Genomic measures of inbreeding in the Norwegian–Swedish Coldblooded Trotter and their associations with known QTL for reproduction and health traits

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Abstract

Background: Since the 1950s, the Norwegian–Swedish Coldblooded trotter (NSCT) has been intensively selected for harness racing performance. As a result, the racing performance of the NSCT has improved remarkably; however, this improved racing performance has also been accompanied by a gradual increase in inbreeding level. Inbreeding in NSCT has historically been monitored by using traditional methods that are based on pedigree analysis, but with recent advancements in genomics, the NSCT industry has shown interest in adopting molecular approaches for the selection and maintenance of this breed. Consequently, the aims of the current study were to estimate genomic-based inbreeding coefficients, i.e. the proportion of runs of homozygosity (ROH), for a sample of NSCT individuals using high-density genotyping array data, and subsequently to compare the resulting rate of genomic-based F (FROH) to that of pedigree-based F (FPED) coefficients within the breed.

Results: A total of 566 raced NSCT were available for analyses. Average FROH ranged from 1.78 to 13.95%. Correlations between FROH and FPED were significant (P < 0.001) and ranged from 0.27 to 0.56, with FPED and FROH from 2000 to 2009 increasing by 1.48 and 3.15%, respectively. Comparisons of ROH between individuals yielded 1403 regions that were present in at least 95% of the sampled horses. The average percentage of a single chromosome covered in ROH ranged from 9.84 to 18.82% with chromosome 31 and 18 showing, respectively, the largest and smallest amount of homozygosity.

Conclusions: Genomic inbreeding coefficients were higher than pedigree inbreeding coefficients with both methods showing a gradual increase in inbreeding level in the NSCT breed between 2000 and 2009. Opportunities exist for the NSCT industry to develop programs that provide breeders with easily interpretable feedback on regions of the genome that are suboptimal from the perspective of genetic merit or that are sensitive to inbreeding within the population. The use of molecular data to identify genomic regions that may contribute to inbreeding depression in the NSCT will likely prove to be a valuable tool for the preservation of its genetic diversity in the long term.

Background

In recent years, there has been a rapid increase in the intensity of selection in many livestock breeding programs with the growing use of elite animals, which ultimately reduces the effective population size (Ne) of some breeds [1–6]. Consequently, a small Ne not only reduces genetic variability, but it also increases the effects of inbreeding (F) and genetic drift, and potentially alters the patterns of runs of homozygosity (ROH) in the long term [3–8]. While such alterations may not necessarily be of concern for large and highly diverse populations, increased homozygosity at loci with a heterozygous
advantage in small native populations reduces furth-
more their genetic diversity [7]. Small populations can
be particularly vulnerable to inbreeding depression since
mating between relatives often decreases individual fit-
ness and can significantly reduce population growth [7, 
9]. Moreover, selection programs, while driving favora-
able alleles to fixation, also allow deleterious alleles to
hitchhike along with favorable mutations. In addition
to this, more intense selection resulting from combi-
ing genomic selection with embryo biotechnologies (e.g.
artificial insemination) not only increases rates of genetic
gain, but can also increase levels of inbreeding [4, 10].

The Norwegian–Swedish Coldblooded trotter (NSCT)
is a domestic breed of horse in Norway and Sweden and
is one of the few remaining descendants of the origi-
nal Nordic coldblooded horse [11]. Since the 1950s, the
breed has been intensively selected for harness racing
performance with estimated breeding values produced
annually since the 1980s [12, 13]. As a result, a remark-
able improvement in the racing performance of NSCT
has occurred during the last half-century. However, this
improved racing performance has also been accom-
panied by a gradual increase in pedigree-based F levels
[14]. Although today NSCT is considered as a relatively
healthy breed, the NSCT breeding industry is well aware
that increased levels of inbreeding are widely known to
increase the expression of recessive deleterious alleles
that are linked to genetic diseases. Historically, inbreed-
ing in NSCT has been monitored by using traditional
methods that are based on pedigree analysis [14, 15].

