J. Dairy Sci. 94:6153–6161
doi:10.3168/jds.2011-4624

Harmful recessive effects on fertility detected by absence of homozygous haplotypes

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ABSTRACT

Five new recessive defects were discovered in Holsteins, Jerseys, and Brown Swiss by examining haplotypes that had a high population frequency but were never homozygous. The method required genotypes only from apparently normal individuals and not from affected embryos. Genotypes from the BovineSNP50 BeadChip (Illumina, San Diego, CA) were examined for 58,453 Holsteins, 5,288 Jerseys, and 1,991 Brown Swiss with genotypes in the North American database. Haplotypes with a length of ≤75 markers were obtained. Eleven candidate haplotypes were identified, with the earliest carrier born before 1980; 7 to 90 homozygous haplotypes were expected, but none were observed in the genomic data. Expected numbers were calculated using either the actual mating pattern or assuming random mating. Probability of observing no homozygotes ranged from 0.0002 for 7 to 10^{-45} for 90 expected homozygotes. Phenotypic effects were confirmed for 5 of the 11 candidate haplotypes using 14,911,387 Holstein, 830,391 Jersey, and 68,443 Brown Swiss records for conception rate. Estimated effect for interaction of carrier service sire with carrier maternal grandsire ranged from −3.0 to −3.7 percentage points, which was slightly smaller than the −3.9 to −4.6 percentage points expected for lethal recessives but slightly larger than estimated effects for previously known lethal alleles of −2.5 percentage points for brachyspina and −2.9 percentage points for complex vertebral malformation. Conception rate was coded as a success only if the gestation went to term or the cow was confirmed to be pregnant. Estimated effect of carrier interaction for stillbirth rate based on 10,876,597 Holstein and 25,456 Jersey records was small. Thus, lethal effects may include conception, gestation, and stillbirth losses. Carrier frequency has been >20% for many years for the confirmed defect in Jerseys and is currently 16% for the defect in Brown Swiss. The 3 defects discovered in Holsteins have carrier frequencies of 2.7 to 6.4% in the current population. For previously known defects, map locations and lack of homozygotes were consistent with the literature and lethal recessive inheritance, but numbers of expected homozygotes for some were small because of low frequency. Very large genotypic and phenotypic data sets allow efficient detection of smaller and less frequent effects. Haplotype tests can help breeders avoid carrier matings for such defects and reduce future frequencies.

Key words: genomics, lethal recessive, haplotype, homozygote

INTRODUCTION

Genotypes and haplotypes for large numbers of individuals provide a new tool for locating lethal recessive genes. Defects that cause embryo loss are difficult to detect without genomic data even with very large sets of phenotypic and pedigree data because of too few observations per estimated interaction (VanRaden and Miller, 2006). With genomic data, lethal recessives may be discovered from haplotypes that are common in the population but are never homozygous in live animals. The method requires genotypes only from apparently normal individuals and not from affected embryos and, therefore, is the opposite of most previous strategies (Lander and Botstein, 1987; Remington and O'Malley, 2000; Charlier et al., 2008; Khatib et al., 2009; Huqun et al., 2010). The new method can discover lethal defects without using any phenotypes. If the numbers of genotyped individuals are large, expected numbers of homozygous haplotypes will also be sufficiently large so that their complete absence likely is not by chance.

Lethal recessives were often discovered in the past from reports of abnormal calves and subsequent breeding trials to confirm inheritance. Most breeders now routinely report reproductive events but might not report individual defects because, if the condition is rare, a consequence might be to decrease the value of their own breeding stock. Breeding companies can combine data and resources to gain power in detecting genetic effects. Breed associations label carriers of known defects but need to know if the inheritance of a defect is

Received June 14, 2011.
Accepted August 31, 2011.
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more complex than simple recessive or if the test may mislabel some animals (Leipold et al., 1990). Defects that affect embryo loss were sometimes discovered from occasional abnormal calves that did reach late gestation or birth (Agerholm et al., 2001, 2006) or from physiological differences between normal and heterozygous cows (Shanks and Robinson, 1989). Instead of relying on breeder reports of abnormalities, embryonic defects may more easily be discovered using fertility traits and genotypes already stored in national or international databases (Veerkamp and Beerda, 2007).

