Use of Marker-Assisted Selection in Babcock Genetics Programs

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Introduction

This paper will review the experiences that Babcock Genetics, Inc. has had in the area of marker-assisted selection. It has always been the goal of Babcock Genetics, Inc. to provide our customers with genetics to allow them to be least cost producers of premium quality products. Initially that definition meant lean gain or lean gain efficiency. Then in 1992 Babcock became a leader in applying molecular genetics to solve meat quality problems by using the HAL-1843™ gene test. Babcock Genetics, Inc. was the first breeding company in 1994 to have all nucleus and multiplier herds guaranteed free of the HAL-1843 gene. Microsatellite markers for lean growth including Gene Markers 3, 7, 11, 13 and 17 followed. Later the RN^- gene test and various SNP's including Gene Markers 19, 23 and 27 followed. This paper will begin with a brief review of our work in the 1990's, followed by a more extensive discussion from the year 2000 and beyond. The main emphasis will be on our meat quality work.

Overview of Babcock Genetics, Inc.

Babcock Genetics, Inc. maintains a closed nucleus herd located north of La Crosse, Wisconsin in an isolated area. Today's Babcock nucleus program is based on 2 maternal lines and 5 sire lines. Two sire lines were derived from a common ancestral Hampshire line and a common ancestral Duroc line. The three remaining sire lines are Duroc synthetic lines and share a common ancestral Duroc line.

The first maternal line is labeled as Landrace synthetic. It is composed of American Landrace, European Landrace and Chester White in declining proportions. The second maternal line is labeled as Yorkshire synthetic. It is composed of American Yorkshire, Large White and a maternal Hampshire line again in declining proportions. The Hampshire contribution was quite small - less than 5%. At the time the initial RN^- tests were completed, a line labeled Hampshire synthetic existed with 80% Hampshire and 20% Duroc ancestry. The line labeled Duroc synthetic is the result of pooling the three Duroc synthetic lines.

The Babcock breeding program for customers is based on a 2-breed rotational replacement gilt program utilizing the Landrace and Yorkshire synthetic lines. Only gilts are mated to the maternal semen and all sows are mated to terminal boars. At present we have three basic programs for our domestic customers, all of which use semen from Duroc synthetic lines. One of these Duroc lines is all black in color, more highly marbling and is regularly exported to Japan. Two Duroc-Hampshire synthetics also exist but are currently in limited use in niche markets.

Early Marker Assisted Selection

Babcock Genetics began marker-assisted selection in 1992 with the use of the HAL-1843 DNA test. The HAL-1843 gene was associated with PSS (Porcine Stress Syndrome) as well as PSE meat (Pale Soft and Exudative). The HAL-1843 gene was having a tremendously negative economic impact in the pork industry and was associated with death loss and poor meat quality. Through testing and population of new multipliers, all Babcock nucleus and multiplier herds were certified free of the undesirable allele of the HAL-1843 gene in 1994. Somewhat surprising to Babcock research staff was that the HAL-1843 undesirable allele was only found in one Babcock line (a Duroc synthetic) and crossbreds from that line. Although animal selection had been practiced using Halothane gas, the low frequency at which the HAL-1843 gene was found was of great interest to Babcock. Because of this low frequency of the HAL-1843 gene, the positive impact of its elimination was probably minimal for our customer base.

The balance of the 1990's was spent in additional basic research projects. This included no-strings-attached gifts to various university programs, financial support to specific projects such as the collaborative Iowa State University – University of Illinois project, which was discussed earlier at this conference. There have also been additional projects specifically involving Babcock Genetics' lines. These Babcock projects have contributed greatly to the proprietary Gene Marker tests previously mentioned.

A lean growth microsatellite marker project was begun in mid 1999. The project (paternal half-sib) was begun to find genetic markers linked to QTL for lean growth. The goal was to further improve one of Babcock's terminal sire lines. Table 1 summarizes the microsatellite markers selected from this project. Markers 3 and 13 are highly significant, consistent across sire families and have an allelic substitution effect at or greater than one standard deviation. Marker 7 has only 2 alleles whereas the others have more than 2 alleles. These markers and others are or will be undergoing further fine mapping.

