Lethal Haplotypes and Candidate Causal Mutations in Angus Cattle

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Abstract

Background: If unmanaged, high rates of inbreeding in livestock populations adversely impact their reproductive fitness. In beef cattle, historical selection strategies have increased the frequency of several segregating fatal autosomal recessive polymorphisms. Selective breeding has also decreased the extent of haplotypic diversity genome-wide. By identifying haplotypes for which homozygotes are not observed but would be expected based on their frequency, developmentally lethal recessive loci can be localized. This analysis comes without the need for observation of the loss-associated phenotype (e.g., failure to implant, first trimester abortion, deformity at birth). In this study, haplotypes were estimated for 3,961 registered Angus individuals using 52,545 SNP loci using findhap v2, which exploited the complex pedigree among the individuals in this population.

Results: Seven loci were detected to possess haplotypes that were not observed in homozygous form despite a sufficiently high frequency and pedigree-based expectation of homozygote occurrence. These haplotypes were identified as candidates for harboring autosomal recessive lethal alleles. Of the genotyped individuals, 109 were resequenced to an average 27X depth of coverage to identify putative loss-of-function alleles genome-wide and had variants called using a custom in-house developed pipeline. For the candidate lethal-harboring haplotypes present in these bulls, sequence-called genotypes were used to identify concordant variants. In addition, whole-genome sequence imputation of variants was

performed into the set of 3,961 genotyped animals using the 109 resequenced animals to identify candidate lethal recessive variants at the seven loci.

Conclusions: Selective breeding programs could utilize the predicted lethal haplotypes associated with SNP genotypes. Sequencing and other methods for identifying the causal variants underlying these haplotypes can allow for more efficient methods of management such as gene editing. These two methods in total will reduce the negative impacts of inbreeding on fertility and maximize overall genetic gains.

Keywords: Inbreeding; Autosomal Recessives; Lethal Haplotypes; Phasing; Imputation

Background

The implementation of a national animal evaluation system in U.S. registered Angus cattle has generated estimates of genetic merit that are used to evaluate and select elite seedstock. Selection on individual traits and indices of traits has resulted in the genetic improvement of multiple traits, such as growth rate, carcass quality and calving ease [1, 2]. At the same time, artificial insemination has increased the utilization of certain paternal lineages. Selective breeding in livestock is known to contribute to the enrichment of deleterious alleles carried by highly utilized sires and also to increase the overall levels of relatedness among individuals. Many numerically important breeds, such as Holstein, Jersey, Nordic Red, and Angus, with extensive use of artificial insemination have recently found autosomal recessive lethal loci at moderate frequencies and that significantly impact fertility [3]. In recent decades several defects that are effectively lethal such as, Neuropathic hydrocephalus, Arthrogryposis Multiplex and Osteoperosis have been propagated within international Angus populations [4, 5]. A striking feature of these defects is their high prevalence in the U.S. registered Angus population, despite their severely deleterious phenotypic presentation [4]. This suggests that it is possible for recessive loci to have major negative impacts on fertility and overall performance without their early detection [3-5]. Consequently, recessive loci causing embryonic loss early in gestation could exist at relatively high population allele frequencies. These loci can reach high frequencies due to drift following severe population bottlenecks, due to their propagation by the extensive use of popular sire lines via artificial insemination, or due to linkage to beneficial alleles at strongly selected loci

72 [7]. The extent of the impact that these alleles have on fertility and fitness in
73 livestock is unknown, particularly because most of the reproductive process is
74 unmonitored. However, when these impacts become large, they can be detected by
75 genomic analysis of the population. In this study, we examined the inheritance of
76 haplotypes genome-wide in U.S. registered Angus cattle to reveal candidate
77 haplotypes that may harbor variants that cause embryonic lethality.

78 The dynamics of inbreeding depression in closed populations can be
79 described statistically, but genomic analysis can reveal their biological basis. The
80 accumulation and impacts of inbreeding are a function of the effective population
81 size, the initial genetic load of deleterious sites, mating patterns, strength of
82 selection and the extent or range of linkage disequilibrium within the genome [8].
83 Our work focuses on sites for which fitness = 0 when homozygous for a deleterious
84 allele. Consequently, identity by descent at loci harboring loss of function (LOF)
85 alleles can significantly impact fitness in populations that are accumulating
86 inbreeding [9]. What is unclear, is how much inbreeding is tolerable and how
87 common are recessive lethal alleles in livestock. Recent investigations in inbred
88 human populations suggest that high levels of relatedness are needed to
89 significantly impact fitness. Counterintuitively, up to a certain high threshold,
90 parental relatedness appears to be beneficial for fitness [10]. An investigation into
91 the North American human Hutterite population estimated that an average of 0.29
92 recessive lethal variants exist per haploid human genome, and that the primary
93 force that removes these alleles from a population is drift [11]. Simulations by these
94 authors also revealed that the majority (57.4%) of recessive deleterious variants

95 that were segregating in the founder population were not observed in the modern
96 population. Of the remaining recessive variants, only 8.23% had phenotypic effects.
97 This result is important, because it suggests that in a population such as the U.S.
98 registered Angus breed, a high proportion of latent recessive lethal variants could
99 still be segregating without their having been detected by breeders.

100 A recent study in U.S. Holstein dairy cattle using genotyped trios found that
101 expected inbreeding coefficients of offspring (obtained by simulated matings of
102 actual parental genotypes) were slightly lower than the realized inbreeding
103 coefficients, suggesting that increased inbreeding was not a constraint on viability of
104 offspring [12]. This result, along with the knowledge of the nature of several existing
105 recessive defects in the U.S. Holstein population suggests that the frequency of
106 lethals may not be sufficiently high to produce an observable impact on the
107 population when the average inbreeding coefficient is only 3.53%. These findings
108 are also consistent with a recent study of dog domestication and breed formation
109 that found that the major factor underlying the enrichment of deleterious variation
110 in modern breed dogs was severe population bottlenecks, and not recent inbreeding
111 [13].