The objectives of this study were to (1) locate potential new lethal recessive alleles from genotypes by examining haplotype inheritance, (2) estimate effects of those haplotypes on phenotypes for conception and stillbirth rates by interaction of carrier status of service sire with carrier status of maternal grandsire (**MGS**), and (3) demonstrate ability of those methods to trace inheritance and estimate effects of previously known lethal defects in Holsteins, Jerseys, and Brown Swiss.

MATERIALS AND METHODS

Marker genotypes obtained using the BovineSNP50 BeadChip (Illumina, San Diego, CA) were examined for 58,453 Holsteins, 5,288 Jerseys, and 1,991 Brown Swiss in the North American database as of April 2011. Marker genotypes from the GoldenGate Bovine3K Genotyping BeadChip (Illumina) for 24,341 Holstein, 4,549 Jersey, and 121 Brown Swiss additional animals (mostly females) were used in inferring haplotypes, but only animals with BovineSNP50 genotypes were used in subsequent analyses to ensure highest accuracy. Haplotypes were obtained with version 2 of the Fortran program findhap.f90 (VanRaden, 2011). Compared with version 1 (VanRaden et al., 2011b), accuracy was improved by using multiple segment lengths instead of just 1 segment length, including population and pedigree haplotyping in 1 loop rather than 2 separate loops, and checking for haplotypes inherited from genotyped great-grandparents instead of just parents and grandparents. The program first examined haplotypes of length 600 markers, then 200 markers, and finally output haplotypes of $\leq$75 markers for further analysis. The resulting haplotypes were mostly 4 to 7 Mbp in physical distance, which is about 4 to 7% of the mean length of a bovine chromosome or about 4 to 7 cM; thus, 4 to 7% of segments would include a new crossover per generation.

Haplotypes that had the highest frequency within their breed but that were never homozygous were examined first to maximize power of detection and because of their potential importance. Several bulls had >1,000 genotyped progeny, but their new haplotypes created by recent crossovers had no opportunity to become homozygous because they were not yet present in the general population of mates. Also, those new haplotypes were not likely to be lethal if they were simply a mixture of 2 ancestral haplotypes that were not lethal. Haplotypes were not examined if the earliest carrier ancestor was too recent (born after 1980). Pedigrees were examined to check if the haplotypes had a logical inheritance pattern across generations within families.

Expected numbers of homozygous individuals for any particular haplotype were calculated in 2 ways. The "simple" method used the number of individuals genotyped divided by 4 and multiplied by the square of the carrier frequency. Random mating of all members of the population across time was assumed for the simple method. The second method used the actual mating pattern that created the genotyped individuals in the population and was the number of carrier service sire $\times$ carrier MGS matings divided by 4. For the "mating" method, allele frequencies for maternal granddams were assumed to equal those for MGS. The requirement that the haplotype exist for several generations was an attempt to ensure that the assumption for grandparent allele frequencies would be approximately true. Expectations from the simple method may be overestimated if inbreeding was avoided or underestimated if allele frequencies changed across time; expectations from the mating method may be underestimated if some service sires and MGS were not genotyped.

Phenotypes were examined using April 2011 data and official models for USDA genetic evaluations for sire conception rate (**SCR**; Kuhn and Hutchison, 2008; Norman et al., 2008) and for stillbirth rate (Cole et al., 2007) except that models also included an interaction for haplotype carrier status of the sire of the embryo or newborn calf (service sire) with carrier status of the cow sire (embryo MGS). That interaction included 9 subclasses for the 3 possible conditions of each bull: haplotype carrier, noncarrier, or not genotyped. Solutions were obtained using programs of Misztal et al. (2002). Numbers of observations differ for the 2 traits because more producers record fertility than stillbirth and only the last 4 yr of SCR records are included.

Conception rate was coded as a success only if the gestation went to term or the cow was confirmed to be pregnant and remained so. That definition of success differs from most other national evaluations that use nonreturn rate, in which success is just the lack of a subsequent insemination. Total numbers of conception rate observations were 14,911,387 for Holsteins, 830,391 for Jerseys, and 68,443 for Brown Swiss. The model for official USDA evaluations for conception rate includes random effects for service sire, age of service sire, dam genetic (with relationship matrix), and dam

permanent environment; fixed effects for herd-year-season-parity-registry status of dam, state-year-month of breeding, interval between breedings, parity of dam, and AI organization-year-age of bull; and regressions on dam age, milk yield, and inbreeding of the service sire and the embryo. Mean conception rates were 31% for Holstein, 37% for Jersey, and 27% for Brown Swiss cows. Corresponding rates for heifers were 55, 50, and 43%, but heifer records are not yet included in SCR or in the current study.