While informative, the NSCT industry understands that
this classical metric likely underestimates inbreeding
within the breed and does not account for the fact that
homozygosity at some regions may, in principal, be more
or less desirable than at other regions. Two animals that
have the same level of inbreeding, may display drastically
different unfavorable effects of inbreeding. Even with an
extensive and complete pedigree, realized inbreeding lev-
els will likely differ from pedigree-based F levels due to
recombination and Mendelian sampling, which is then
compounded by the fact that, although the base animals
in a pedigree are considered unrelated, they are more
often than not, related.

Consequently, the NSCT industry has actively sup-
ported a shift towards using genomic data for F calcula-
tions in the breed, thus allowing for diversity across the
entire genome as well as at specific regions to be evalu-
ated and monitored, and providing not only a more accu-
rate assessment of inbreeding within the breed, but also
a much more detailed assessment. As such, the aims of
the current study were to provide genomic-based F coef-
ficient estimates (\(F_{ROH}\)) for a sample of NSCT using
a high-density genotyping array and to compare the
rate of \(F_{ROH}\) to that of classical pedigree-based F (\(F_{PED}\))
within the breed. Common ROH within the breed were
also assessed for overlaps with previously characterized
quantitative trait loci (QTL) for health and reproduction
traits in the horse, thus providing a first look at genomic
regions and traits that may warrant industry intervention
in the future.

Methods
Pedigree data
Complete pedigree information on all raced and unraced
NSCT were provided by the trotter associations in both
Norway and Sweden (Det Norske Travelskap and Svensk
Travsort). The pedigree consisted of 112,195 individuals
with a median pedigree depth of 15 generations.

Collection of samples
In total, 566 individuals born between 1 January 2000
and 31 December 2009 were selected for this study based
on the following criteria: (1) each horse had to have
participated in at least one race during its lifetime; this
restriction was implemented to allow for a broader use of
the data in future analyses that will explore racing per-
fomance traits within the breed; (2) hair and/or blood
samples had to be readily accessible from the pedigree
registration authorities in either Norway (Department of
Basic Sciences and Aquatic Medicine, Norwegian Uni-
versity of Life Sciences) or Sweden (Animal Genetics
Laboratory, Swedish University of Agricultural Sciences);
and (3) a sufficient amount of sample material had to be
available to ensure high DNA quality standards.

DNA isolation
DNA was extracted from hair roots using a standard
procedure of hair preparation. Briefly, 186 μL of Chelex
100 Resin (Bio-Rad Laboratories, Hercules, CA) and 14
μL of proteinase K (20 mg/mL; Merck KgaA, Darmstadt,
Germany) were added to each sample. This mix was incu-
bated at 56 °C for 2 h and proteinase K was inactivated
for 10 min at 95 °C. For DNA preparation from blood,
DNA from 350-μL blood samples were extracted by using
the Qiasymphony instrument and the Qiasymphony DSP
DNA mini kit (Qiagen, Hilden, Germany).

Genotyping and quality control
Prior to quality control (QC), the dataset consisted of
individuals that were genotyped with the 670K Axiom
equine genotyping array (\(n=473\)) and the 670K+ Axiom
equine genotyping array (\(n=93\)). Data from the two
arrays were subsequently merged based on SNP name,
chromosome number and position, which yielded a com-
bined SNP dataset of 611,888 SNPs for 566 horses (SNPs
located on chromosomes X and Y were excluded during this process). Then, QC was performed with the PLINK v1.07 software. SNPs were screened based on minor allele frequency (MAF > 0.01), Hardy–Weinberg equilibrium \((p > 0.0001)\), and genotyping rate (>0.95) with data that did not conform to these criteria and individuals with missing genotypes (>15%) being removed. Descriptive data for the sample of horses used in the analyses are in Table 1.

Inbreeding coefficient and runs of homozygosity

Inbreeding coefficients \((F_{\text{PED}})\) were calculated based on the complete pedigree of the breed using the Contribution, Inbreeding \((F)\), Coancestry v1.0 software, which uses a modified algorithm of Sargolzaei et al. [16] to compute inbreeding coefficients that is a fast and accurate tool for \(F_{\text{PED}}\) calculations.