112 Given experiences in other breeds and species, we hypothesize that there
113 may be many more recessive lethal alleles in the U.S. registered Angus population
114 than have previously been detected. To identify these, we implemented a method
115 that was first described by VanRaden [14]. A sample of 3,961 genotyped Angus
116 cattle, primarily bulls extensively used in artificial insemination that were members
117 of a pedigree containing 117,212 identified individuals, was analyzed to identify

118 haplotypes that were expected to be observed but that were not actually observed
119 in the homozygous state. The pedigree spanned more than 60 generations with the
120 earliest ancestor born in 1836, and with the earliest genotyped animal born in 1955
121 [2]. The analysis assumed that the haplotypes harbored fully penetrant lethal alleles
122 that would preclude the viability and genotyping of an animal homozygous for the
123 haplotype. Within this pedigree, haplotypes that were identical by descent were
124 repeatedly sampled, allowing for the detection of autosomal recessive lethal alleles.

125 The adoption of high-density array-based genotyping in commercial beef and
126 dairy cattle populations is rapidly increasing, due to the utility of genomic selection
127 [15] and as a consequence a large number of trios, patrios (sire, maternal grandsire
128 and son) and half-sib families have now been genotyped. Whole-genome sequencing
129 of influential population members and the use of genotype imputation will allow the
130 identification of lethal alleles without the observation of the phenotype responsible
131 for the loss [16]. Characterizing the number and identity of these variants will
132 provide a deeper understanding of the biological and quantitative underpinnings of
133 inbreeding depression. It will also enable enhanced management of animal
134 reproduction, as these variants can be identified in any genotyped animal.

135 Methods

136 *Genotypes and Animals*

137 BovineSNP50 BeadChip (Illumina, San Diego, CA)[17] data for 3,993 registered
138 Angus animals born between 1955 and 2012 and representing 63 generations were
139 available for analysis. Genotypes had been filtered to retain data with a call rate of ≥

140 90% and minor allele frequency (MAF) ≥ 0.01 [2]. Pedigrees had also been validated
141 by homozygous transmission incompatibility rates estimated as the frequency of
142 loci for which the parent and offspring were alternate homozygotes. Pedigree
143 relationships were expunged when the incompatibility rate exceeded 1.5%, and
144 individuals who did not match their recorded parents or offspring were removed
145 from the pedigree. After filtering, 52,545 loci and 3,961 animals remained. These
146 sites were next phased and missing genotypes were imputed using findhap (v2)
147 based upon a combination of simple haplotype frequency sorting and the use of all
148 available pedigree information [18].

149 *Population and Pedigree Based Haplotype Analyses*

150 Haplotypes were defined by 20-marker sliding windows genome-wide. Evidence for
151 a lethal allele within a haplotype was evaluated using two statistics. The first was
152 based on population frequency. For each haplotype in each window, frequency was
153 estimated and the number of individuals in the population that were homozygous
154 for the haplotype was tallied. When there was an absence of homozygotes, the
155 likelihood of this occurrence was calculated under a model that assumed carriers to
156 be randomly mating and selective neutrality of the haplotype.

157 In the second analysis, the actual matings within the pedigree were used to
158 estimate the expected number of homozygotes for any given haplotype based upon
159 the number of matings involving carriers and the assumption of selective neutrality.
160 The pedigree for the genotyped animals was parsed to identify patrios, defined here
161 as families for which genotypes were available for the offspring, sire and maternal

162 grandsire. Maternal genotypes were rarely available as DNA was primarily sourced
163 from cryopreserved semen [2]. This family structure allowed the testing of
164 segregation distortion when both the sire and maternal grandsire were
165 heterozygous for the same haplotype. For each sliding window of 20 markers if a
166 haplotype was never observed in the homozygous state and had a sample frequency
167 of greater than 2%, the number of families for which the sire and maternal
168 grandsire were both heterozygous was counted. The probability of observing at
169 least one homozygote was then calculated based on the count of patrios and the
170 assumption of selective neutrality. The probability that no homozygous *hh*
171 haplotypes are observed in the progeny of C patrios when both the sire and
172 maternal grand-sire are *Hh* heterozygotes and the *h* haplotype does not harbor a
173 selected deleterious allele is $(0.875 - 0.25q)^C$ where q is the frequency of the *h*
174 haplotype in the population. There were 2,480 patrios represented in the sample.
175 Regions of the genome with an identified deficit of homozygotes for a specific
176 haplotype were examined for underlying genes using pybedtools and the UMD3.1
177 genome assembly annotation run 104 [14, 15].

178 *Generation of Sequence Data*

179 To further analyze the variation within the genomic regions harboring haplotypes
180 that were deficient for homozygotes, the whole-genome sequences of 109 animals
181 from this population were examined [20]. These animals were selected for
182 sequencing based upon their impact on the breed assessed by the expected numbers
183 of genome equivalents (progeny have 0.5, grandprogeny have 0.25, etc) present
184 within registered descendants in the population. They were sequenced with paired-

185 end 2 x 100 bp sequence reads to an average of 27X depth of coverage of the
186 UMD3.1 assembly with Illumina Genome Analyzer, GAII, HiSeq 2000 or 2500
187 instruments from two libraries with 350 bp and 550 bp average fragment sizes.
188 FastQC was used to analyze the quality of the reads [21]. Exact duplicates were
189 removed, and adapters were trimmed using a custom in-house Perl script. All
190 remaining reads were error corrected using QuorUM [22]. Newly created duplicates
191 (due to the trimming of low quality ends and correction of errors) and reads shorter
192 than 35 bp were removed and the final data set was aligned to the UMD3.1
193 reference genome assembly using NextGENe 2.4.1 (SoftGenetics, LLC, State College,
194 PA) alignment software. Reads were required to have a matching segment at least
195 35 bases long and 95% overall match, and a maximum of 2 bases of mismatch across
196 the whole alignment. Up to 1000 alignments of equal likelihood were allowed
197 genome-wide. NextGene 2.4.1 was also used for variant calling.
198 The sequence-derived variants for the 109 bulls were used for two purposes;
199 identification of variants shared amongst animals identified as carriers for the
200 putatively lethal BovineSNP50 20 SNP haplotypes, and for the imputation of the
201 entire genotyped population to whole-genome sequence variation.