Stillbirth or perinatal mortality was coded as died within 48 h or lived and was evaluated with a threshold model. Solutions on the underlying scale were converted back to the observed scale with the same formula used for official USDA evaluations but were expressed as a difference from the official mean of 8%. The actual mean across all parities was 6.6%, whereas the official mean is the average of 11% for first-parity births and 5% for later-parity births. Number of stillbirth observations was 10,876,597 for Holsteins. Jersey and Brown Swiss do not have official USDA stillbirth evaluations because of fewer records, but stillbirth results were provided from unofficial Jersey data for 1 candidate haplotype that had >500 carrier service sire × carrier MGS matings. The official USDA model for stillbirth evaluations includes random effects for service sire and MGS (correlated, with relationship matrix) and herd-year and fixed effects for service sire and MGS birth years, year-season of calf birth, and parity-sex.

To compute standard errors, both conception rate and stillbirth were analyzed with fixed models in PROC GLM of version 9.2 of SAS (SAS Institute Inc., Cary, NC). Effects of herd-year-season subclasses were absorbed, and other environmental effects and carrier interaction were included in the model. The noncarrier × noncarrier subclass was set equal to 0 because it had the largest number of observations, and the other subclasses such as carrier × carrier were expressed as deviations from that subclass. Haplotype effects were not reported if numbers of carrier × carrier matings were <500 because standard errors were >1.5% and lethal effects could not be reliably detected. Stillbirth standard errors were multiplied by 1.21 (8%/6.6%) to adjust for the difference between official and observed means. The standard errors were approximate and may have underestimated true standard errors because the main effects for service sire and dam of embryo could not be included in standard error computations.

Probabilities of observing 0 homozygotes when n are expected can be obtained by 2 formulas analogous to those used to obtain the expectations. For the simple method that assumed random mating, probability equals (1 – carrier frequency squared/4) raised to the power of the number of genotyped animals. For the method that used the actual mating pattern, probability equals 0.75 raised to the power of the observed number of carrier service sire × carrier MGS matings. The null hypothesis of a normal haplotype can be rejected in favor of the alternative hypothesis of lethal if that probability is sufficiently small. Probabilities for the mating method were 0.0002 for 7 and 10^{-45} for 90 expected homozygotes. Probabilities for the simple method were similar. Small probabilities were desired because the genomic data included thousands of haplotypes that could have had 0 homozygotes by chance, whereas only the 11 haplotypes with the greatest number of expected homozygotes were selected.

Previously known lethal or semi-lethal recessives were examined to determine if those also lacked homozygous haplotypes and had phenotypic effects consistent with those previously reported. Conditions examined were bovine leukocyte adhesion deficiency (**BLAD**; Shuster et al., 1992), brachyspina (Agerholm et al., 2006), complex vertebral malformation (**CVM**; Agerholm et al., 2001), deficiency of uridine monophosphate synthase (**DUMPS**; Shanks and Robinson, 1989), and syndactyly or mulefoot (Leipold et al., 1969) in Holsteins and spinal muscular atrophy (**SMA**; el-Hamidi et al., 1989) and bovine progressive degenerative myeloencephalopathy or weaver (Leipold et al., 1973) in Brown Swiss. Limber legs (Lamb et al., 1976) and rectovaginal constriction (Leipold et al., 1990) in Jerseys and arachnomelia (Drögemüller et al., 2010) in Brown Swiss had too few genotyped carriers for further study. For some lethal conditions, homozygotes might be found if genotyping is done before death occurs. Locations of causative mutations are known for BLAD, CVM, and DUMPS but not for the others.

Inheritance of known recessives was traced and effects were estimated using 2 strategies. The first was to use only bulls already officially tested as carriers or noncarriers. The second was to identify haplotypes containing the defective allele and assign carrier or noncarrier status based on those haplotypes. The first strategy can be more precise by using only the confirmed mutation and previously tested animals that do not have BovineSNP50 genotypes. The second strategy can increase power by using additional animals that have BovineSNP50 genotypes and carry the haplotype but have not yet been tested by the official test (such as females). Haplotypes containing known defects were identified by having higher frequencies in carriers compared with the general population.