Inconsistency between ROH-defining criteria in various industries and breeds has been shown to convolute the comparison of studies over time and across population samples [1–6, 17–22]. Since the criteria to define a ROH continue to remain ambiguous, in our study, we applied a wide range of ROH-defining criteria. Runs of homozygosity were defined in PLINK v1.07 using the sliding windows approach through the \texttt{homozyg} command. The details of each applied threshold setting are in Table S1 (see Additional file 1: Table S1). Genomic inbreeding coefficients \((F_{\text{ROH}})\) were estimated for each threshold setting by dividing the summed length of all ROH (per individual) by the length of the genome (2,242,879,462 bp) covered with SNPs. Pearson correlation coefficients between \(F_{\text{PED}}\) and \(F_{\text{ROH}}\) were determined using the statistical software R [23]. Paired t-tests between all \(F_{\text{ROH}}\) were also performed.

To better identify population-wide ROH in the breed, custom scripts in R were applied to ROH data from the threshold setting that resulted in the highest correlation between \(F_{\text{PED}}\) and \(F_{\text{ROH}}\). These scripts were used to determine which regions of the genome were shared in at least 95% of individuals in the sample [23]. Ultimately, we chose the threshold setting that resulted in the highest correlation between \(F_{\text{PED}}\) and \(F_{\text{ROH}}\) since it did not allow the capture of longer ROH that would subsequently be more beneficial when evaluating previously associated QTL, but it also yielded a more conservative estimate of inbreeding within the breed (i.e. an estimate that was more likely to be skewed upwards than downwards). Homozygous regions that were present in at least 95% of the sampled NSCT were then compared to previously reported QTL for reproduction and health traits in the horse (downloaded from the horse QTL database; [24]) using bed file comparisons in BEDOPS [25].

Results

After QC, 360,977 autosomal SNPs and 566 horses were available for analyses. Summary statistics, stratified by country of birth, for \(F_{\text{PED}}\) are in Table 2. \(F_{\text{PED}}\) and \(F_{\text{ROH}}\) of Norwegian born horses were higher than those of Swedish born horses, although the highest \(F_{\text{PED}}\) estimate was found for a Swedish born horse. Median \(F_{\text{PED}}\) and \(F_{\text{ROH}}\) for the entire cohort of sampled horses, stratified by year, are shown in Fig. 1. Inbreeding in the NSCT population during the 2000–2009 period increased by 1.48 and 3.15% based on \(F_{\text{PED}}\) and \(F_{\text{ROH}}\) estimates, respectively. Average \(F_{\text{ROH}}\) (%) ranged from 1.78 to 13.95% (see Additional file 1: Table S1). Correlations between \(F_{\text{PED}}\) and all \(F_{\text{ROH}}\) estimates were significant \((P<0.001)\) and ranged from 0.27 to 0.56 (see Additional file 2: Table S2) and Fig. 2. The threshold settings as defined below resulted in the highest correlation \((R = 0.5629)\) between \(F_{\text{PED}}\) and \(F_{\text{ROH}}\):

- Size of the sliding window in SNPs: 50 SNPs.
- Minimum length in kb that a run must have to be called as a ROH: 500.
- Minimum number of SNPs that a run must have to be called as a ROH: 100.
- Number of heterozygous SNPs allowed in a ROH: 1.
- Number of missing calls allowed in a ROH: 5.

Table 1 Descriptive data on the genotyped horses

| Sex            | Number |
|----------------|--------|
| Intact males   | 56     |
| Females        | 222    |
| Geldings       | 288    |
| Country of birth |      |
| Norway         | 265    |
| Sweden         | 301    |
| Year of birth  |        |
| 2000           | 25     |
| 2001           | 60     |
| 2002           | 72     |
| 2003           | 53     |
| 2004           | 55     |
| 2005           | 40     |
| 2006           | 60     |
| 2007           | 70     |
| 2008           | 63     |
| 2009           | 68     |
| Total          | 566    |
Table 2 Descriptive results, stratified by country of birth, for average inbreeding coefficient ($F_{\text{PED}}$) and average genomic inbreeding coefficient ($F_{\text{ROH}}$) for a sample of raced Norwegian–Swedish Coldblooded trotters born between 1 January 2000 and 31 December 2009

| Country of birth | 25th percentile | Median | Mean | 75th percentile | Max |
|------------------|----------------|--------|------|----------------|-----|
| Norway FPED (%)  | 0.96           | 5.18   | 6.18 | 7.38           | 14.35 |
| $F_{\text{ROH}}$ (%) | 1.98          | 8.86   | 10.12| 11.76          | 14.39 |
| Sweden FPED (%)  | 1.19           | 4.50   | 5.42 | 6.86           | 17.04 |
| $F_{\text{ROH}}$ (%) | 1.61          | 7.39   | 9.03 | 10.98          | 13.56 |

* Results based on the $F_{\text{ROH}}$ across all threshold settings

- Pruned for linkage disequilibrium: No.
- Minimum density to consider a ROH: 1 SNP per 50 kb.
- Maximum gap allowed between two SNPs: 100 kb.

