourth as many

Y-linked genes as autosomal genes and only one-third

as many as X-linked genes (Tucker and Lundrigan,

1995). In most farm-animal breeding programs, such

as in Holsteins, the female-to-male breeding sex ratio is

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and other livestock species have not been addressed.

Genes on the mammalian Y chromosome are believed
to be essential for maleness, spermatogenesis, and male

fertility. Recent studies on the bovine Y chromosome
gene copy number variations (CNV; Hamilton et al.,

2009, 2011, 2012; Yue et al., 2013, 2014) have revealed

that CNV of several multicopy gene families, including

PRAMEY (preferentially expressed antigen in melanoma, Y-linked),

HSFY (heat-shock transcription factor, Y-linked), and ZNF280BY (zinc finger protein

280B, Y-linked), are associated with fertility traits in

Holstein bulls (Yue et al., 2013, 2014), indicating that

the bovine Y chromosome has important influences on

bull fertility.

The objective of this study was to investigate the

effective population size of the Holstein Y chromosome

and the effects of limited Y chromosome lineages on

male reproduction and traits important for the future

of the breed in North America.

MATERIALS AND METHODS

Retrieval of Paternal Pedigree Information

of Holstein Bulls in North America

Holstein bull registration numbers, names, and birth
dates in the United States and Canada that were en-
rolled in the National Association of Animal Breeders
were retrieved following the August 2013 national ge-
netic evaluations of the Council on Dairy Cattle Breeding
(CDCB; https://www.cdcb.us/eval.htm). Paternal
pedigree information of 62,987 bulls was available from
CDCB files beginning from the 1940s. Beyond the
1940s, the paternal pedigrees were manually checked
based upon online family tree searches available at
http://www.holsteinusa.com/. In cases in which the
sire information was missing from online family trees,
manual inspection of a physical HHB collection main-
tained by Lawrence Specht (The Pennsylvania State
University, University Park) was used to complete the
pedigree analysis.

Determination of Founders and Ancestors

for Holstein Bulls

We used founders and ancestors to specify the contri-
bution of an individual Y chromosome in the paternal
pedigree. Bulls that were born in the 1960s, when the
national genetic evaluation program was implemented
and AI technology was widely adopted, are referred
to as AI-era “founders.” For “ancestors,” we refer to
those sires that were born in the Netherlands and were
imported to the United States before the 1900s, or sires
that were traced back to 1880 based on the available
HHB records.

In addition, we retrieved pedigrees of 220,872 Hol-
stein-Friesian bulls from 35 different countries, evalu-
ated by the International Genetic Evaluation Service
through the multiple-trait across-country evaluation
(MACE) of Interbull (http://www.interbull.org/ib/
terbull) as of December 3, 2013, which is henceforth
referred to as the Interbull database. As the paternal
pedigree information was not available for most of the

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bears in the Interbull database before the 1950s, we evaluated the AI-era founder sires, but ancestors were not analyzed.

Genotypic and Phenotypic Data

The copy number of 3 Y-linked gene families, \textit{PRAMEY}, \textit{HSFY}, and \textit{ZNF280BY} was used to present the genotype of the particular Y chromosome, as these 3 gene families are amplified and spread over 95\% of MSY (Chang et al., 2013). The CNV of 257 Holstein AI bulls were determined previously (Yue et al., 2013, 2014). These bulls were grouped according to AI-era founder. The median copy number (MCN) of the Y-linked genes and the animal reproductive records (see below) were compared among different groups. In addition, bulls were also grouped based on either their generation number to their ancestors (with the ancestor being the 0 generation) or their relationship as full-/half-siblings.