Crossover haplotypes can be useful in refining the map location and in gaining power by including more animals that possess the defective portion but not the full haplotype. Crossovers were identified directly by findhap.f90 when the source parent was genotyped or

were identified as any other haplotype that had at least 50% overlap (identical SNP) with the original haplotype. For brachyspina, the original haplotype 459.95 from Sweet-Haven Tradition (registration number 1682485) was combined in analyses with 13 crossover haplotypes containing a shorter 50-marker region where the defect was known to be located. Haplotype test results delivered to the industry may not always be conclusive when new crossovers appear within known defective regions.

RESULTS AND DISCUSSION

Eleven haplotypes had from 7 to 90 expected homozygotes but none observed. Table 1 lists those potentially lethal haplotypes ranked by descending number expected from the mating method. Mean carrier frequency for genotyped animals is listed as well as birth year of the earliest genotyped ancestor with that haplotype (but rounded to the nearest decade to prevent easy identification of the ancestor). Defects are identified by segment number and haplotype within segment (e.g., 355.10 denotes segment 355, haplotype 10). Previously known defects and their carrier frequencies for genotyped animals are listed in Table 2. Map locations are from the UMD 3.0 *Bos taurus* genome assembly (Center for Bioinformatics and Computational Biology, 2010).

Estimated effects on conception and stillbirth rates are in Table 3 for possibly defective haplotypes and in Table 4 for previously known defects. Expected decrease in conception rate for a simple recessive, which is calculated from the mean conception rate for cows and a one-eighth mortality ratio, was 3.9 percentage points (31/8) for Holsteins and 4.6 percentage points (37/8) for Jerseys. However, the model also included main effects of sire and maternal grandsire and a regression on embryo inbreeding, all of which removed a proportion of the observed carrier interaction effect.

Of the 11 haplotypes rated as most detectable and important to investigate using only information from the genotype database, large harmful effects on conception rate were confirmed for 5 of those using the phenotype database. Those 5 had effects on conception rate of −3.0 to −3.7 percentage points (Table 3) compared with −2.9 percentage points for CVM and −2.5 percentage points for brachyspina (Table 4). The other 6 that did not have large fertility effects might be explained by chance, problems with the marker map, a marker with non-Mendelian inheritance (e.g., due to copy number variation), or deaths occurring after birth (such as for BLAD). Segment 369 contained a large number of haplotypes, indicating a possible map problem, but correlations and frequencies of genotypes within the segment did not seem unusual. After introducing stricter marker edits, 2 different candidate haplotypes appeared and 2 dropped out of the top 11, but the 5 haplotypes with confirmed effects remained.

Conception rates for the 5 new and 2 previous defects were significantly negative and were consistent with the hypothesis of lethal recessive (SE ranged from 0.2 to 1.7%; Tables 3 and 4). One other potentially lethal haplotype (369.1) also had a significant ($P < 0.01$) negative effect (−0.9% ± 0.3%), but its effects were not large enough to be considered lethal. The same families that had haplotype 21.337 also had nearby segment 23.51 with the same inheritance pattern, number of expected homozygotes, and effects on conception rate and still-

Table 1. Location and carrier frequency for potentially lethal haplotypes in genotyped animals and birth year of earliest genotyped carrier

Potentially lethal defect[1]	Breed	Chromosome	Haplotype		Homozygotes, n			Earliest carrier birth year
			Map location,[2] Mbp	Mean carrier frequency, %	Simple (expected)[3]	Mating (expected)[4]	Observed	
355.10	Jersey	15	13–18	23.4	72	90	0	1962
369.1	Holstein	15	72–76	1.8	5	46	0	1952
133.74	Holstein	5	58–66	4.5	30	23	0	1962
62.7	Jersey	2	116–121	6.0	5	21	0	1960
424.49	Brown Swiss	19	7–16	15.1	11	14	0	1970
175.5	Holstein	7	3–9	2.5	9	10	0	1953
186.139	Jersey	7	58–62	4.2	2	10	0	1974
21.337	Holstein	1	92–97	4.6	30	9	0	1975
514.8	Jersey	25	7–11	8.9	10	9	0	1960
183.13	Brown Swiss	7	41–47	14.0	10	9	0	1972
218.61	Holstein	8	90–95	4.7	33	7	0	1968

[1]Defects are identified by DNA segment number and haplotype within segment (e.g., 355.10 indicates segment 355, haplotype 10).

[2]UMD 3.0 genome assembly (Center for Bioinformatics and Computational Biology, 2010).

