# Proposed Strategy for Selection Against Recessive Genetic Defects Through a Combination of Inbreeding and DNA Markers

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## Introduction

Over the past few years, several genetic defects have reached substantial frequencies in major breeds of beef cattle and have heightened the awareness of cattle breeders of serious recessive defects. Now it is practical to develop diagnostic tests for these defects relatively rapidly because of improvements in genomics technology. However, most of the recent emphasis on DNA testing in beef cattle has focused on improvement of quantitative traits through selection with essentially no emphasis on development of a systematic approach to identify and eliminate genetic defects before they become serious problems. Planned inbreeding could be a useful tool for accomplishing this objective.

Two of the processes for improving livestock populations recognized by pioneers in animal breeding were selection and mating systems. In recent decades, almost all of the attention has focused on selection. Recent research on mating systems tends to focus on methods of minimizing inbreeding or optimizing the balance between selection and inbreeding with the assumption that inbreeding is undesirable.

Jay Lush (1973), Gordon Dickerson (1973), and others recognized a number of theoretical advantages of inbreeding and put considerable effort into designing mating systems that utilized inbreeding effectively. A number of inbreeding experiments were conducted in livestock from the 1930s through the 1960s and linebreeding was practiced to a considerable extent, particularly within the Hereford breed of cattle (Brinks and Knapp, 1975). Without the benefit of today's technology, the general conclusion of these early experiences in inbreeding livestock was that the negative consequences of inbreeding were too great and the efficiency of overcoming these consequences through progeny testing was too low to make inbreeding feasible, particularly for species with low reproductive rates and long generation intervals. In the 1950s and 1960s, recessive genetic defects had major effects on the beef seedstock industry. From the 1960s to the 1980s, the emphasis in livestock mating systems research shifted to the use of heterozygosity to counteract the effects of inbreeding. In the 1970s and 1980s, the use of crossbreeding increased rapidly and in the 1990s, composite breeding systems gained favor. In the 2000s, the beef industry seems to be returning to straightbreeding. A potential consequence is that recessive defects may again increase in impact on commercial beef production.

For decades, the beef industry has asked geneticists for tools that could make beef cattle less variable and more genetically consistent. The usual answer from geneticists has been that (aside from cloning, which has it own set of problems), the only means to improve consistency would require levels of inbreeding that are far above what is economically feasible.

Much of the variation in livestock may be due to individuals falling below the normal range of phenotypes. Our hypothesis is that many of these nonconformities are due to recessive genetic defects. Most of these defects are likely to be sub-clinical: they are severe enough to result in production losses, but not severe enough to take the

animals out of the normal production chain. Most of these probably occur at low frequencies within one or a few breeds, but there may be a very large number of them and their total economic impact on the beef industry is likely to be quite large. The theory that much genetic variation is caused by very many rare recessive defects is gaining support in the field of human genetics (Goldstein, 2009), although it is still the subject of debate.

Recessive defects that occur at low frequency individually contribute very little to additive genetic variance and as a result are not selected against efficiently. Because these defects are clustered within breeds, they are less likely to occur in crossbred animals than in purebreds; this may be a primary cause of heterosis and also for the observation that variances in composite populations are typically less than or equal to variances in the purebred populations from which the composites were derived (Gregory et al., 1999).

Occasionally, a rare recessive defect carried by an influential sire will increase in frequency either due to chance or because carriers or the sire line have some favorable characteristic. Because breeders generally avoid mating of close relatives, such a defect may go unnoticed until it reaches a relatively high frequency and then suddenly causes a crisis.

We propose a combination of a mating system and a selection scheme that could make it practical for livestock breeders to systematically reduce recessive defects and resulting nonconformities. This scheme could result in less variation and increased performance. Because these defects would be recessive, it would only be necessary to eliminate them from one side of the pedigree, so the benefit would be immediate. Furthermore, the removal of many of the recessive defects (along with a system to remove remaining ones as they are uncovered) could make it feasible to produce highly inbred lines of cattle, from which somewhat more uniform groups of commercial cattle could be produced. The basic DNA marker technology to map and track the defects that would be identified currently exists, but new types of DNA testing services would be required to implement the strategy effectively.

**Proposed Strategy**

The basic idea is to eliminate recessive defects from the population by identifying them in the influential sires in the population and select against them in the descendants of these sires. Once this has been done, the defects should vanish rapidly in the elite segment of the population and the occurrence of defects in the whole population will begin to diminish steadily. This approach has been used effectively in the dairy industry to eliminate defects (e.g., BLAD) that were at high enough frequencies to be recognized as problems and for which DNA or physiological tests for carriers were available.