202 *Examining Carrier Sequence Data*

203 Within the genomic regions identified from the marker data as containing candidate
204 lethal haplotypes, all variants identified in our sequenced sample were analyzed for
205 carrier concordance, implemented using python scripts. This involved examination
206 of variants that were observed as heterozygous in all bulls predicted to be carriers

207 of the homozygote deficient haplotype. The sample size for the sequenced animals
208 was not sufficient to run the population allele frequency or patrio analyses.

209 *Imputation of BovineSNP50 Genotypes to Whole Genome Sequence Variation*

210 We imputed the BovineSNP50 genotypes for this population to whole-genome
211 sequence level variation in order to identify potential lethal variants, using the 109
212 sequenced animals as the reference population. We selected 24,974,785 SNPs from
213 the full set that had been identified in these animals. We first included SNPs found in
214 the 109 sequenced Angus bulls that were biallelic and located within gene
215 boundaries (777,432), UTRs (33,379) or that were splice site variants (636,492).
216 These variants spanned the allele frequency spectrum but were identified at high
217 sequence coverage. To enable imputation we also included 23,527,482 variants that
218 had been independently identified in run 5 of the 1000 Bull Genomes project [23].
219 These variants represent filtered, high quality segregating sites identified from the
220 whole genome sequences of 1,578 animals from multiple taurine breeds including
221 Angus. Genotypes called for the 24,974,785 variable sites genome-wide in the 109
222 registered Angus bulls were used as the reference set for whole-genome sequence
223 imputation of the BovineSNP50 data using Fimpute [24].

224 The imputed genotypes were individually analyzed for the absence of
225 homozygotes for alleles present within each of the candidate haplotyped loci using
226 the frequency and pedigree approaches. We first identified high frequency variants
227 for which no homozygous individuals were predicted. The pedigree analysis was
228 also performed for all candidate variants identified in the frequency analysis.

229 Variants identified by either of these processes were characterized using the variant
230 effect predictor release 79 [25].

231 Results

232 *Identification of Putative Lethal Haplotypes*

233 We identified seven haplotypes with a pattern of inheritance in U.S. registered
234 Angus cattle that suggests that they each harbor an autosomal recessive lethal allele
235 (Table 1). Using a binomial distribution for the number of observed homozygotes in
236 the progeny of C patrios, we calculated the probability of observing no homozygotes
237 when each haplotype was selectively neutral. We selected haplotypes as putatively
238 harboring autosomal recessive lethals when the probability of observing no
239 homozygotes was less than 0.02. This threshold for statistical significance provides
240 considerable confidence that the lack of homozygosity for these haplotypes did not
241 occur by chance alone.

242 *Sensitivity to Window Size*

243 We evaluated the sensitivity of identification of these marker-based haplotypes to
244 window size and concluded that a window size of 20 contiguous BovineSNP50
245 markers was appropriate for capturing the haplotypic diversity within the
246 population (Figure 1). This window size appears to discriminate between
247 haplotypes that are identical by descent (IBD) and those that are identical by state
248 (IBS). Our analysis shows that the number of common haplotypes detected rises as
249 window size increases, but begins to plateau at 20 markers. Considering the
250 moderate marker density of the BovineSNP50 (1 SNP per 50 kb), haplotypes that

251 are defined by only two markers are assumed to be IBS for the purposes of analysis
252 but likely actually represent a number of distinct haplotypes at the level of genome
253 sequence. As the window size increases, the likelihood increases that two
254 haplotypes found in different individuals that are IBS are also IBD and are thus
255 concordant at the level of sequence variation. However, with large window sizes,
256 recombination may lead to a lethal variant being present on more than one
257 haplotype, thus decreasing the power of the analysis. Indeed, as window size
258 increases, the overall rate of individuals homozygous for any haplotype declined.
259 The window size selected for this study appears to achieve an appropriate balance
260 of genome-wide homozygosity and rate of occurrence of high frequency haplotypes
261 (Figure 1). Slight changes in the haplotype window size did not affect the detection
262 of the 7 haplotypes reported in Table 1.

263 We were also able to validate IBD status of these regions by an examination
264 of the sequence data generated for animals that were predicted to be carriers of
265 identical haplotypes. For each of the loci predicted to harbor recessive lethal
266 haplotypes (Table 1), we identified the bulls among the 109 sequenced animals that
267 were predicted to be carriers of each putative lethal haplotype and computed the
268 pairwise rate of opposing homozygous sequenced sites between all pairs of carrier
269 animals. For example, for the haplotype on chromosome 29, with 16 predicted
270 carriers we made 120 pairwise comparisons using all sequence called variant
271 genotypes within the haplotype coordinates (Figure 2). Amongst individuals that
272 share a common haplotype, the alternate homozygote rate is related to the error
273 rate for sequence-based genotype calls for heterozygotes. The rate observed in our