[3]Number of individuals genotyped divided by 4 and multiplied by square of carrier frequency.

[4]Number of carrier service sire × carrier maternal grandsire matings divided by 4.

Table 2. Location and carrier frequency of previously known defects for genotyped animals and birth year of earliest genotyped carrier

			Haplotype		Homozygotes, n			
Known defect[1]	Breed	Chromosome	Map location,[2] Mbp	Mean carrier frequency, %	Simple (expected)[3]	Mating (expected)[4]	Observed	Earliest carrier birth date
CVM (75.28)	Holstein	3	42 (40–46)	1.6	4	23	0	1963
Brachyspina (459.95)	Holstein	21	20–25	6.0	21	22	0	1974
SMA (511.4)	Brown Swiss	24	58–63	9.2	4	3	0	1953
BLAD (32.1)	Holstein	1	145 (141–146)	0.8	1	2	0	1952
Weaver (105.9, 106.10)	Brown Swiss	4	45–56	2.6	0	1	0	1966
Mulefoot (370.37)	Holstein	15	76–82	0.1	0	0	0	1961
DUMPS (13.125–16.94)	Holstein	1	55–73	0.01	0	0	0	1957
Arachnomelia	Brown Swiss	5	62	—	—	—	—	—

[1]DNA segment number and haplotype within segment (e.g., 355.10 indicates segment 355, haplotype 10) shown inside parentheses; CVM = complex vertebral malformation, BLAD = bovine leukocyte adhesion deficiency, mulefoot = syndactyly, SMA = spinal muscular atrophy, weaver = bovine progressive degenerative myeloencephalopathy, and DUMPS = deficiency of uridine monophosphate synthase.

[2]UMD 3.0 genome assembly (Center for Bioinformatics and Computational Biology, 2010).

[3]Number of individuals genotyped divided by 4 and multiplied by square of carrier frequency.

[4]Number of carrier service sire × carrier maternal grandsire matings divided by 4.

birth as found for 21.337. Thus, the map location of 21.337 is indicated in Table 1, but a longer region of 15 Mbp might contain the defect because a large chromosomal segment remained intact. Similarly, adjacent haplotypes 218.61 and 219.154 were inherited together and had similar properties over a 10-Mbp region.

Estimated subclass effects for an example haplotype (218.61) are shown in Table 5 for the 9 interactions of carrier status of service sire with carrier status of MGS. The main focus of this study was the difference between carrier × carrier and noncarrier × noncarrier matings because most other subclasses (e.g., carrier × not tested) differed only a little from noncarrier × noncarrier matings. Some other small differences did appear to be significant ($P < 0.01$), but mainly because standard errors were very small because of many observations.

Estimated effects on stillbirth rate (Tables 3 and 4) were positive (more stillbirths), as expected, for all haplotypes except 355.10. However, those effects include some effect of inbreeding because it is not included in the official model. Haplotype 21.337 had the largest but not significant ($P \geq 0.05$) effect of only 1.8 percentage points, which was slightly larger than the CVM effect of 1.4 percentage points. Stillbirth effects

Table 3. Effects of carrier service sire × carrier maternal grandsire interaction on conception and stillbirth rates for potentially lethal defects

		Conception rate, %			Stillbirth rate, %		
Potentially lethal defect[1]	Breed	Matings	Effect	SE	Calves	Effect	SE
355.10	Jersey	52,449	−3.7	0.2	1,612	−0.4	0.8
369.1	Holstein	30,531	−0.9	0.3	20,642	0.1	0.2
133.74	Holstein	24,555	−3.1	0.3	11,905	0.7	0.3
62.7	Jersey	999	−0.3	1.6	NA[2]	NA	NA
424.49	Brown Swiss	964	0.4	1.5	NA	NA	NA
175.5	Holstein	2,777	−1.5	0.9	1,061	0.5	0.9
186.139	Jersey	3,049	1.5	0.9	NA	NA	NA
21.337	Holstein	3,252	−3.0	0.8	896	1.8	1.0
514.8	Jersey	5,356	0.3	0.7	NA	NA	NA
183.13	Brown Swiss	936	−3.4	1.5	NA	NA	NA
218.61	Holstein	14,114	−3.2	0.4	7,510	1.0	0.3

[1]Defects are identified by DNA segment number and haplotype within segment (e.g., 355.10 indicates segment 355, haplotype 10).

[2]Not applicable.