Out of these 257 bulls (Yue et al., 2013, 2014), 140 bulls were from Select Sires Inc. (Plain City, OH), which had records in scrotal circumference (SC), age-adjusted relative scrotal circumference (RLSC), postthaw motility (PTM), incubated motility (IM), percentage of normal sperm (PNS), and percentage of intact acrosome (PIA). The remaining 117 bulls were from Semex Alliance (Guelph, ON, Canada) and had nonreturn rate (NRR) records. In addition, the sire conception rate (SCR) of active AI bulls (1,509 in total) as of December 2013 was retrieved from the CDCB database (https://cdcb.us/eval/summary/scr_menu.cfm). To have a more reliable estimation, bulls (488) with SCR reliability $\geq$90\% were included in the analysis.

Data Analysis

One-way ANOVA was applied to analysis of the AI-era founder effect on reproductive traits, the association between CNV of Y-linked genes, and the number of generations between bulls and their ancestor. Pair-wise comparisons of mean value were conducted by Student's $t$-test. The MCN of Y-linked genes were compared by the nonparametric Mann-U test. The $P$-values for the multiple comparisons were adjusted by Bonferroni correction (Dunn, 1961); $P < 0.05$ is considered a significant difference.

RESULTS AND DISCUSSION

Two Y Chromosome Lineages Survived in Current Holsteins in North America

In total, 62,897 bulls born between 1950 and 2013 were retrieved, and were divided into 7 groups based upon their decade of birth. As shown in Figure 1, the number of bulls increased from 1950 to 1990. Only 846 AI bulls were born in the 1950s in the CDCB database. The number of bulls increased rapidly from the 1970s to the 1990s and reached a peak of 17,447 in 1990 to 1999, followed by a slight decrease in the 2000s. In total, 4,325 bulls were born in the first 3 yr (2010–2012) of this decade. If the trend for this decade remains roughly the same, we estimate that there will be about 14,410 bulls at the end of this decade (Figure 1, dashed line). However, dairy producers have only been able to genomically test their own bulls since 2013, and it is likely that the number of bulls with official genomic evaluations will increase in the coming decade.

It is well known that the effective population size ($Ne$) of the Holstein cattle population has been reduced in the last half-century because of intensive artificial selection and application of AI technology. There was an increase in inbreeding estimates of 0.57\% for Holsteins born in 2014 before the August 2014 CDCB genetic evaluation compared with those born in 2010 (https://www.cdcb.us/eval/summary/inbrd.cfm).

With an assumption of a 4-yr generation interval for North American Holsteins, we estimated that the $Ne$ ($Ne = 1/2\Delta F$, where $\Delta F = 0.0057$, which is the rate of inbreeding per generation) was less than 100.

Upon analysis of the North American Holstein pedigrees, all 62,897 bulls born between 1950 and 2013 were descendants of only 4 ancestors: 886HBB (Hullemann, 3/27/1881), 711HBB (Neptune H, 3/23/1880), 716HBB (Netherland Prince, 4/1/1880), and 608HBB (Jacob, 2/25/1880). Of these, 56\% (35,220) and 43.75\% (27,516) were the descendants of 886HBB and 711HBB, and only 0.04\% (27) and 0.21\% (134) were the descen-
dants of 716HHB and 608HHB, respectively. The latter 2 ancestors do not have any living descendants, and the most recent bull in these pedigrees was born on 7/12/2002, with registration no. HOUSA132946113. His father (Brigeen Model Paragon, HOUSA1413083) was born on 12/7/1961. Therefore, the generation interval was 40 yr between the son and his father.

This suggests that only 2 independent Y chromosomes have survived in North American Holsteins. Theoretically, the extremely small effective population size of males (or Y chromosomes) has reduced variation at Y-linked loci (relative to X-linked and autosomal loci) because of stochastic bottleneck effects (Nei et al., 1975; Tucker and Lundrigan, 1995). Practically, the effect of this extremely small Ne of males on Holstein breeding program, especially on the male reproductive traits, is unknown. We believe that the extreme lack of genetic diversity of Y chromosome could be a limiting factor toward improvement in Holsteins for male fertility traits that are influenced by the Y chromosome.