Table 1: Microsatellite Markers from Lean Growth Project

Marker	Trait	P value	Good Allele Frequency	Allelic Substitution Effect
Marker 3	Average Backfat	0.0038	0.11	-0.15 inches
Marker 7	Loin Muscle Area	0.0413	0.73	0.05 square inches
Marker 11	Loin Muscle Area	0.0123	0.11	0.11 square inches
Marker 13	Days to Market	0.0008	0.86	-11.6 days to market
Marker 17	Days to Market	0.0331	0.11	-10.1 days to market

RN^- Marker Assisted Selection

In 2000 we began utilizing the RN^- gene test in all Babcock pure lines. Table 2 shows the initial gene frequencies of the RN^- allele in each of the two maternal lines, the ancestral Hampshire synthetic and three pooled Duroc synthetic lines.

Table 2: Initial Gene Frequency of RN^- Allele by Line

Babcock Line	Frequency Found	Frequency Expected[1]	Difference
Landrace Synthetic	0.09	0.00	0.09
Yorkshire Synthetic	0.38	0.03 - 0.04	0.34
Hampshire Synthetic	0.70	0.48 - 0.56	0.18
Duroc Synthetics	0.17	0.12 - 0.14	0.04

[1]Expectation based on reported results by ancestral breed frequencies known at that time and Babcock line composition

All lines were found to have frequencies higher than expected. Prior to 2000, published research was based on tests for glycolytic potential (GP) and not gene markers. Researchers assumed that this work indicated only the Hampshire breed possessed the RN^- allele and any high GP animals in other breeds were often ignored.

The two major surprises were the Landrace synthetic and the Yorkshire synthetic. The Landrace synthetic is an all white animal with all ancestral breeds being white. Admittedly a Hampshire boar or boars could have sometime in the last 40 years "jumped the fence" either at Babcock or at a source of sows or semen. However, that possibility is no more likely to have happened to Babcock than to anyone else. The question becomes "Is Babcock's assumption of little or no Hampshire influence correct or is the industry's assumption of a RN^- free Landrace breed correct?" I suspect the idea that the Landrace breed is not as RN^- free as is assumed contributes to this discrepancy.

The results of the Yorkshire synthetic were even more surprising. The frequency of the RN^- allele was 10 times what was expected. That line does have approximately 5% Hampshire genetics in its ancestry and that explains 3-4% of the frequency. This discrepancy is even more unlikely than the Landrace discrepancy.

A literature review has shown additional information. Previous research showed pigs in the high GP range in both Landrace and Yorkshire breeds with RN^- allele frequencies of 0.045 and 0.017 respectively (Enfält *et al*. 1997). Similarly research by Stoller *et al*. (2003) showed RN- allele frequencies based on DNA testing to be 0.033 for a line of purebred Berkshires, 0.037 for a line of purebred Durocs and 0.071 for a high-lean, terminal commercial crossbred line based on a cross of Landrace-Yorkshire females and a synthetic sire line with no Hampshire ancestry. This helps explain discrepancies in allelic frequencies for both the Landrace and Duroc synthetic lines but provide little information in understanding the extent of the Yorkshire synthetic discrepancy.

In spite of the unexpected frequencies found, especially in the Yorkshire synthetic, by November 2000 Babcock was delivering domestically only RN^- free boars of all lines. The use of the Hampshire synthetic line had been discontinued prior to RN^- gene testing. All Babcock controlled boar studs were essentially free of the RN^- gene by July of 2001.