Table 4. Effects of carrier service sire × carrier maternal grandsire interaction on conception and stillbirth rates for previously known Holstein defects and the haplotypes that contain those defects

Known defect[1]	Conception rate, %			Stillbirth rate, %		
	Matings	Effect	SE	Calves	Effect	SE
CVM[2]	9,387	−2.9	0.5	31,320	1.4	0.2
(75.28)[3]	1,954	−1.4	1.0	9,987	1.6	0.3
Brachyspina[2]	748	−1.9	1.7	176	−0.4	2.3
(459.95)[4]	26,143	−2.5	0.3	12,499	−0.2	0.3
BLAD[2]	304	1.0	2.6	9,887	0.1	0.3
(32.1)[4]	75	1.0	5.3	1,813	0.9	0.7

[1]DNA segment number and haplotype within segment (e.g., 355.10 indicates segment 355, haplotype 10) shown inside parentheses; CVM = complex vertebral malformation; BLAD = bovine leukocyte adhesion deficiency.

[2]Analysis used only officially tested sires and maternal grandsires.

[3]Haplotype test included only the copies of 75.28 that traced to Penstate Ivanhoe Star.

[4]Haplotype test included crossover haplotypes known to contain the lethal region.

for haplotypes 218.61 and 133.74 were not as large but were significant ($P < 0.05$) because of more observations. Positive effects may indicate that at least some of the lethal effects are in mid to late gestation; therefore, economic costs are larger because cows may be culled or have long intervals between calves. Jersey haplotype 355.10, which had the largest effect on conception rate, did not have a significant effect on perinatal mortality. For some other lethal recessives, stillbirth and calf loss after 48 h past birth may help explain why decrease in conception rate was slightly less than expected. A lethal with all loss occurring at birth or within 48 h would have an expected increase of 11.5% [(100% − mean stillbirth rate)/8] for carrier service sire × carrier MGS matings, and no estimated effects were that large.

Previously reported lethal defects all had their locations confirmed, and none had homozygous haplotypes. Official test results for brachyspina for 600 bulls (Georges et al., 2010) exactly matched results of this study when the 13 less-frequent crossover haplotypes were included with the original. Phenotypic effects on conception rate were confirmed for brachyspina by haplotype test and for CVM by official test. The haplotype test showed that CVM is a fairly recent mutation inherited from the bull Penstate Ivanhoe Star (1441440). Other copies of this same haplotype in the bulls Irvington Pride Admiral (1237057), Hilltop Apollo Ivanhoe (1399824), Ideal Fury Reflector (1381027), and Paclamar Astronaut (1458744) did appear in homozygous descendants and did not reduce conception rate. The stage of gestation where embryo loss occurs was examined for the new defects (VanRaden et al., 2011a) similar to a previous study of the timing of embryo losses caused by CVM (Berglund et al., 2004).

The lethal effect of BLAD occurs only months after birth (Shuster et al., 1992); in this study with fewer observations, BLAD had no detectable effects on conception or stillbirth rates. The new defect at haplotype 133.74 in Holsteins is at the same location as arachnomelia (Drögemüller et al., 2010) in Brown Swiss. A lethal effect was not found and was not expected for the signal transducer and activator of transcription 5A gene at 43,728,692 bp on chromosome BTA19 (Btau_4.2 genome assembly; National Center for Biotechnology Information, 2011) because that defect was reported to be maternally imprinted and only semi-lethal (Khatib et al., 2009).

Trends in carrier frequency for genotyped animals across time are plotted in Figure 1 for the new haplotypes with confirmed phenotypic effects and also for haplotype 369.1, which had a smaller but significant ($P < 0.01$) effect. The carrier frequency for Holstein hap-

Table 5. Numbers of observations and effects of carrier status of maternal grandsire on conception rate (%) within carrier status of service sire for haplotype 218.61

Carrier status of service sire	Carrier status of maternal grandsire					
	Carrier		Noncarrier		Not tested	
	Obs,[1] n	Effect	Obs, n	Effect	Obs, n	Effect
Carrier	14,114	−3.2 ± 0.4	350,271	0.3 ± 0.1	267,542	0.5 ± 0.1
Noncarrier	365,594	−0.5 ± 0.1	6,880,546	0.0 ± 0.0	6,283,334	0.6 ± 0.0
Not tested	13,655	−1.4 ± 0.4	285,967	−0.5 ± 0.1	450,364	0.4 ± 0.1

[1]Observations.