The Number of Founder Bulls in North American Holsteins and in the Interbull Database

In the present study, we analyzed the population structure of paternal pedigrees for those Holstein bulls that were born in and after the 1960s, and that participated in the AI program, because both genetic evaluation and AI became routine practices in the dairy industry in the 1960s. We started with the current descendants of the 2 living Y chromosome lineages and traced their male relatives born in the 1960s, referred as AI-era founders in this study; 1,821 founders were present initially (Figure 2). To investigate how these founders were lost over the past 50 yr, we counted the number of remaining founders by decade (1960 to 2010) under the condition that the founder had live male descendant(s) in that particular decade. Otherwise, the founder and its lineage were considered lost. As shown in Figure 2, the number of remaining AI-era founders decreased dramatically from the 1960s to the 2010s in North America. Only 3 AI-era founders, HOUSA1427381 (Pawnee Farm Arlinda Chief, 5/9/1962), HOUSA1441440 (Penstate Ivanhoe Star, 01/20/1963) and HOUSA1491007 (Round Oak Rag Apple Elevation, 08/30/1965), were present in the 2010s. Among these 3 founders, HOUSA1427381 and HOUSA1441440 shared the same ancestor (866HHB).

We further analyzed the proportion of the population that traced to the 3 AI-era founders identified in the 2010s (Figure 3). Because these 3 bulls were born in the 1960s, we calculated the percentage of their descendants in North American Holsteins after 1960, which gradually increased (Figure 3). By the 1970s, the contribution of these 3 paternal lines was 30.92%. It reached over 80% by the 1980s and 1990s (Figure 3), which coincided with the dramatic increase in the number of AI bulls during the same period (Figure 1). Two of these 3 founders became dominant in the 2010s, with 48.78% (2123/4352) of all bulls in the Pawnee Farm Arlinda Chief pedigree, and 51.06% (2222/4352) in the Round Oak Rag Apple Elevation pedigree. The contribution of the third founder (Penstate Ivanhoe Star) gradually decreased, from 10% in the 1990s to only 0.16% (7/4352) in the 2010s.

We performed a similar analysis on 220,872 Holstein-Friesian bulls in the Interbull database. As indicated in Figure 2, the number of founders in Interbull decreased from the 1960s to the 2010s with a very similar trend to that found for North American Holsteins. By the 2010s, 5 founders survived. Besides the 3 founders that were present in North America, we identified 2 additional founders in the Interbull database; namely, Roybrook Telstar (Reg. No: HOCAN288790, 12/13/1963) and Paclamar Astronaut (Reg. No: HOUSA1458744, 01/19/1964). Roybrook Telstar is a descendant of 711HHB, and Paclamar Astronaut is a descendant of 866 HHB. Our analyses suggest that, most likely, all of
today’s Holstein bulls across the world are descendants of these 2 ancestors: 886HHB and 711HHB.

It appears that the vast majority of male AI-era founders (>99%) in Holsteins (Figure 2) were eliminated from the breeding populations in the past 50 yr (1960 to 2010). However, very little information is available on the reasons behind the elimination of these Y chromosome lineages. The Y-lineage of the Penstate Ivanhoe Star could be lost from the Holstein breed if the declining trend is maintained. Therefore, Penstate Ivanhoe Star provides a unique opportunity for us to investigate how a Y-lineage can be lost so quickly in just a few decades (Figure 3). It does not appear that the Penstate Ivanhoe Star lineage has been lost due to inferior genetic merit. Based upon current genetic evaluations (https://www.cdcb.us), Penstate Ivanhoe Star is similar in Lifetime Net Merit (−$421) to Round Oak Rag Apple Elevation (−$412) and is superior to Pawnee Farm Arlinda Chief (−$537).