274 predicted carriers within our 6 testable haplotype regions was similar to the rate for
275 sire-son pairs. One region (Chr 1) had only one sequenced predicted carrier and
276 could not be evaluated. The majority of the carrier pair comparisons with rates of
277 opposing homozygous sequenced sites >0.01 were caused by the inclusion in the
278 analysis of 3 individuals that had been sequenced to averages depths of < 10X.
279 Within the haplotype, opposing homozygous sequenced site rates were markedly
280 lower for predicted carriers than for randomly selected individuals. When predicted
281 carriers for a lethal haplotype were analyzed for a randomly selected 20 marker
282 region elsewhere in the genome, they had opposing homozygote rates that were
283 similar to those of the unrelated sire pairs. This indicates that the haplotypes
284 generated from the BovineSNP50 data successfully identified genomic regions that
285 were IBD at the level of the genome sequence. We also observed that this method is
286 sensitive to the depth of sequence coverage due to the inaccurate identification of
287 heterozygous loci as being homozygous when the alternate allele was never
288 sequenced. In total, 16 animals had depths of sequence coverage of < 10X and most
289 of the remaining animals (70) had > 20X. For a Sire-Son pair, low sequence coverage
290 for at least one member of the pair led to a rate of homozygous inconsistency that
291 was increased by an order of magnitude.

292 *Analysis of Sequence Variation and Candidate Genes in Bulls Predicted to be Lethal*
293 *Haplotype Carriers*

294 To identify the causal variants underlying these putatively lethal haplotypes, we
295 first directly examined resequencing data from bulls that were predicted to be
296 carriers of the lethal haplotypes. Among the 109 sequenced bulls up to 21 animals

297 were predicted to be carriers of each of the BovineSNP50 putatively lethal
298 haplotypes reported in Table 1. Within these seven genomic windows, we identified
299 candidate variants that were never homozygous in the 109 sequenced bulls.
300 However, none were observed to be exclusively heterozygous in the predicted
301 carrier animals. That is, all of the alleles found to be heterozygous in all of the
302 predicted carriers were also observed to be heterozygous in animals that did not
303 carry the predicted lethal haplotype. A recessive lethal variant could be
304 heterozygous in animals that were not carriers of the putatively lethal haplotype if
305 the mutation is sufficiently old that recombination has occurred relative to the
306 haplotype on which the mutation occurred. Table 1 reports, for each genomic region
307 predicted to harbor a lethal haplotype, the number of variants that were
308 heterozygous in all predicted haplotype carriers and that were never found to be
309 homozygous in any of the 109 resequenced bulls. For instance, on chromosome 15,
310 an intronic variant in *SSRP1* was found in 9 of the 10 sequenced bulls that were
311 predicted to carry the putatively lethal haplotype, and in 25 of all 109 sequenced
312 Angus bulls, but was never found to be homozygous. Although the variant is
313 intronic, with no expected impact on splice site variation, it resides in an interesting
314 candidate gene as *SSRP1* is essential for mouse embryonic development [26].
315 This analysis was also conducted after excluding the 16 animals sequenced to
316 low average sequence depth of coverage (< 10X). Genotypes for these animals are
317 likely to contain false positive homozygotes at many true heterozygous sites. This
318 resulted in the detection of additional concordant loci but again none were exclusive
319 to the predicted carriers. The chromosome 29 locus had 12 additional variants

320 identified that were located in genes such as *CAPN*, *NRXN2*, *PACS1* and *PP25B*.
321 However, none of these mutations are in exons or had other interpretations in
322 Variant Effect Predictor (VEP) and all 12 were heterozygous in 18 animals, including
323 4 that were not predicted by their marker data to be carriers. Thus, the region
324 appears to comprise a large consistent haplotype with no one particular variant
325 being simply implicated as causal for lethality. The locus on chromosome 4 had 118
326 variants identified as being heterozygous in haplotype carriers following the
327 removal of the low sequence coverage animals, but none were predicted to alter
328 protein amino acid sequences.
329 The locus on chromosome 1 for which we predicted a lethal haplotype
330 contains only one gene, *GBE1* that encodes a protein that catalyzes the branching of
331 glycogen, the main form of energy storage in the body [24]. There was only one
332 sequenced animal in our population predicted to carry this disease and further
333 analysis of the unique heterozygotes within this gene were therefore not feasible.
334 *Analysis of Imputed Sequence Variation in 3,961 Registered Angus*
335 When analyzed in the 3,961 animals, 2,504 of the 24,974,785 imputed variants had
336 a MAF ≥ 2%, no predicted homozygotes, and were found in ≥ 30 double-carrier
337 patrios. Of the 24,974,785 variants, 147,764 had a deleterious consequence
338 predicted by their SIFT scores [30], but only 4 were among the 2,504 candidate
339 autosomal lethal alleles. Two of these sites were in *LOC521645*, an olfactory
340 receptor gene (near, but not within the chromosome 15 locus, Table 1), and another
341 was in *LOC100336589* on chromosome 18. The fourth was on chromosome 21 in
342 *GEMIN2*, for which a recessive lethal embryonic phenotype occurs in mouse

343 knockouts and is associated with the survival motor neuron complex [31]. This
344 allele had a frequency of 7.8% following imputation into the 3,961 animals and was
345 observed in 55 double-carrier patrios without producing a homozygous progeny.
346 Twelve variants within *GBE1*, none of which were predicted to be
347 deleterious, were at a MAF ≥ 2%, had no homozygotes predicted, and were found in
348 ≥ 30 double-carrier patrios, with one variant present in 133 double-carrier patrios.
349 However, only one of the sequenced animals was predicted to carry this haplotype
350 making it difficult to use the data for the sequenced animals to exclude any
351 heterozygous sites within this animal's diplotype from candidacy for lethality. The
352 haplotype on chromosome 1 spanning *GBE1* was the only predicted lethal to contain
353 any of the 2,504 candidate variants identified in the analysis of the imputed whole
354 genome sequence data.