lotype 369.1 declined rapidly from 10% in 1955 through 1975 to only 0.4% by 2010, and carrier frequency for Holstein haplotypes 133.74 and 218.61 increased to almost 10% by 1985 but decreased to 3 to 5% by 2010. That pattern suggests strong selection against those haplotypes, probably because of selection for daughter pregnancy rate (**DPR**) or other fitness traits. By contrast, haplotypes 183.13 in Brown Swiss and 218.61 in Holsteins continued to increase in frequency to 16% and 6.4%, respectively, primarily because of a few very popular sires of sons. Jersey haplotype 355.10 has had high carrier frequency of 20 to 25% for 40 yr. For each of the 5 new defects, 2.7 to 20.7% of elite animals in the current population are carriers. In each breed, many additional lethal defects may exist at frequencies too low to be detected. Carrier frequencies can increase rapidly if present in popular bulls but can also decrease rapidly with testing and selection, and probabilities of inheritance can be adjusted for the loss of homozygotes (Van Doormaal and Kistemaker, 2008).

Selection against carriers will improve future fertility only slightly. A carrier sire mated at random to females in the breed will reduce average conception rate by carrier frequency divided by 4. For example, if average conception rate is 31% and carrier frequency is 5%, the average loss from using a carrier sire is $31\%(0.05)/4 = 0.39$ percentage points, which is small compared with normal variation among animals for conception rate traits. Standard deviations of total sire effects or transmitting abilities are 2.3 percentage points for SCR, 2.9 percentage points for cow conception rate (**CCR**), and 2.4 percentage points for heifer conception rate (**HCR**). Many carrier animals have positive fertility evaluations because they possess other favorable genes.

Conception rates will increase by <1 percentage point by eliminating any of the haplotypes from the population. The largest additive effect for haplotype 355.10 is only the service sire carrier × MGS carrier effect multiplied by twice the carrier frequency (Table 1) or −1.7 percentage points [3.7%(2)(0.234)]. Complete elimination of 355.10 would increase Jersey CCR by only 0.5 percentage points from the current mean of 37% to 37.5% [$37\%(1 + 0.234^2/4)$]. The increase for HCR would be larger because the success rate is higher but would increase only from 50.0 to 50.7%. For the other 4 defects, additive effects and increases from selection are smaller because carrier frequencies are smaller. Current carrier frequencies range from 2 to 21% for the 5 haplotypes.

Economic effects of the haplotypes are small and the expected losses are already included to some degree in released rankings for SCR, CCR, HCR, and DPR. Of those, net merit includes only DPR and thus does not fully account for economic effects of the haplotypes on fertility. Net merit does include service sire and daughter stillbirth rates. Therefore, continued selection for net merit with some attention to SCR, CCR, and HCR is recommended instead of direct selection against carriers. Because inheritance is recessive and carrier status will be reported for genotyped bulls and cows, avoid-