Penstate Ivanhoe Star was a carrier of loss-of-function mutations for both complex vertebral malformation (CVM) and bovine leukocyte adhesion deficiency (BLAD; Shuster et al., 1992; Thomsen et al., 2006). Breeders have eliminated descendants that were carriers of these loss-of-function mutations, particularly bulls entering AI, which has likely reduced the influence of the Penstate Ivanhoe Star in Holstein sire lines. Although unfavorable loss-of-function mutations may have contributed significantly to the decline of the Penstate Ivanhoe Star Y lineage, not all male descendants were carriers of CVM and BLAD and this may not be the sole reason for the decline. Random genetic drift may also have played a critical role due to the small Ne in Holsteins, which would suggest that populations with a large census population can be vulnerable to a loss of genetic diversity due to drift in some instances.

There is a lack of knowledge of Y chromosome genes and their functions, which also contributes to lack of attention paid to the effect of selection on Y. Recent studies in the bovine and other mammalian Y chromosome sequence and genes signify the importance of Y chromosome in male reproduction and other functions (Chang et al., 2013; Yue et al., 2013; Bellott et al., 2014; Cortez et al., 2014) and provide a molecular basis for functional characterization of the Y chromosome and identification of Y-linked markers for male selection.

**The Relationship Between the Founders and Their Y Chromosome Variations in North American Holsteins**

The bovine MSY is composed of 3 regions: X-degenerate, Y-transitional, and Y-ampliconic (Chang et al., 2013). The Y-linked genes in the ampliconic region have multiple copies and their CNV have been found to be associated with scrotal circumference and male fertility in Holsteins (Yue et al., 2013, 2014). To investigate the relationship between the AI-era founders and their Y chromosome variations, we analyzed the CNV of 3 Y-linked gene families, PRAMEY, ZNF280BY, and HSFY, among 257 Holstein bulls (Yue et al., 2013, 2014). These bulls were born between 1995 and 2007. All but 3 bulls investigated were the descendants of the 3 founders discussed above. The remaining 3 bulls were descendants of a fourth founder, HOUUSA1428104 (Sunnyside Standout-twin, 03/06/1962; Table 1). No living animal found in this founder’s lineage is found in the current Holstein population. Statistical analysis indicated that the MCN of the 3 Y-linked genes was not significantly different among these Y chromosome lineages ($P > 0.05$), although copy number varied significantly among individuals within each lineage (Table 1; Yue et al., 2013, 2014).

We further analyzed the CNV of the Y-linked genes among different generations within each Y chromosome lineage. We grouped the 257 bulls based on the number of generations from their ancestor. As shown in Table 2, bulls in the 711HHB lineage belong to the 19th to 21st generations, whereas bulls in the 866HHB lineage belong to the 22nd to 25th generations. The MCN of one Y-linked gene family (ZNF280BY) was significantly different ($P < 0.05$) among different generations in the 711HHB lineage, whereas the MCN of all 3 gene families studied were significantly different ($P < 0.05$) among generations in the 866HHB lineage. However,
we did not observe any association of the MCN with the increase (or decrease) of the generation numbers (Table 2).

Out of 257 bulls tested, 79 were half-siblings fathered by 10 sires (Table 3). Although the MCN of the 3 Y-linked genes varied within and between different half-sib families, most of these variations were not significant ($P > 0.05$). Only 2 half-sibling families (HOUSA220977 and HOUSA122358313) had a significant difference in the MCN of $PRAMEY$ ($P = 0.009$).

Our previous studies indicated that CNV of the bovine Y-multicopy genes were directly linked to the number of repeat units in the Y chromosome ampliconic region (Chang et al., 2013), which contain palindrome-like sequences and harbor mirror-image-like gene pairs (Yang et al., 2011). Research on human Y-linked palindromes suggested that gene conversion and interchromatid exchanges could be the source of CNV in the MSY ampliconic region (Lange et al., 2009). Although the MCN of Y-linked genes were found to be associated with the formation and selection of cattle breeds (Yue et al., 2013, 2014), the significant variations among individuals and the fluctuation of MCN among generations within each Y-lineage observed in this work suggest that the Holstein Y chromosome has not been a target for directional selection in the Holstein breeding program. In future studies, the use of variations in the Y chromosome X-degenerate region, where genes are single copy, might demonstrate stronger relationships among bulls in the same lineage than the Y-ampliconic region, where genes are multicopy (Chang et al., 2013) because there is thought to be reduced genetic diversity of the Y chromosome in the X-degenerate region.