355 Discussion

356 Due to the repeated sampling of parental gametes and the sharing of haplotypes
357 across families in this extensive Angus pedigree, we were able to powerfully test the
358 fitness consequence of many haplotypes. The use of artificial insemination increases
359 selection intensity by allowing relatively few individuals to sire large numbers of
360 progeny and this population has historically been strongly selected for growth and
361 calving ease [2]. Four bulls in this dataset were each represented in over 100 of the
362 2,480 genotyped patrios, suggesting that intense selection can drive deleterious
363 alleles carried by these bulls to a relatively high frequency. In some cattle
364 populations, allele frequencies of up to 20% have been observed for some recessive

365 lethal haplotypes [6]. These common recessive lethals have the greatest impact on
366 population fitness, but as genotyping becomes more pervasive, rare recessive lethal
367 haplotypes will be detected and the available genotypes should allow for their
368 management.

369 *Management Strategies*

370 Angus breeders have historically selected against recessive lethal alleles which
371 manifest as fatal calf defects in this population [4]. Registered animals are tested for
372 known defects and registration is prevented for young animals that test to be
373 carriers. As new deleterious alleles including those causing early embryonic loss are
374 discovered, this approach is likely to be untenable. The seven new putatively
375 autosomal recessive lethal haplotypes may now be predicted in hundreds of
376 thousands of genotyped animals [32] and many more deleterious loci may be
377 discovered as the number of genotyped animals increases and new lineages rise to
378 prominence that contain yet to be detected recessive alleles. Management must shift
379 from registration exclusion to a means of incorporating marker diagnostics into
380 genomic selection and mate selection protocols.

381 A mate selection procedure has been implemented in the MateSel software
382 that applies a linear weight against either the number of recessive alleles present in
383 the progeny generation (LethalA) or the number of homozygous progeny (LethalG)
384 [33]. In a simulation with a higher count (N=100) for the number of lethal alleles
385 segregating in a population than identified in this study, it was not possible to
386 sufficiently weight in either selection scheme to eliminate embryonic mortality. This
387 scenario may approach reality as the number of identified deleterious recessive

388 alleles causing calf defects as well as embryonic loss increases. These simulations
389 also suggest that implementing the LethalG strategy is a more efficient means of
390 achieving genetic gain while reducing the frequency of recessive alleles.

391 Cole (2015) suggested an alternative strategy in which parent average
392 breeding indexes are adjusted for lost progeny by adding a cost associated with
393 recessive haplotypes [34]. This method could be extended to account for the joint
394 impact of multiple linked or unlinked segregating loci to reduce the frequency of
395 deleterious alleles. Counterintuitively, this study also found a zero to inverse
396 relationship between the embryo's realized inbreeding coefficient and its
397 probability of being homozygous for an allele responsible for a recessive disorder.
398 This suggests that the goal of reducing the long-term rate of accumulation of
399 inbreeding in breeding programs may not impact the rate of embryonic loss due to
400 the action of recessive lethal alleles. This is consistent with the observation from
401 studies of embryonic loss and coancestry in humans [8].

402 *Managing False Positives*

403 Managing selection based on these results requires certainty about the lethal
404 haplotype's effect. In this study, a total of 12,020 haplotypes were found genome-
405 wide (including partially overlapping haplotypes) that occurred at a MAF ≥ 2% but
406 were never found as homozygotes. Assuming random mating, a haplotype at this
407 frequency is expected to have, on average, only 1.5 homozygotes in our Angus
408 sample. However, the existence of non-random mating within this population could
409 substantially decrease the likelihood of observing homozygotes, and explain why so
410 many of these regions were observed. The patrio analysis directly incorporates the

411 matings that created the population to detect deviations from selective neutrality in
412 progeny genotypes. However, this approach is limited by the availability of
413 genotyped patrios. In breeds where insemination and pregnancy records are more
414 detailed, these have been crucial for validating putative lethal haplotypes[14].

415 In the absence of these data, both the MateSel approach and the index
416 adjustment could be adapted to account for the uncertainty of the lethality of the
417 predicted allele or haplotype. In addition to validated haplotypes, and the high
418 confidence haplotypes that we observe here, this approach could enable the
419 incorporation of haplotypes into the selection scheme that appear to be lethal but
420 that are at low frequency in the population. There are now Angus pedigrees in
421 which hundreds of thousands of animals have been genotyped world-wide and an
422 analysis of these data would likely improve the resolution of lethal haplotype
423 detection and could also identify many rarer variants [15]. If these lethal alleles are
424 individually rare but each individual carries many of them, recessive lethals could
425 affect a substantial portion of pregnancies.

426 *False Negatives*

427 Even with adequate sample sizes to ensure statistical power, there are limitations to
428 the methods that we have employed. The approaches employed will only detect
429 recessive alleles that are perfectly concordant with a BovineSNP50 haplotype. If a
430 recent autosomal recessive lethal mutation has occurred on a common haplotype,
431 the population will comprise haplotypes harboring either the lethal mutation or the
432 wild type allele and homozygotes that include the wild type allele will be observed.