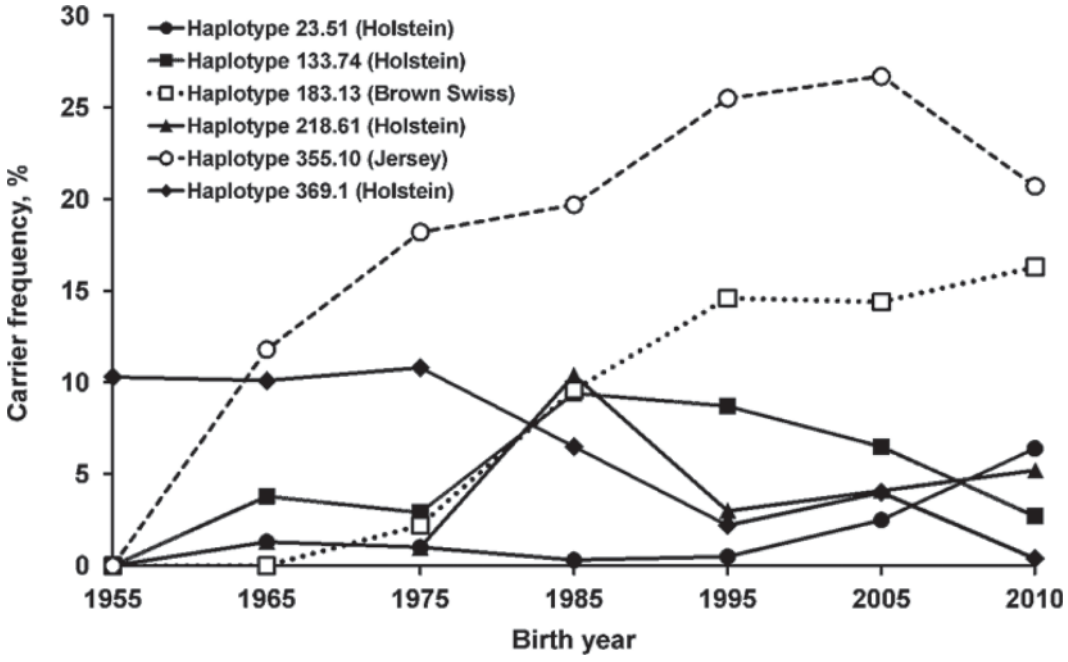

Figure 1. Genotyped carrier frequency for potentially lethal haplotypes by birth year (haplotype 23.51 indicates segment 23, haplotype 51).

ance of carrier × carrier matings using computerized mating programs will be an effective and less costly method to improve fertility.

The haplotype numbering system used in research is not ideal to report confirmed defects to breeders. Instead, breed association staff proposed sequentially numbering confirmed defects within each breed, coded with the first letter of the breed name, followed by an "H" for haplotype, and the sequential defect number within breed. Following this proposal, reported defect names and original defect numbers are JH1 = 355.10, HH1 = 133.74, HH2 = 21.337, HH3 = 218.61, and BH1 = 183.13. The US breed associations (Holstein, Jersey, and Brown Swiss) each began reporting carrier status for those new defects in August 2011 for all animals with BovineSNP50 genotypes. Further details on inheritance, economic effects, use of crossovers for fine mapping, and accuracy of detection methods were reported by VanRaden et al. (2011a).

Genomic testing can reveal carriers of haplotypes for new defects or for previously known defects that should be confirmed by testing the causative mutation if known, or breeders can label animals as carriers or noncarriers directly from the haplotype test without further testing expense if accuracy is high. Once identified, carriers should be mated to noncarriers to avoid nonadditive effects. Different lethal recessives were discovered for each breed, which supports the well-known fact that crossbreeding improves fertility. The ease of finding potentially lethal recessives was a surprise, but their presence in all breeds was probably not a surprise. Traditional approaches of maintaining diversity within breeds and avoiding close inbreeding can also help breeders improve fertility.

CONCLUSIONS

Recessive defects causing embryo loss can be discovered from the absence of homozygous haplotypes. Eleven candidate haplotypes were identified, and phenotypic effects were confirmed for 5 of those by estimating the interaction of service sire carrier status with MGS carrier status in the USDA national evaluation for SCR. Estimated effects of those 5 new defects (3 for Holsteins, 1 for Jerseys, and 1 for Brown Swiss) were slightly larger than for the previously known defects CVM and brachyspina. Carrier frequencies for the 5 new defects ranged from 2 to 21% in genotyped animals born since 2008. The number of observed carrier × carrier matings for conception rate ranged from 936 to 52,449 (Table 3). Because those lethal haplotypes have been in the breeds for several generations, preventing a carrier ancestor from appearing in the pedigree of both parents would be difficult even with computerized mate assignment to reduce inbreeding. Use of haplotypes to avoid carrier matings is possible, but use of the actual QTL would be more accurate and simpler if the causative mutations could be found. Large genotypic and phenotypic databases make the discovery of potentially lethal defects possible.

ACKNOWLEDGMENTS

The Council on Dairy Cattle Breeding and its member organizations contributed genotypes, phenotypes, and pedigrees used in this research. The Bovine Functional Genomics Laboratory (ARS, USDA, Beltsville, MD) and private breeders contributed genotypes and DNA from early ancestors that made inheritance of the defects easier to trace. Additional genotypes and pedigrees from Canada, Germany, Austria, and Switzerland increased the power of haplotype detection. The authors thank J. R. O'Connell (University of Maryland School of Medicine, Baltimore), C. W. Wolfe (American Jersey Cattle Association, Reynoldsburg, OH), T. J. Lawlor (Holstein Association USA, Brattleboro, VT), and members of the National Association of Animal Breeders biochemical defects committee for helpful discussions, as well as M. E. Tooker for computing assistance and S. M. Hubbard for technical editing (Animal Improvement Programs Laboratory, ARS, USDA, Beltsville, MD).

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