The Founder Effect on Reproductive Traits in North American Holsteins

When reproductive traits, including SCR, NRR, SC, RLSC, PTM, IM, PNS, and PIA, were compared, we found no significant difference ($P > 0.05$) among the 3 different founder Y-lineages (Table 4). Although we could not exclude the potential effect of the relatively small sample size available for this study on the lack of statistical significance, Y-linked gene CNV were overlapped among the 3 Y-lineages (Table 1), which may contribute to the lack of differences among founder lines. Previous analysis on these male reproductive traits with the CNV of the Y-linked genes indicated that the CNV were significantly associated with SC, RLSC, PNS, SCR, and NRR, regardless of the founder effect on the productive traits (Yue et al., 2013, 2014), suggesting that these associated male reproductive

### Table 1

<table>
<thead>
<tr>
<th>Founder</th>
<th>No. of bulls</th>
<th>$PRAMEY$</th>
<th>$ZNF280BY$</th>
<th>$HSFY$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOUSA1427381</td>
<td>88</td>
<td>10.8 ± 0.3</td>
<td>225.3 ± 4.3</td>
<td>204.7 ± 3.1</td>
</tr>
<tr>
<td>HOUSA1441440</td>
<td>27</td>
<td>11.8 ± 0.7</td>
<td>225.2 ± 7.6</td>
<td>199.0 ± 4.6</td>
</tr>
<tr>
<td>HOUSA1491007</td>
<td>139</td>
<td>12.2 ± 0.3</td>
<td>236.9 ± 6.9</td>
<td>205.4 ± 2.7</td>
</tr>
<tr>
<td>HOUSA1428104</td>
<td>3</td>
<td>8.8 ± 2.0</td>
<td>199.5 ± 8.1</td>
<td>203.7 ± 12.3</td>
</tr>
</tbody>
</table>

$^{1}$PRAMEY = preferentially expressed antigen in melanoma, Y-linked; $ZNF280BY$ = zinc finger protein 280B, Y-linked; $HSFY$ = heat-shock transcription factor, Y-linked.

$^{2}$No significant difference ($P > 0.05$) was found among these founder bulls.

### Table 2

<table>
<thead>
<tr>
<th>Ancestor</th>
<th>Generation</th>
<th>No. of bulls</th>
<th>$PRAMEY$</th>
<th>$ZNF280BY$</th>
<th>$HSFY$</th>
</tr>
</thead>
<tbody>
<tr>
<td>711HHB</td>
<td>19</td>
<td>5</td>
<td>10.9 ± 0.9</td>
<td>225.3$^{ab}$ ± 11</td>
<td>205.0 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>46</td>
<td>12.0 ± 0.4</td>
<td>236.9$^{a}$ ± 3.7</td>
<td>204.1 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>87</td>
<td>12.2 ± 0.3</td>
<td>222.8$^{b}$ ± 4.1</td>
<td>205.6 ± 4.3</td>
</tr>
<tr>
<td>866HHB</td>
<td>22</td>
<td>24</td>
<td>11.4$^{ab}$ ± 0.6</td>
<td>228.5$^{b}$ ± 4.3</td>
<td>205.2 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>36</td>
<td>10.7$^{ab}$ ± 0.6</td>
<td>226.1$^{a,b}$ ± 6.5</td>
<td>198.6$^{b}$ ± 5.4</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>41</td>
<td>11.8$^{a,b}$ ± 0.4</td>
<td>237.4$^{a}$ ± 3.3</td>
<td>208.2$^{b}$ ± 3.4</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>10</td>
<td>9.9$^{a,b}$ ± 0.9</td>
<td>199.9$^{a,b}$ ± 6.4</td>
<td>184.1$^{b}$ ± 11.5</td>
</tr>
</tbody>
</table>

$^{a,b,A,B}$Different lowercase (uppercase) superscript letters indicate a significant difference at $P < 0.05$ ($P < 0.01$) among different generations within an ancestral lineage.