Whereas the above settings resulted in the highest correlation between $F_{\text{PED}}$ and $F_{\text{ROH}}$, a similarly strong correlation ($R = 0.5594$) was obtained from the analysis of the pruned data with the same threshold settings except that the minimum number SNPs that a run must have to be called as a ROH was set to 50 SNPs.

Paired $t$ test between all $F_{\text{ROH}}$ yielded significant differences for most of the $F_{\text{ROH}}$ threshold settings with only 35 (1.49%) comparisons resulting in no significant difference (see Additional file 3: Table S3). Variations in sliding window size, minimum length in kb and minimum number of SNPs of a run to be called as a ROH clearly altered $F_{\text{ROH}}$. The influence of different threshold settings on ROH length and ultimately on $F_{\text{ROH}}$ is illustrated in Figures S1 and S2 (see Additional file 4: Figure S1 and file 5 Figure S2).

By applying the threshold settings that resulted in the highest correlation between $F_{\text{PED}}$ and $F_{\text{ROH}}$, the average
percentage of a single chromosome covered in ROH ranged from 9.84 to 18.82% (Table 3 Column D). Comparisons of ROH between individuals yielded 1403 regions that were present in at least 95% of the sampled horses (Fig. 3). The length of these regions ranged from 1 bp to 935 kb and overlapped with 35 previously characterized QTL for reproduction and health traits (see Additional file 6: Table S4). A visual representation of overlapping regions is in Fig. 3 with a brief description of each overlapped QTL in Table 4. QTL associated with osteochondrosis accounted for 48.6% of the overlapped QTL with only one of the 35 QTL being associated with fertility (QTL 103450, located on Equus caballus chromosome (ECA) 1).

Discussion

As expected based on previous studies in other species, the realized \( F_{ROH} \) in the NSCT population tended to be slightly higher than the \( F_{PED} \) estimates [1–7, 17, 26]. However, in our study, applying strict threshold settings regarding the number of heterozygous SNPs or missing calls allowed in a ROH significantly reduced correlations between \( F_{PED} \) and \( F_{ROH} \) and drastically altered the ability to capture longer ROH. Since size and frequency of ROH provide evidence for relatedness within and between populations, as well as details on distant and recent ancestry, the ability to capture consistently long ROH is essential for the integration of genomic data into breeding evaluation and preservation protocols for the NSCT breed [4–6, 18–20]. Shorter ROH (<1 Mb) tended to be more easily detected regardless of the ROH criteria applied, but longer ROH (>10 Mb) were more difficult to capture when no heterozygous SNPs or missing calls were allowed in a ROH and at least 100 SNPs were required for a run to be called as a ROH. Although this seems logical since a true ROH does not include any heterozygous SNPs, the high-density equine genotyping array contains more than 670,000 SNPs. Even a genotyping error rate of only 1% could yield 6700 possibly incorrectly genotyped SNPs. Since these incorrectly genotyped SNPs, which are likely attributable to poor sample quality in the current study, tend to be randomly scattered across the entire genome, individual horses can be disproportionately affected simply by chance.

Nevertheless, regardless of the ROH threshold settings applied, \( F_{ROH} \) in the NSCT breed appears to have steadily increased between 2000 and 2009. While the overall inbreeding level within the breed is slightly
underestimated based on classical metrics, the upward trend of inbreeding level revealed by the $FPED$ calculations is clearly supported by the $FROH$ estimates and likely warrants additional exploration by the NSCT breeding industry—particularly in relation to the difference in inbreeding levels between Norwegian born horses and Swedish born horses (Table 2). Furthermore, it is important to note the difference in $FPED$ between the entire population and the sample of individuals used in our study (Fig. 1). Generally speaking, inbreeding is expected to increase by 1% per generation (i.e. 7–9 years in the NSCT). This 1% increase in inbreeding level is clearly seen in the $FPED$ values for the whole population, but is not so obvious for the sample of individuals analyzed here, for which a ~1.5% increase was observed instead of the expected 1% over the same time period. Consequently, since the sample of individuals used in our study included only raced horses, although, not certain, it is plausible that the population of raced NSCT is perhaps slightly more inbred than the unraced population.