The approach currently in use for reducing genetic defects applies only to those (such as curly calf, marble bone, and dwarfism) recognized as being important problems within the population. It requires considerable research and development expenditure to identify either the causative mutation(s) or markers in close enough association with them to test individuals throughout the breed without the need for pedigree analysis. Then, candidates to become elite sires in the breed are tested for the defect until it reaches a low frequency and is considered unimportant. Testing sire candidates could be a considerable expense.

The proposed strategy is to breed young sires identified for progeny testing to enough females to produce 25 to 50 progeny. Based on their progeny performance, those sires expected to be used extensively in AI will be selected to produce inbred progeny, by breeding those sires to their daughters to produce an average of 16 to 32 inbred progeny. This will result in an average of two to four affected progeny for any recessive defect carried by their sire because 1/8 of progeny from sire-daughter matings are expected to be homozygous recessive for each allele.

This system should detect defects that would otherwise be unlikely to be detected. It begins with systematic inbreeding to influential sires to uncover their recessive alleles. This step is needed to eliminate most of the recessive defects in the population. However, sires carrying recessive defects are not discarded. In fact, considering sub-clinical defects, there may be few sires in the current population that carry no recessive defects. Instead, moderate selection is applied to the relatives of the sires in which defects are discovered. Affected progeny are used to identify DNA markers flanking the defect.

Simulation results show that two or three affected progeny out of an inbred family of 16 should be sufficient to map a recessive defect to a small enough region of the genome to make it practical to select against the defect in descendants and collateral relatives of the sire in which it was mapped. For example, on average, sire-daughter matings producing 2 affected and 14 unaffected progeny would provide sufficient information to map the defect to an average of about 3.2 distinct regions comprising about 1.1% of the genome. On average, three affected and 13 unaffected progeny are sufficient to map a defect to an average of about 1.6 regions totaling about 0.6% of the genome. These genomic regions are few enough in number and small enough in length to respond rapidly to marker-assisted selection. A method that accommodates ambiguity in a manner analogous to whole genome selection (Meuwissen *et al*., 2001) could generate a selection tool incorporating marker data from multiple chromosomal regions.

Mapping efficiency increases with the number of inbred progeny produced. If cost were not an issue, it would be possible to design mating strategies that would precisely map a very high percentage of the defects in the elite sires in one breeding season. However, this is not necessary and even if it was successful, the next generation of sires would still need to be tested to find the new set of defects introduced by their dams. It would be considerably more efficient to test less stringently and spread the cost over more sires and more generations. After applying the strategy for several generations, only a small proportion of the original recessive defects would remain.

The optimum number of inbred progeny to produce is currently unknown. There is a trade-off between mapping efficiency and cost. The number of inbred animals produced per sire to be tested should be sufficient to have a reasonable probability of producing at least two affected progeny for any recessive defect that the sire carries. An expected value of more than about four affected progeny per defect seems to add more expense than it does benefit, assuming that the breeder incurs substantial costs with larger numbers of sire-daughter matings. This suggests that the optimal number may be between 16 and 32 inbred progeny, but it could be higher if the cost of additionally sire-daughter matings is not great. However, there are a number of variations on the basic mating plan that could reduce the number of sire-daughter matings required.

The inbred progeny that would be produced by this system should be viewed as valuable contributors to the next generation of the herd, not as byproducts of little value. There would undoubtedly be challenges in marketing them, but perhaps also great opportunities for innovative and skilled marketers. Inbred animals that do not express defects are actually less likely, on average, to carry recessive defects than outbred animals. The progeny of these inbred animals would be slightly less variable than the progeny of outbred cattle. However, there would be greater genetic variation among the inbred cattle; on the surface, this may seem undesirable, but it actually increases the likelihood of finding a star. The difference in value between a good bull and a great one is usually much larger than the difference in value between a cull and a really bad cull.

Furthermore, the inbred progeny would have already been genotyped for most of any defects carried by their sire and 75% of the inbred progeny's germplasm would have been inherited from the sire. Therefore, the risk of undiscovered defects would be much lower in the inbred progeny than in outbred cattle and any defects that had already been discovered could be managed through appropriate matings.

Therefore, after progeny testing, the best of the inbred bulls that were generated through one generation of sire-daughter progeny testing could be ideal candidates for being tested in the same way themselves. Testing in successive generations would greatly increase the mapping efficiency. Any defects that were not adequately mapped in the previous generation would almost certainly be well-mapped in the next generation. Moreover, assuming selection against defects had occurred, the son would be expected to have only a fraction as many newly discovered defects as his sire. Continuing this process over several successive generations should produce bulls in which virtually all readily identifiable recessive defects were discovered.