433 In this case, very large sample sizes are required to detect deviations from the
434 number of homozygotes expected under Hardy-Weinberg equilibrium. This could
435 also involve restricting the analysis to different pedigree lineages for an IBS
436 haplotype. This may explain why we failed to identify any of the recent recessive
437 genetic defects found in the Angus breed [5]. In recent years, the alleles responsible
438 for these defects have frequencies that have ranged from 3-9% [4]. However, the
439 regions of the genome that harbor these alleles were not detected in the marker-
440 based haplotype analysis and the causal variants were not detected in the analysis
441 of the imputed sequence data. The likely cause of this is that the haplotypes we
442 examined did not always indicate the presence of the defective allele. For instance,
443 the mutation causing Neuropathic Hydrocephalus, originated in bull G A R Precision
444 1680 born in 1990 [35]. Consequently, there are both wild-type and deleterious
445 versions of the BovineSNP50 haplotype on which the mutation arose segregating in
446 the genotyped population. When larger sample sizes become available, it would be
447 useful to repeat these analyses and test for homozygote deficiency rather than
448 complete absence.
449 We have also not attempted to model mutations in loci with parent of origin
450 affects, such as imprinting associated defects [36]. SNP array genotypes identify
451 large heterozygous deletions as being homozygous for the alleles present on the
452 non-deletion chromosome, which may prevent the identification of carriers for the
453 deletion. This type of mutation has previously been associated with lethal recessive
454 diseases in cattle [7]. Additionally, lethal alleles with incomplete penetrance will not

455 be captured by our analyses. Other errors in genotyping, phasing or imputation also
456 likely contribute to reductions in power to detect lethal haplotypes.

457 *Using sequence data*

458 One potential solution to the limitations of array genotype data is to analyze
459 sequence-derived and/or imputed genotypes. These data may help with the
460 management of putative recessive lethal alleles. True causal variants can be tracked
461 more effectively than marker haplotypes. When the causative alleles have been
462 identified, gene editing may also present an efficient means of reducing the genetic
463 load of elite sires in a manner that is complementary to the current breeding system
464 [37].

465 Sequence data also has potential advantages for the detection of recessive
466 loci. If appropriately processed to capture SNPs, large and small indels and
467 structural variants, they directly represent the pool of all recessive deleterious
468 alleles. However, in practice, sample sizes have been small and the identification of
469 large indels, particularly insertions, and complex structural variants has been
470 challenging. Our analysis of sequence data failed to identify any candidate causative
471 mutations in the marker-based haplotypes that were predicted to be lethal. The
472 sample size for the sequenced animals was not sufficient to conduct the frequency
473 or pedigree analysis with the genome-wide sequence variants. Furthermore, we did
474 not attempt to analyze many types of complex variation, such as large indels or
475 structural variants [38]. Large structural variants are enriched for deleterious
476 variation but can be complex to analyze with short read data [39]. Alternative

477 analyses of the sequence data to identify these variants or the use of methods which
478 generate longer reads may be necessary to capture the causative variants.
479 In this study we did not detect a particular variant within a putative
480 haplotype that was likely to cause a recessive lethal phenotype. However, the gene
481 within the region on Chromosome 1, *GBE1* appears quite promising. Mutations in
482 this gene produce recessive phenotypes in mammals including horse, mouse and
483 human [24, 25]. In the U.S. Quarter Horse population, phenotypes created by
484 homozygotes for *GBE1* mutations ranged from stillbirth to early failure to thrive,
485 with death never occurring later than 18 weeks of age [28]. Mouse knockout
486 analysis revealed few visible or biochemical phenotypic effects in heterozygotes.
487 Monitoring of embryonic development in homozygous knockouts revealed that
488 deformities only occurred late in gestation and led to stillbirth or death shortly after
489 birth. Mice with a construct with low GBE1 activity incorporated into their genome
490 to replace the wild type allele demonstrated poor metabolic performance, and the
491 accumulation of polyglucosan [29]. None of the mice with limited GBE1 function
492 lived beyond 39 weeks, while all control mice survived the trial. While not all of the
493 recessive *GBE1* genotypes in other species have resulted in embryonic loss, the
494 reduced growth associated with homozygosity for these mutations makes it unlikely
495 that an affected animal would be selected as a sire or dam. They would therefore be
496 highly unlikely to be included in our genotyped sample of Angus cattle. However,
497 identifying homozygous calves from those produced by mating carriers and
498 assaying their GBE1 functionality might be possible.

499 *Mapping Candidate Variants without Sequencing*

500 Rather than generating expensive sequence data for identifying recessive lethals,
501 two strategies might be useful: assay development and imputation. Novel variants of
502 all classes that are detected by sequencing can readily be incorporated onto
503 commercial genotyping platforms. This expedites fine-mapping within a known
504 lethal haplotype in a commercial population. Variants that had predicted deleterious
505 functional impacts based on bioinformatic analysis would also be excellent
506 candidates for inclusion on commercial genotyping platforms.

507 Imputation accuracy, particularly for rare variants, may not be sufficient to
508 identify candidate segregating recessive lethals. We previously analyzed the
509 accuracy of imputation using variants from Run4 of the 1000 bull genomes project
510 as a reference for our Angus BovineSNP50 genotyped population using the same
511 imputation methods that were used in this study [20]. Our 109 sequenced animals
512 had their BovineSNP50 genotypes imputed to the 1000 Bull Genomes Run4
513 sequence reference set as well as variants called directly from their whole-genome
514 sequences. Comparing these two sets of genotypes revealed correlations between
515 genotypes in the range of 80-90% for common variants, and 60-80% for variants
516 with MAF ≤ 10% [20]. We would expect the causal variants underlying these lethal
517 haplotypes to fall in the rare, more inaccurately imputed frequency class. This
518 greatly complicates the utility of imputation for this application, and the results of
519 imputation presented here are not appropriate for application to breeding
520 decisions. Had an interesting candidate locus with a plausible biological mechanism
521 emerged, it would have been a good target for further confirmation and possibly
522 immediate use. More sophisticated sequence imputation methods that provide

523 higher imputation accuracies across the allele frequency spectrum are now
524 becoming available. These have been used in human studies to identify individuals
525 that are homozygous for rare variants with predicted deleterious effects [16].
526 Applying these methods to livestock populations with extensively described and
527 genotyped pedigrees is feasible, and may prove useful for fine-mapping within
528 candidate haplotypes.