Although NSCT is not currently considered an at risk breed, it represents unique Norwegian and Swedish genetic resources and is present on the department of agriculture’s list of horse breeds that should be preserved [27]. The NSCT industry has historically been at the forefront regarding the application of emerging

| Chromosome | Average ROH (%) |
|------------|-----------------|
|            | A  | B  | C  | D  | E  | F  | G  | H  |
| 1          | 10.08 | 12.67 | 14.89 | 15.05 | 13.59 | 13.58 | 7.35 | 11.34 |
| 2          | 9.40  | 11.67 | 13.89 | 14.08 | 12.02 | 12.02 | 8.62 | 10.74 |
| 3          | 9.18  | 11.07 | 12.66 | 12.74 | 12.27 | 12.24 | 10.02 | 9.11  |
| 4          | 10.09 | 12.27 | 13.82 | 13.99 | 13.16 | 13.17 | 11.50 | 10.61 |
| 5          | 10.38 | 12.28 | 14.28 | 14.46 | 13.06 | 13.05 | 10.62 | 11.10 |
| 6          | 8.30  | 10.34 | 12.58 | 12.69 | 10.57 | 10.57 | 8.21  | 10.23 |
| 7          | 10.41 | 12.23 | 14.29 | 14.48 | 13.12 | 13.07 | 9.53  | 9.58  |
| 8          | 8.61  | 10.07 | 11.02 | 11.05 | 10.43 | 10.45 | 11.73 | 8.93  |
| 9          | 9.25  | 10.88 | 12.90 | 13.05 | 11.67 | 11.65 | 10.15 | 8.94  |
| 10         | 9.90  | 12.09 | 14.11 | 14.29 | 13.05 | 13.05 | 11.04 | 9.87  |
| 11         | 9.29  | 11.00 | 12.81 | 12.84 | 11.73 | 11.70 | 11.76 | 8.21  |
| 12         | 11.10 | 12.76 | 14.74 | 14.79 | 12.77 | 12.73 | 15.28 | 11.02 |
| 13         | 12.96 | 14.70 | 15.64 | 15.76 | 14.43 | 14.43 | 14.28 | 13.06 |
| 14         | 8.96  | 10.81 | 12.24 | 12.34 | 11.34 | 11.31 | 9.85  | 9.81  |
| 15         | 10.40 | 12.08 | 13.36 | 13.52 | 12.66 | 12.68 | 11.43 | 10.96 |
| 16         | 13.10 | 15.72 | 17.43 | 17.58 | 16.45 | 16.45 | 12.72 | 14.17 |
| 17         | 9.19  | 10.88 | 11.94 | 12.01 | 10.65 | 10.63 | 12.94 | 10.72 |
| 18         | 7.23  | 8.74  | 9.78  | 9.84  | 8.80  | 8.81  | 9.56  | 8.30  |
| 19         | 9.95  | 12.08 | 13.04 | 13.19 | 11.51 | 11.53 | 13.29 | 11.15 |
| 20         | 8.11  | 9.66  | 11.44 | 11.54 | 9.69  | 9.69  | 8.43  | 9.91  |
| 21         | 9.86  | 11.69 | 12.67 | 12.68 | 11.36 | 11.36 | 13.65 | 11.23 |
| 22         | 11.34 | 13.04 | 13.89 | 13.96 | 12.34 | 12.32 | 16.26 | 12.59 |
| 23         | 8.29  | 9.58  | 10.42 | 10.58 | 9.16  | 9.22  | 14.82 | 8.78  |
| 24         | 10.13 | 11.70 | 12.87 | 12.89 | 11.20 | 11.19 | 15.51 | 11.28 |
| 25         | 12.33 | 13.44 | 13.87 | 13.88 | 12.24 | 12.45 | 19.26 | 12.93 |
| 26         | 12.48 | 14.68 | 15.46 | 15.56 | 12.97 | 12.98 | 16.99 | 14.06 |
| 27         | 11.11 | 12.65 | 13.64 | 13.59 | 12.01 | 12.00 | 18.84 | 12.35 |
| 28         | 10.02 | 11.60 | 12.39 | 12.44 | 10.91 | 10.97 | 14.89 | 11.07 |
| 29         | 11.28 | 12.96 | 14.24 | 14.40 | 12.45 | 12.43 | 17.81 | 12.05 |
| 30         | 11.43 | 13.11 | 14.24 | 14.48 | 11.08 | 11.08 | 23.12 | 12.27 |
| 31         | 14.51 | 17.03 | 18.55 | 18.82 | 16.25 | 16.25 | 22.79 | 16.81 |