$^{1}$PRAMEY = preferentially expressed antigen in melanoma, Y-linked; $ZNF280BY$ = zinc finger protein 280B, Y-linked; $HSFY$ = heat-shock transcription factor, Y-linked.
traits could be improved if the CNV of the Y-linked genes were used as markers for male selection in Holstein breeding program.

**CONCLUSIONS**

This study found that almost all AI bulls in Holsteins worldwide are descendants of 2 ancestor bulls born in 1880. These 2 ancestral Y-lineages have been continued in North America through 2 dominant AI-era founders, Pawnee Farm Arlinda Chief and Round Oak Rag Apple Elevation, who contributed 48.78% and 51.06% of bulls in the 2013 Holstein population, respectively. Analysis of CNV in Y-linked gene families demonstrated that the Y-ampliconic region can diverge quickly, but the rapid decline in Y-lineage founders since the 1960s has likely eliminated much previous Y-ampliconic variation, and genetic diversity in the X-degenerate region is likely to be severely limited. The loss of Y-lineages was likely a consequence of the combination of the intensive artificial selection and the use of AI technology, and was a by-product of the intensive artificial selection, as the Y chromosome has never been a direct target for selection in Holsteins. The neglect of the Y chromosome in cattle breeding program may be altered by the recent progress in bovine and other mammalian Y chromosome studies, but only if traits important to dairy production are found to be associated with Y chromosome variation.

**ACKNOWLEDGMENTS**

We thank Lawrence W. Specht (professor emeritus of dairy science, Penn State University, University Park) for help finding pedigree information in the Holstein-Friesian herd book, and Tom Lawlor (geneticist and research director at Holstein Association USA Inc., Brattleboro, VT), for supplying the Interbull pedigree information.

### Table 3. The median copy number (±SE) of Y-linked genes among different half-sibling families in North American Holsteins

<table>
<thead>
<tr>
<th>Sire</th>
<th>No. of sons</th>
<th>PRAMEY</th>
<th>ZNF280BY</th>
<th>HSFY</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOCAN5429693</td>
<td>6</td>
<td>10.7 ± 0.8</td>
<td>240.5 ± 6.8</td>
<td>208.8 ± 6.8</td>
</tr>
<tr>
<td>HOCAN5470579</td>
<td>8</td>
<td>12.7 ± 0.5</td>
<td>241.5 ± 8.3</td>
<td>209.2 ± 5.3</td>
</tr>
<tr>
<td>HOCAN6026421</td>
<td>8</td>
<td>11.7 ± 1.1</td>
<td>240.5 ± 7.6</td>
<td>209.9 ± 7.6</td>
</tr>
<tr>
<td>HOCAN10705668</td>
<td>6</td>
<td>11.7 ± 1.7</td>
<td>232 ± 9.3</td>
<td>189.2 ± 10.6</td>
</tr>
<tr>
<td>HODEU254210</td>
<td>6</td>
<td>14.5 ± 1.5</td>
<td>203 ± 15.3</td>
<td>216.4 ± 9.1</td>
</tr>
<tr>
<td>HOUSA2290977</td>
<td>12</td>
<td>12.9 ± 0.5</td>
<td>231.1 ± 5.6</td>
<td>218.5 ± 4.4</td>
</tr>
<tr>
<td>HOUSA17013604</td>
<td>6</td>
<td>10.6 ± 1</td>
<td>228.7 ± 5.8</td>
<td>213.6 ± 7.9</td>
</tr>
<tr>
<td>HOUSA122358313</td>
<td>10</td>
<td>9.6 ± 0.8</td>
<td>211 ± 14.3</td>
<td>186.6 ± 14.8</td>
</tr>
<tr>
<td>HOUSA123066734</td>
<td>12</td>
<td>12.4 ± 0.8</td>
<td>220.5 ± 12.4</td>
<td>215.9 ± 7.2</td>
</tr>
<tr>
<td>HOUSA129890008</td>
<td>5</td>
<td>9.4 ± 1.7</td>
<td>224.6 ± 12.6</td>
<td>181.8 ± 7.7</td>
</tr>
</tbody>
</table>