529 Conclusions

530 We identified 7 potentially recessive lethal haplotypes segregating in the U.S.
531 registered Angus population that open opportunities for improving breeding
532 success and increasing the mean fitness of the population. These haplotypes have
533 been propagated throughout Angus lineages represented in a set of 3,961 genotyped
534 animals and were not observed in homozygous form. The phenotypic effects of
535 these haplotypes have not been directly observed, but may be inconspicuous such as
536 in the event of early embryonic loss or may be unreported defects leading to the loss
537 of the calf. Efforts to identify causal mutations with a clear molecular impact from
538 sequence data were unsuccessful but interesting candidate genes such as *GBE1* were
539 identified. Further validation of the impact of these haplotypes on fertility and the
540 direct observation of calves that are homozygous for these haplotypes could reveal
541 interesting biology. Our capacity to detect these loci will continue to improve as
542 increasingly large numbers of animals are genotyped and sequenced. The quality of
543 the reference genome assembly and methods for characterizing and imputing
544 structural variants are also improving and will improve the quality of this type of

545 analysis. Eventually, we will identify dozens of deleterious recessive loci, and can
546 use chip-based genotyping to manage matings, track alleles through lineages and
547 potentially use gene editing to remove them from elite animals.
548

549 **Declarations**

550 *Ethics approval and consent to participate*
551 The genotype data described in this manuscript was previously analyzed or
552 collected from commercially generated animal semen; as such no ethics or animal
553 welfare approval was required.

554 *Consent for publication*
555 Not applicable

556 *Availability of data and material*
557 Genotypes are available to scientists interested in non-commercial research upon
558 signing a Materials Transfer Agreement (MTA). All sequence data will be deposited
559 under NCBI Bioproject Accession PRJNA343262.

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565 Food and Agriculture.

566 *Authors' contributions*

567 JLH JED and RDS JFT designed and conducted the study. JFT and RDS collected
568 samples and processed the genotypes. RDS processed NGS data. JLH conducted
569 bioinformatic analyses of genotypes and sequence variants. JLH and JFT wrote the
570 manuscript. JFT RDS and JED edited the manuscript.

571 *Competing Interests*

572 Authors have no competing interests to declare.

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579

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705

706 Table 1: Chromosomal regions predicted to harbor lethal haplotypes identified in the analysis of the BovineSNP50 data.

707

Chr	Location (Mb)	Length (Mb)	Haplotype Frequency[1]	Number of Patrios[2]	Probability[3]	Sequenced Carriers	Concordant Variants	Concordant In High Coverage
1	27.7-29.0	1.3	0.023	39	0.0042	1	4	4
4	82.5-84.0	1.5	0.076	127	2.66E-09	21	9	118
8	62.0-63.0	1.0	0.023	35	0.0074	5	1	1
12	60.0-61.2	1.2	0.032	46	0.0014	12	0	0
15	82.3-83.1	0.8	0.038	31	0.011	10	1	1
17	46.5-47.5	1.0	0.045	49	0.00076	15	2	2
29	43.0-44.2	1.2	0.044	118	3.22E-08	16	3	13

708 [1]Haplotypes estimated for 20 contiguous SNP loci.

709 [2]Number of families out of 2,480 for which the sire and maternal grandsire were both heterozygotes for the haplotype.

710 [3]Probability of observing no homozygous progeny if the haplotype is selectively neutral.

