Use of Molecular Markers to Determine Parentage in Multiple Sire Pastures

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Summary

The purpose of this research was to determine the parentage of animals generated by multiple sire technique. Test of paternity was performed on 63 Angus animals, belonging to 29 families within the Rhodes breeding project, using multiplexed microsatellites. Paternity was determined for 23 families. One of the two possible sires was excluded with multiple markers for 19 families and with a single marker for four families. One of the possible sires was excluded for two families but DNA was not available for the other possible sire. Thus it wasn't possible to confirm paternity. Four families had both possible sires excluded. In these six cases, it's necessary to collect blood again and redo the paternity test in order to confirm the results, especially if it's an important animal to be registered. This study demonstrated the importance of performing a paternity test in breeding populations in order to reach the maximum expected annual genetic gain especially for herds that employ multiple sires.

Introduction

Many national cattle evaluation programs use mixed models to compute animal expected progeny differences (EPDs) for economically important traits. These methodologies incorporate pedigree or relationship matrix information in order to improve the accuracy of genetic prediction. Therefore, errors in paternity have a detrimental effect on populational genetic parameter estimations as well as on individual animal EPDs predictions. Identification errors can be as high as 20% of animals registered in various countries, which drastically reduces the annual genetic gain with in a population (Geldermann et al., 1986; Beechinor & Kelly, 1987; Ron et al., 1995).

The relationship between individuals can be determined by paternity test, which can be performed with various genetic and molecular markers, including blood type, biochemical, RFLP, DNA-fingerprinting and microsatellites. Microsatellites are codominant and highly polymorphic even in endogamic populations. Thus, these characteristics make it possible to determine the origin of the parental alleles there by accurately identify parentage errors.

Tests of paternity are necessary in genetic breeding programs under various situations, such as verifying the validity of breeder provided information, and identifying the paternity of animals generated through multiple-sire pasture mating. Therefore, the objective of this report was to illustrate the feasibility of using parentage tests to correctly identify animals generated by multiple sire mating.

Material and Methods

The current study included 63 young Angus bulls and heifers from 29 families. These bulls and heifers were part of the Iowa State University beef cattle breeding project. This project is designed to develop two lines, one selected primarily to increase retail product yield and the other to improve meat quality. Mature cows and replacement heifers were bred artificially with semen from industry sires and young bulls raised on the farm. Recycling Females were inseminated a second time whenever identified by farm personnel. Following pregnancy diagnosis pregnant and open cows were run with cleanup bulls in multi-sire pens.

Traditionally parental identification has been determined based on the comparison between the calculated conception date, which is back calculated based on birth date and the AI date. In some cases, it's not possible to determine paternity because the calculated conception date lies in between the AI date and possible natural mating date. For those cases a test of paternity is recommended in order to avoid misidentification.

Animals were tested for paternity using microsatellites markers. Genomic DNA was purified from blood samples and the genotypes were determined. The genotypes were used to calculate the number of alleles, frequencies and heterozygosity for each marker as well as the probability of paternity for each animal (Dodds et al., 1996).

A parentage test was performed by comparing the offspring and parents genotypes in order to verify the compatibility between the genotypes. Each animal has two alleles; one should be inherited from the sire and the other from the cow. If neither of the two offspring's alleles for a single marker were present in the possible sire that animal were cannot be the sire. This is called single marker exclusion (SME). Exclusion based on more than one marker is considered multiple marker exclusion (MME). Even if no exclusion were found after testing multiple markers, there is still a possibility of genotypic compatibility by chance between two unrelated animals. Thus, the probability that a given sire is the father of a given offspring should be greater than 99%. The probability of paternity is calculated based on the frequencies of alleles inherited from the sire. This

means, the lower the frequency of the allele inherited from the sire, the lower is the probability of having the genotypes compatible by chance, and therefore the higher the probability of paternity.

Results And Discussion

Calves were genotyped for 15 markers with an average of 4.9 alleles per marker and an average heterozygosity of 61.26%. The number of alleles and heterozygosity for each marker are presented in Table 1.