Table 3  Average percentage of the genome, stratified by chromosome, covered by runs of homozygosity (ROH)

| Chromosome | Average ROH (%) |
|------------|-----------------|
| A          | 50snp_500 kb    |
| B          | 50snp_500 kb    |
| C          | 50snp_500 kb    |
| D          | 50snp_500 kb    |
| E          | 50snp_500 kb    |
| F          | 50snp_500 kb    |
| G          | 50snp_500 kb    |
| H          | 50snp_500 kb    |

A = 50snp_500 kb _100snp_0_0; B = 50snp_500 kb _100snp_0_2; C = 50snp_500 kb _100snp_1_2; D = 50snp_500 kb _100snp_1_5; E = 50snp_500 kb _15snp_0_1; F = 50snp_500 kb _50snp_0_1; G = 50snp_500 kb _50snp_1_2; H = pruned_50snp_500 kb _50snp_0_1 (FORMAT: "sliding window size","minimum length (kb) for a run to be called as a ROH","minimum number of SNPs for a run to be called as a ROH","number of heterozygous SNPs allowed in a ROH","number of missing calls allowed in a ROH")
genetic technologies in racehorses, and is currently providing $F_{PED}$ estimates, as well as estimated breeding values (EBV) for breeders and owners to use as part of their criteria for determining sire/dam pairing [28, 29]. While this information has undoubtedly proved valuable over the last half-century, genomic information provides the opportunity to manage NSCT breeding more effectively—particularly if it is used to produce genomic EBV. In addition, the use of genomic information to determine both inbreeding levels and relationships between individuals is also likely to have a knock-on effect on performance, increasing the accuracy of the industry’s current EBV and therefore increasing the industry’s ability to improve the performance and health of their horses.

As with other species and breeds, opportunities exist for the NSCT industry to develop software programs that provide breeders with easily interpretable feedback on regions of the genome that are suboptimal from the perspective of genetic merit or that are sensitive to inbreeding within the population. Overall, 1403 common ROH regions were identified within the sample of raced horses used here. There were few overlaps with known QTL for health and reproduction traits, which indicates that perhaps only a small percentage of these regions may warrant concern, at this time [24]. Whereas multiple ROH regions ($n=17$) contained QTL that are associated with osteochondrosis (OC) [30–33], it is possible that homozygosity in these regions may be optimal rather than detrimental when one considers the widely heralded robustness of the breed and that only raced horses were evaluated in our study. It is likely that both the draught horse origins of NSCT and the breeding industry’s emphasis on continued production of robust, tractable horses through artificial selection, have resulted in the breed displaying a strong resistance to the development of OC with increasing homozygosity in specific areas of the genome over time. A similar observation can also be made for the common ROH that overlap with QTL associated with recurrent exertional rhabdomyolysis (RER), which is another condition rarely seen in NSCT [34]. However, additional research is required to confirm this.

Increased inbreeding within a population also tends to impact fertility traits unfavorably; however, only one of the common ROH regions overlapped with a known QTL related to reproduction [35], which suggests that, at present, poor fertility may not be a major concern in the NSCT breed. Nevertheless, it is strongly recommended that future genomic studies in this breed.
should consider the inclusion of data on fertility traits, since it will likely prove to be highly beneficial in subsequent efforts to preserve the breed’s genetic variability in the long term [5, 36].