Traditional progeny testing programs for recessive defects in cattle have typically been based on sire-daughter matings. In some breeds, 35 such progeny with none affected (corresponding to a 99% probability of detection) are required in order for a sire to be considered "free of defects".

Under traditional progeny testing programs, a sire could not be evaluated until he was old enough to have produced grandprogeny. Therefore, selection could take either of two forms: increase the use of sires that tested clean late in their lives or breed each of the sires tested to some elite females and then use family selection to eliminate grandsons of bulls discovered to have a defect from consideration for use as sires. The former would increase generation interval considerably, but the latter would cause the 75% of grandsons that did not inherit the observed defects to be culled unnecessarily. DNA testing will make a third alternative possible: identify which of the sons or grandsons of a progeny-tested sire actually inherited a defect from him and which did not. This will make it unnecessary to choose between the two alternatives described above.

DNA testing makes it unnecessary to cull all potential sires which are carriers of defects. Obviously, the intent is to eliminate the defects and that implies selection. But, if an individual is otherwise outstanding, there is no need to cull it only for a genetic defect. Instead, genetic defects become simply additional traits to be considered in the selection and mating schemes. Selection against the defects may also be applied in collateral relatives of the sire in which they were identified and mapped. Matings can be planned to reduce the likelihood of affected progeny. This approach should not affect

generation interval substantially because selection is applied to descendants of the sire in which the defect is discovered instead of to the sire himself.

This approach would require a new class of DNA testing services that are distinctly different from the services currently offered by DNA testing companies offering products to the beef industry. The Illumina BovineSNP50 BeadChip[1] (**50K**) provides a powerful tool for identifying markers flanking defects from affected progeny. However, it will probably not be cost effective to run the 50K chip across all of the unaffected progeny. There likely would be utility in four to eight sets of 384 SNP that, collectively, comprised an evenly spaced set of the most highly informative SNP on the 50K chip.

However, it seems inevitable that, at some point, some level of customization of marker sets would be more efficient than off-the-shelf marker sets. There would be a trade-off between the total amount of lab work to be performed and the amount of customization that could be accommodated in the work flow. There would be great value in a genotyping platform with low initial cost and high reliability of setting up new assays.

After narrowing down the part of the genome in which the defect could reside, the next step would be to develop a multiplexed assay (comprised of SNP flanking each defect) for use in tests to allow selection of descendants of the sire.

## Discussion

Although it should be obvious that breeds and the beef industry might benefit greatly, individual breeders will need a considerable incentive in order to bear the expense and risk of putting their best sires through this process. We propose that these sires could be given a special designation such as “Tested for genetic defects”.

This designation and logo could be applied as soon as the matings to daughters were completed; they would not require that the progeny be born. The designation would not imply that the sire was free of defects, just that he had been through the testing process. The process should be structured to encourage sire owners to report any defects, no matter how sub-clinical. The sire owners’ greatest concern should be that someone else would find and map a defect in a descendant of his sire, trace it back to the sire, and realize that the defect should have been identified in an earlier generation.

The process would work best if breed associations took an active role in it. They should be responsible for issuing the designation “Tested for genetic defects” and assuring that the requirements for it were met on schedule and all results reported. They should probably require and store digital photographs (and perhaps blood samples) of all inbred progeny produced.

The benefits and ease of use of this approach would increase substantially if the process ever became standard operating procedure for sires that were to become highly influential in their breeds. For example, 16 to 32 inbred progeny will not always be enough to map a defect to a single region of the genome, but if the same defect is observed in the inbred progeny of a closely related sire, the two families combined

[1] Reference herein to any specific commercial products by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government, and shall not be used for advertising or product endorsement purposes.

should almost always be sufficient to map it to a single region. In fact, even unaffected inbred progeny of closely related sires can refine the mapping of a defect. Furthermore, if a defect is mapped to multiple regions of the genome in one sire, and a similar defect is mapped in an unrelated sire, it would be reasonable to concentrate selection on the region(s) that potentially contain the defect in both sires.

This approach could rejuvenate the “art” of livestock breeding and visual inspection. The success of the method depends on the correct identification of recessive defects and the correct identification of individuals that are expressing those defects. In many cases, this will be done most effectively by visual inspection. For severe defects, it will not be especially challenging. But, the greatest long term benefit of the system is more likely the removal of sub-clinical defects and this may be quite challenging. Ideally, it would involve penning all of the inbred descendants of a particular sire together and looking for small sets of individuals that share anomalies, structural unsoundnesses, or causes of unthriftiness that appear to have a common physiological basis. The real benefit of the system is likely to be the removal of these subclinical defects whose identification may be quite challenging. For this reason, the system will be most effective if as many as are practical of the inbred progeny of a particular sire are contemporaries in time, location, and management.