711

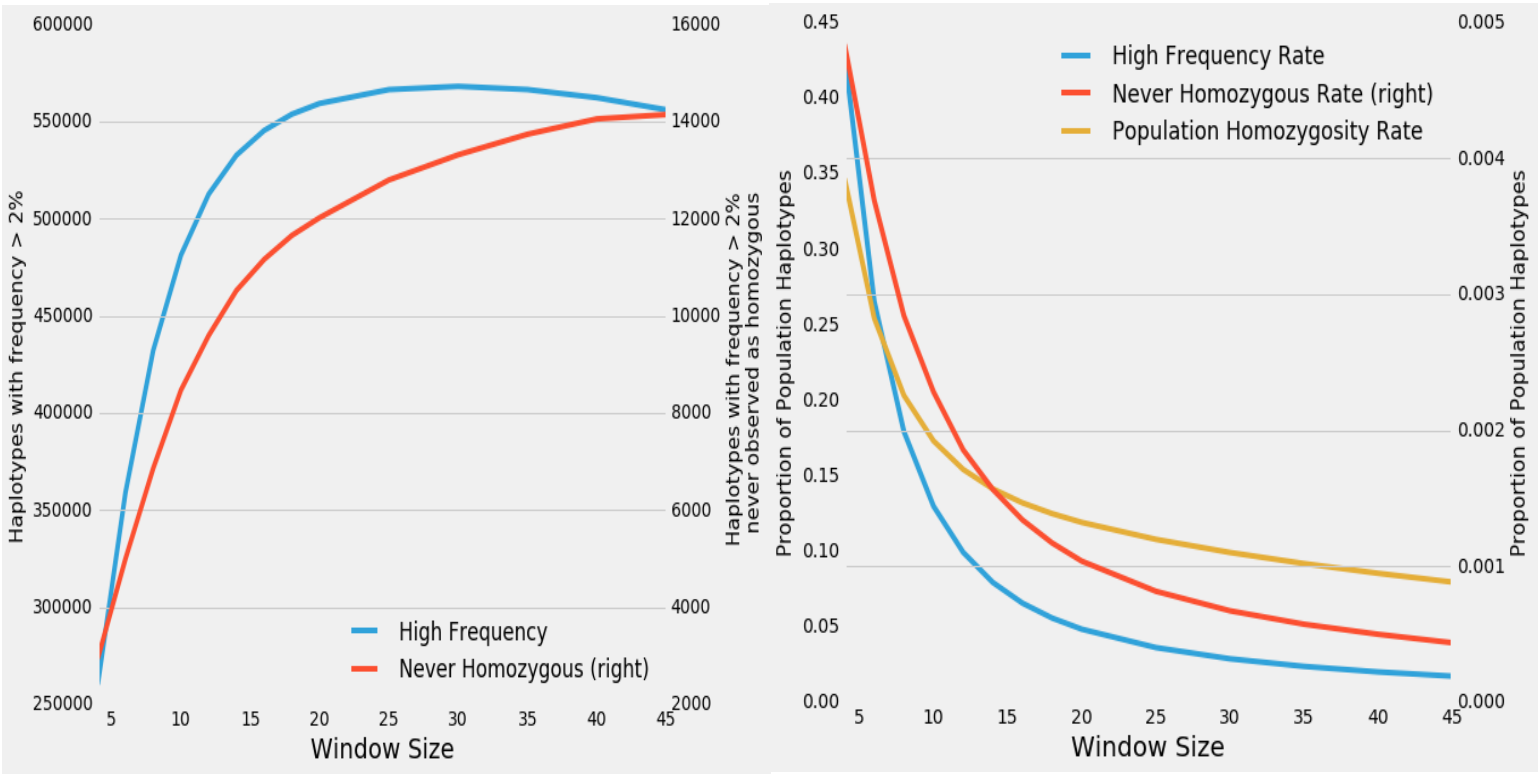

Figure 1: Effect of window size on haplotypic diversity and lethal haplotype detection. A) As the size of the window expands, many more distinct haplotypes are detected genome-wide. However, fewer of the newly detected haplotypes are common as window size increases, and the number of common haplotypes that are never observed as being homozygous asymptotes. B) Rate of homozygosity, which is the percentage of individuals that are homozygous for any haplotype, is high for small window sizes but quickly declines. The assumption that phased marker homozygosity implies identity by descent underlies the population frequency and patrio tests for haplotype lethality.

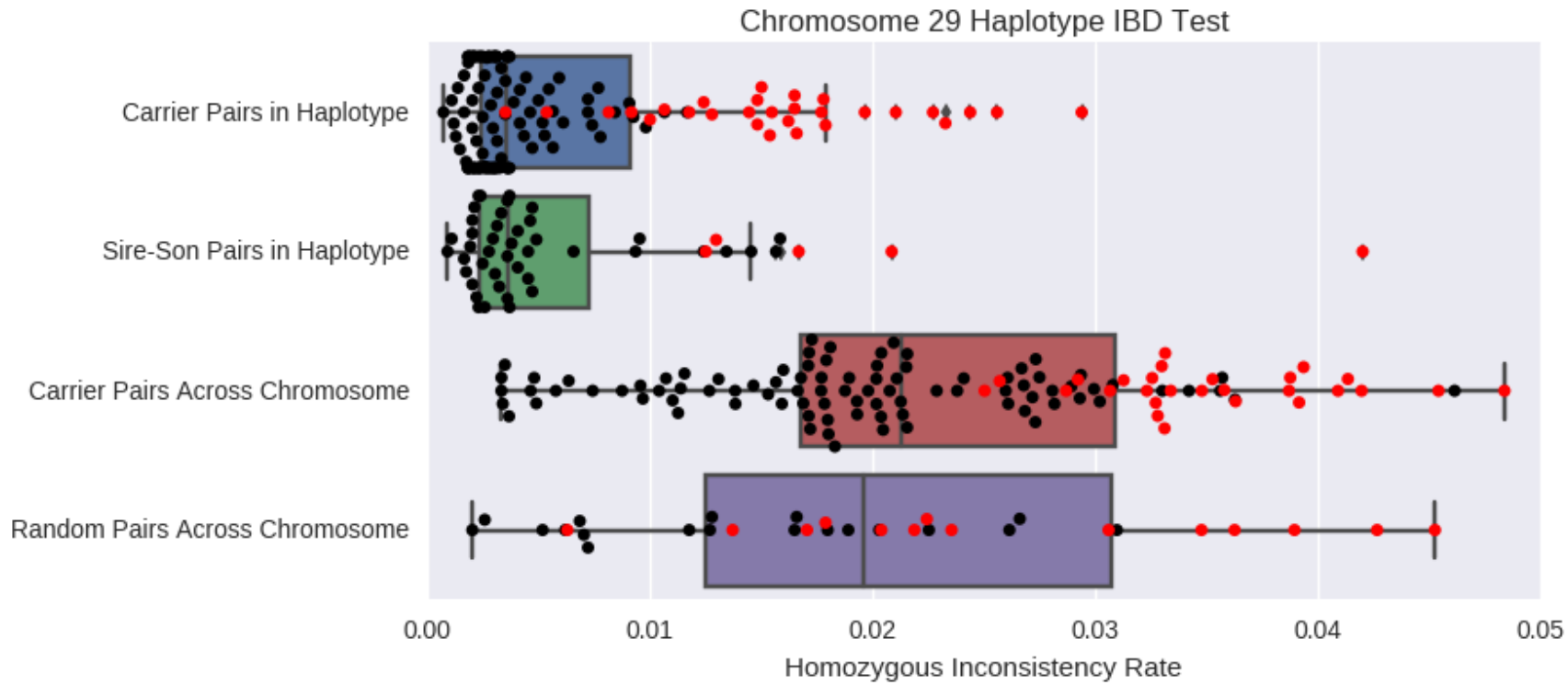

Figure 2: Validating the sequence level IBD status of bulls predicted to be carriers of a BovineSNP50 lethal haplotype using sequence data. Homozygous inconsistency rates are calculated pairwise amongst predicted carriers, sire-son pairs and randomly sampled animals (not 1st degree relatives). Two different regions are compared: within the tested chromosome 29 haplotype and across the entirety of chromosome 29. The discordance rate among of pairs (shown in red) that contain one animal with low coverage (<10x) is greatly elevated.