Conclusions
In the current study, both $F_{\text{PED}}$ and $F_{\text{ROH}}$ were calculated for a sample of raced NSCT with $F_{\text{ROH}}$ resulting in higher inbreeding coefficients, and both methods showing a gradual increase in inbreeding between 2000 and 2009. Stricter ROH threshold criteria regarding the number of heterozygous SNPs and missing calls allowed in a ROH significantly reduced correlations between $F_{\text{PED}}$ and $F_{\text{ROH}}$ and noticeably altered the chances of capturing long ROH. While the exact reasons behind this decrease in correlations are not known with certainty, the established associations between classical $F$ estimates and recent inbreeding within a pedigree (characterized by long ROH) in other species provide some insight. Since retaining genetic variation is important to allow populations to adapt to changing environments, the integration of genomic data into their EBV and the use of molecular

| QTL ID  | Symbol | Trait name                      | Chr | Start position (bp) | End position (bp) |
|---------|--------|---------------------------------|-----|--------------------|------------------|
| 103450  | MOTSCT | Number of motile sperm          | 1   | 53958169           | 53958209         |
| 32144   | OSTEOD| Osteochondrosis                 | 2   | 11816213           | 21391792         |
| 32142   | OSTEOD| Osteochondrosis                 | 2   | 12910010           | 21391792         |
| 32146   | OSTEOD| Osteochondrosis                 | 2   | 12910010           | 21391792         |
| 32145   | OSTD   | Osteochondrosis dissecans       | 2   | 13028376           | 22500086         |
| 32143   | OSTEOD| Osteochondrosis                 | 2   | 18664801           | 19717761         |
| 32147   | OSTEOD| Osteochondrosis                 | 2   | 18664801           | 23235964         |
| 29325   | OSTEOD| Osteochondrosis                 | 3   | 105163057          | 105163097        |
| 29326   | OSTEOD| Osteochondrosis                 | 3   | 105546982          | 105547022        |
| 29327   | OSTEOD| Osteochondrosis                 | 3   | 105830605          | 105830645        |
| 29306   | SUSBITE| Insect bite hypersensitivity    | 4   | 43000811           | 43945687         |
| 37902   | OSTD  | Osteochondrosis dissecans       | 5   | 77424966           | 77425006         |
| 29287   | SUSBITE| Insect bite hypersensitivity    | 11  | 22016942           | 22985500         |
| 29268   | RHOD  | Rhodococcus equi infection      | 14  | 3055253            | 3055293          |
| 29315   | SUSBITE| Insect bite hypersensitivity    | 15  | 20012397           | 20994475         |
| 29316   | SUSBITE| Insect bite hypersensitivity    | 15  | 32000266           | 32987009         |
| 29035   | GPT   | Guttural pouch tympany          | 15  | 53093059           | 53093099         |
| 29111   | GPT   | Guttural pouch tympany          | 15  | 65298904           | 65298944         |
| 29067   | GPT   | Guttural pouch tympany          | 15  | 78013499           | 78013539         |
| 28922   | OSTEOD| Osteochondrosis                 | 16  | 1299549            | 5389006          |
| 29002   | OSTD  | Osteochondrosis dissecans       | 16  | 1299549            | 5389006          |
| 28933   | OSTEOD| Osteochondrosis                 | 16  | 5228939            | 5496903          |
| 28999   | OSTD  | Osteochondrosis dissecans       | 16  | 5228939            | 5496903          |
| 28927   | OSTD  | Osteochondrosis dissecans       | 16  | 22275834           | 22702331         |
| 28937   | OSTEOD| Osteochondrosis                 | 16  | 22275834           | 22702331         |
| 29006   | OSTEOD| Osteochondrosis                 | 16  | 22275834           | 22702331         |
| 29338   | RER   | Recurrent exertional rhabdomyolysis| 16  | 29314251           | 29314291         |
| 29277   | RER   | Recurrent exertional rhabdomyolysis| 16  | 29349222           | 29349262         |
| 29337   | RER   | Recurrent exertional rhabdomyolysis| 16  | 29349222           | 29349262         |
| 29320   | SUSBITE| Insect bite hypersensitivity    | 19  | 21037979           | 21977304         |
| 29298   | SUSBITE| Insect bite hypersensitivity    | 20  | 41031989           | 41982509         |
| 37895   | SUSBITE| Insect bite hypersensitivity    | 20  | 41530793           | 42603867         |
| 37896   | SUSBITE| Insect bite hypersensitivity    | 20  | 41530793           | 42603867         |
| 28920   | SARRESI| Equine sarcoïds                | 23  | 16126529           | 41049520         |
| 28921   | SARRESI| Equine sarcoïds                | 25  | 24227654           | 30109054         |
data to identify both genomic regions contributing to inbreeding depression and pedigree errors will likely prove invaluable as the NSCT industry moves forward in its conservation and selection efforts.