Different uppercase superscript letters within the same gene family represent significant difference at $P < 0.01$.

1PRAMEY = preferentially expressed antigen in melanoma, Y-linked; ZNF280BY = zinc finger protein 280B, Y-linked; HSFY = heat-shock transcription factor, Y-linked.

### Table 4. Reproductive performance of the 3 dominant AI-era founder bulls in North American Holsteins

<table>
<thead>
<tr>
<th>Trait (%)</th>
<th>No. of bulls $^2$</th>
<th>n</th>
<th>Mean ± SE</th>
<th>n</th>
<th>Mean ± SE</th>
<th>n</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC (cm)</td>
<td>140</td>
<td>40</td>
<td>40.55 ± 0.61</td>
<td>18</td>
<td>39.60 ± 0.77</td>
<td>81</td>
<td>39.51 ± 0.37</td>
</tr>
<tr>
<td>RLSC</td>
<td>140</td>
<td>40</td>
<td>100.78 ± 1.00</td>
<td>18</td>
<td>99.00 ± 1.64</td>
<td>81</td>
<td>98.15 ± 0.87</td>
</tr>
<tr>
<td>PTA</td>
<td>140</td>
<td>40</td>
<td>77.24 ± 0.30</td>
<td>18</td>
<td>76.93 ± 0.93</td>
<td>81</td>
<td>78.12 ± 0.26</td>
</tr>
<tr>
<td>IM</td>
<td>140</td>
<td>40</td>
<td>33.78 ± 0.38</td>
<td>18</td>
<td>33.25 ± 0.94</td>
<td>81</td>
<td>34.45 ± 0.33</td>
</tr>
<tr>
<td>PNS</td>
<td>140</td>
<td>40</td>
<td>78.67 ± 1.21</td>
<td>18</td>
<td>74.99 ± 1.98</td>
<td>81</td>
<td>79.27 ± 0.83</td>
</tr>
<tr>
<td>PIA</td>
<td>140</td>
<td>40</td>
<td>79.85 ± 0.53</td>
<td>18</td>
<td>79.37 ± 0.90</td>
<td>81</td>
<td>80.46 ± 0.33</td>
</tr>
<tr>
<td>NRR</td>
<td>117</td>
<td>44</td>
<td>66.00 ± 0.71</td>
<td>9</td>
<td>58.56 ± 1.20</td>
<td>58</td>
<td>65.15 ± 0.65</td>
</tr>
<tr>
<td>SCR</td>
<td>488</td>
<td>250</td>
<td>1.26 ± 0.10</td>
<td>6</td>
<td>1.72 ± 0.28</td>
<td>232</td>
<td>1.13 ± 0.09</td>
</tr>
</tbody>
</table>

$^1$SC = scrotal circumference; RLSC = age-adjusted relative scrotal circumference; PTM = postthaw motility; IM = incubated motility; PNS = percentage of normal sperm; PIA = percentage of intact acrosome; NRR = nonreturn rate; SCR = sire conception rate.

$^2$Three different populations of bulls (140, 117, and 488) in North American Holsteins were used in the present study.
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REFERENCES