Table 3 shows the allelic substitution effects as measured by the RN^-/rn^+ genotype minus the rn^+/rn^+ normal genotype. The first data column lists substitution effects for all Babcock synthetic lines pooled except the Hampshire synthetic. No Babcock Hampshire synthetic pigs are included in the analysis because so few rn^+/rn^+ pigs of that line had been slaughtered at the time this data was generated. Line by allele interactions were both significant for ultimate ham pH but not for ultimate loin pH. Line by allele interactions were significant for L*, b* and drip loss but allele was not a significant source of variation for these three traits. The final three columns list allelic substitution effects taken from the literature for purebred Hampshire and Hampshire sired crossbred pigs.

Table 3: The Allelic Substitution Effects of the RN^- Allele (RN^-/rn^+ minus rn^+/rn^+)

Synthetic Line	All Lines Pooled Except Hampshire	Hampshire	Hampshire	Hampshire
Trait	Babcock	Literature[1]	Literature[2]	Literature[3]
pH_μ ham	-0.110**	N/A		
pH_μ loin	-0.084**	-.26***	-.12 / -.22***	-0.07***
Color[4]	0.037	-0.75*		-0.25
Firmness[4]	-0.146	-0.43		-0.35**
Marbling[4]	-0.157*	-0.58		-0.14
L*	0.551	4.21***	1.4 / 4.9***	1.09*
a*	0.339***	-0.04		
b*	0.413	1.75***		
Drip loss %	1.64	2.35***		0.22

[1]Hamilton *et al*. 2000
[2]Ellis *et al*. 1998 presented results of several experiments
[3]Ohio State, 2001
[4]NPPC 1-6 subjective scores
L*, a*, b* measurements taken with various equipment including Minolta and CIELAB
* Significance <0.05
** Significance <0.01
*** Significance <0.001

Current Proprietary SNP Work

Prior to the utilization of the RN^- gene test, Babcock began a project searching for various meat quality markers. In 2003 we confirmed from our data the existence of a single nucleotide polymorphism, which we have designated as Marker 19. This SNP is segregating in all Babcock lines. Refer to Table 4 for results of Marker 19. Early measurements indicated a higher effect than contained in Table 4. Additional data is being accumulated as Marker 19 is in use. Response differences between the maternal and paternal lines are of particular interest.

Table 4: Allelic Frequency and Substitution Effects of Marker 19

Line	Good Allele	Allelic Substitution Effects			
	Frequency	Loin pH	Ham pH	Drip Loss	Color[1]
Landrace synthetic	0.15	0.04		-0.2%	0.32*
Yorkshire synthetic	0.14	0.04			0.48
Hampshire-Duroc synthetics	0.77	0.09	N/A	N/A	N/A
Duroc synthetics	0.38		0.04	-1.2%	
All lines	0.24		0.04	-0.7%	0.25*

[1]NPPC 1-6 subjective scores
*significance <0.05
If * is not present, significance <0.20
N/A trait not measured

Gene Marker 27 is another SNP verified as a result of the same project as Gene Marker 19. The SNP was segregating only in the Landrace synthetic line. The desirable allele was found to be fixed in all other lines.

Table 5: Allelic Frequency and Substitution Effects of Marker 27

Line	Good Allele	Allelic Substitution Effects			
	Frequency	Loin pH	Ham pH	Drip Loss	Color[1]
Landrace synthetic	0.40	0.04	0.11	-0.63%	0.07
Significance		0.23	0.02	0.09	0.18

[1]NPPC 1-6 subjective scores

Marker 23 is the final marker to be discussed in this paper. It is also a SNP and has a significant (<0.05) affect on number born alive in only the Yorkshire synthetic line. These are preliminary results. We have genotyped several hundred gilts and hope to have several hundred sows genotyped prior to the meeting and will present allelic substitution effects at that time.

Future Work

We need not be reminded that gene markers are most appropriate for those traits difficult to measure in the live animal. That is why we have emphasized meat quality in much of our previous and current work. The emphasis on meat quality will continue but will not necessarily target the same traits. Traits such as tenderness and juiciness are still important to a natural (non-injected) product. Disease markers will be considered as well as additional reproductive traits. Lean growth is not likely to be emphasized in the search for gene markers but markers for feed efficiency certainly could be.

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