**Additional files**

Additional file 1: Table S1. Threshold settings used to define runs of homozygosity in PLINK and the corresponding average \( F_{ROH} \) for a sample of raced Norwegian-Swedish Coldblooded trotters born between 1 January 2000 and 31 December 2009.

Additional file 2: Table S2. Correlation matrix between \( F_{PEO} \) and all \( F_{ROH} \) estimates.

Additional file 3: Table S3. Results of the paired t-test (P-values) between \( F_{PEO} \) and all \( F_{ROH} \) estimates.

Additional file 4: Figure S1. Histograms for run of homozygosity (ROH) lengths based on four different threshold combinations in PLINK v1.07. A: \( 50\text{snp}_{500\text{kb}} \_100\text{snp}_{0\_0} \_1\_5 \) (FORMAT: "sliding window size"="minimum length (kb) to be called as homozygous"="minimum number of SNPs to be called as homozygous"="number of heterozygotes allowed"="number of missing calls allowed").

Additional file 5: Figure S2. Histograms for run of homozygosity (ROH) lengths based on varying window size thresholds in PLINK v1.07. A = \( 50\text{snp}_{500\text{kb}} \_15\text{snp}_{0\_1} \_1\_5 \); B = \( 50\text{snp}_{500\text{kb}} \_50\text{snp}_{0\_0} \_1\_5 \); C = \( 50\text{snp}_{500\text{kb}} \_50\text{snp}_{0\_0} \_1\_D = \text{pruned }50\text{snp}_{500\text{kb}} \_50\text{snp}_{0\_0} \_1 \) (FORMAT: "sliding window size"="minimum length (kb) for a run to be called as a ROH"="minimum number of SNPs for a run to be called as a ROH"="number of heterozygous SNPs allowed in a ROH"="number of missing calls allowed in a ROH").

Additional file 6: Table S4. Homozygous regions of the genome that are shared by at least 95% of the sample of Norwegian-Swedish Coldblooded Trotters (n = 56).

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Authors’ contributions

BDV, KJF, CI, ES, and GL conceived and designed the experiments; KJF, MKR, and KHR contributed to sampling. GL and ES contributed the reagents and DNA extracted the DNA; BDV analyzed the data and drafted the manuscript; KJF, MKR, CI, ES, and GL discussed and contributed to data analysis. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the Swedish Trotter Association (Stockholm, Sweden) and the Norwegian Trotter Association (Oslo, Norway), but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. However, data are available from the authors upon reasonable request and with permission of the Swedish Trotter Association (Stockholm, Sweden) and the Norwegian Trotter Association (Oslo, Norway).

Ethics approval and consent to participate

All experimental procedures and sample collection methods were approved by the Ethics Committee for Animal Experiments in Uppsala, Sweden [Number: C 121/14]. Samples used in this study were already available at either the Animal Genetics Laboratory at SLU in Uppsala, Sweden or the Department of Basic Sciences and Aquatic Medicine at the Norwegian University of Life Sciences in Oslo, Norway, since they had been previously used for parentage testing. Permission to use the samples was granted from the Swedish Trotting Association and the Norwegian Trotting Association (the owners of the samples per the rules/guidelines of the industry).

Consent for publication

Not applicable.

Competing interests

The authors have the following interests: GL is a co-inventor on a granted patent concerning commercial testing of the DMRT3 mutation: A method to predict the pattern of locomotion in horses. PCT EP 12,747,875.8. European patent registration date: 2011-05-05, US patent registration date: 2011-08-03. There are no further patents, products in development, or marketed products to declare.

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