It seems likely that the subclinical defects uncovered by sire-daughter mating could be similar to many of the defects that caused many of the inbred lines discussed in Brinks and Knapp (1975) to become non-viable.

Breeders would need to develop the skill of identifying sub-clinical defects, but this skill would be built upon skills that successful breeders already possess: an understanding of Mendelian genetics, visual inspection, and a detailed memory of individuals produced in previous generations. This latter skill would become particularly important after the system had been applied for several generations.

As breeders began to examine inbred progeny grouped by sire, it is likely that they would see minor abnormalities that had occurred sporadically in their herds for many years, but which were never previously recognized as recessive defects because of the population structure in which they occurred. In many cases, it would not be possible to determine unambiguously whether a particular anomaly was a recessive genetic defect or not. However, if the same anomaly appeared again in a closely related sire, combining the two families might make it completely clear that the anomaly was recessive.

The efficiency of mapping would decrease substantially if it was not clear which inbred progeny were affected and which were not. The numbers of inbred progeny discussed previously would likely be insufficient in this case. However, testing a network of related sires would likely make it feasible to identify and map ambiguous defects.

Undesirable recessive genes that reduce performance for economically important traits without any visually distinguishable characteristics will be much more difficult to identify correctly and, consequently, much more difficult to map. Furthermore, interactions between different genes will undoubtedly mask the expression of some recessive genes, which will greatly complicate the identification and mapping of such genes. Nonetheless, sire-daughter matings may be a useful tool in managing these potentially important sources of variation through quantitative approaches. If the proposed strategy became common for highly influential sires, it might be possible to

estimate sire-specific inbreeding depression as part of the genetic evaluation process. Selection against it might improve average performance and uniformity of non-inbred animals. Furthermore, if this practice became common, it would be very useful to account for inbreeding depression in the models used to compute expected progeny differences (**EPD**).

If the proposed system is widely practiced for several generations, it should become common that most of the sires in a young sire's pedigree will have already been tested. At this point, there should be few surprises when the young sire is tested. If sire-daughter progeny testing becomes common enough, this should allow focusing on progressively less obvious sub-clinical defects.

The identification and mapping of embryonic lethal defects could be one of the greatest opportunities for improving the reproductive performance of cattle, although its potential is currently unknown. Mapping these defects is quite challenging because it is generally not practical to obtain DNA from the affected embryos that contain most of the mapping power. However, there is hope that by combining inbred progeny of several closely related sires, they might be mapped. There would be additional opportunity if the inbred progeny were produced by embryo transfer and the live embryos could be sampled for DNA prior to transfer. Obviously, genotyping degenerate embryos during ET may be a practical way to identify and map recessive defects that manifest themselves visibly in embryos by day seven.

One of the greatest impediments to applying the technology described herein would be the need to re-educate breeders to understand genetic defects in the proper context. Breeders get highly emotional about genetic defects. A valuable sire can become almost worthless overnight if he is found to be a carrier of a genetic defect, especially if no DNA test is available for the defect.

Breeders would need to learn to accept the fact that most animals probably carry recessive alleles with effects that are undesirable, to some extent. It is not a matter of "clean" vs. "dirty" pedigrees, but rather a matter of degree. Genetic defects should be managed, together with other economically important traits, as part of a balanced selection and mating program. Perhaps, they should even be included in selection indices. Development of indices would require some additional theoretical development as the weights on different defects in the selection indices should change as selection decreased their frequencies in the overall population.

The economic consequences of genetic defects are real, but they are caused at least as much by emotions as by direct losses in the commercial beef industry. The greatest costs of genetic defects are probably caused by people's overreaction to them.

## Conclusions

We do not know how many defects of various levels of severity would be uncovered by sire-daughter matings of current influential sires. Current DNA testing technology would make it feasible to map any defects that were discovered, provided that sufficient clearly affected progeny were available. It seems clear that, given sufficient investment, this approach could greatly reduce the frequency of severe genetic defects. The potential for the more ambitious benefits such as quantitative selection and reduction of embryonic mortality are enticing but completely speculative.

The systematic reduction of recessive defects through planned inbreeding may be an opportunity to use DNA testing technology in a way that has not yet been

exploited. In addition to reducing the occurrence of lethal and other very severe recessive defects, this approach might improve uniformity and average performance, especially in straightbred production. However, it would require a coordinated effort among elite seedstock breeders, breed associations, and DNA testing companies and would require each of these groups to adopt some new paradigms. If applied only to a few very elite sires, the impact of this approach would be marginal. But, if it became a "rite of passage" that AI sires were expected to go through before becoming highly influential, it could have a very positive impact on the beef industry.

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