By comparing genotypes, we were able to exclude one of the two possible sires with more than one marker (MME) for 19 animals. This is the desired situation and there is no doubt about the true sire. A single marker exclusion (SME) was obtained for four families. This could be due to a new mutation. However, this is a very rare event and once the other potential sire was not excluded we can assume it was the sire. These four families were tested without DNA from the dams. In these cases it was not possible to determine the maternal allele so the sires were evaluated for presence of the two calf alleles, which is less informative and therefore the probability exclusion is lower. Among the 19 families with MME, only four did not have the dam's DNA, again demonstrating the importance of having the dam's DNA to perform the test of paternity. However, the Dam's DNA is not absolutely required (Table 2).

The genotype for animal 1256 was compared to only two of the three possible sires, because DNA was not available for sire N1525. Sires 9027 and 9050 were excluded, so the only possible sire is N1525, but it was not analyzed, thus a definitive determination of parentage was not possible. A similar situation existed for calf 1268. Sire 8102 was analyzed and was not excluded, but the N1444 was not analyzed. The other four families had both possible sires excluded. This could be due to errors during blood collection, tube labeling, data analysis or animal misidentification in the farm. In these cases, it was important to collect blood again and redo the paternity test, especially if it was an important animal. The probability of paternity was calculated for the sires that were not excluded. Probabilities greater than 99% were reached for the majority of the cases when the maternal DNA was available, ranging between 95.6% and 99.96%, demonstrating again the benefits of including the cow DNA in the test. Probabilities ranged from 67.04 to 94.3% when no cow DNA was available for analysis (Table 2).

Examples of single or double exclusions or in which both sires were excluded are presented in Figure 1 and Table 3. Family A is an example of MME, in which animal 9111 was excluded as the possible sire with markers BM434, allele 120, and BM757, alleles 191 and 193. Family B had MME (markers BM6507, allele158, BM2809, allele 160, and BM757, allele191) for both possible sires. Finally, an example of SME was obtained for the family C with marker BM434, alleles 110 and120.

Implications

The results presented here demonstrate a need to routinely determine parentage in breeding programs in order to avoid misidentification. This will maximize the annual genetic gain. The animals with uncertain parentage, generated by multiple sire technique for example, should be tested for paternity in order to be included in the relationship matrix and generate better EPD predictions and populational parameter estimations.

Table 1: Microsatellites marks, Multiplex information, Number of Alleles and Heterozygosity.

Marker	BMS	BMS	BM	BM	HUJ	BMS	BM	CSSM	INRA	BL	ILST	BM	BP	BM	BL
Name	2142	434	2809	6507	246	1675	757	22	6	25	36	103	2	188	1036
Number of Alleles	5	5	4	6	4	4	7	5	5	4	4	6	3	5	7
Heterozygosity	0.79	0.73	0.63	0.56	0.414	0.709	0.714	0.648	0.5775	0.6278	0.579	0.5963	0.57	0.596	0.448

Table 2: Test of paternity results.

Calf ID	Dam ID	Tested Sires ID			True Sire	Probability	Type of exclusion
244	8229	9111	9156		9111	99.94	MME
1264	6003	75A	N0713		N0713	94.3	MME
1265	8824	9073	9067		9607	99.11	MME
1266	6353	9073	9111		9111	67.04	MME
1280	8806	9156	9111		9111	98.9	MME
1284	8088	9009	9027		9027	98.46	MME
1288	6341	9073	2325		2325	94.2	MME
1295	8844	9009	9027		9027	98.53	MME
1302	8708	9111	9156		9111	99.9	MME
1303	8249	9027	9050		9027	95.6	MME
1305	8004	9111	9156		9156	99.73	MME
1307	8897	9027	9050		9050	98.24	MME
1316	6303	9073	4437		4437	93.84	MME
1319	8283	9027	9050		9027	99.96	MME
1325	8727	9027	9050		9027	97.28	MME
1328	8732	9027	9050		9027	99.45	MME
1335	8238	9111	9156		9156	99.45	MME
1341	8836	9111	9156		9111	99.88	MME
1346	8630	9027	9050		9050	97.05	MME
1291	6048	8181	N507		N507	92.73	SME
1293	6411	9111	9073		9111	84.6	SME
1294	6231	9073	N507		N507	74.29	SME
1287	6250	8102	N9067		8102	98.64	SME
1256	8490	9027	9050	N1525	N1525 (NA)	?	MME (NA)
1268	6306	8102	N144		8102 (A)	80.89	N144 (NA)
1212	8860	9027	9050		?	0	BSE
1276	6428	9005	9009		?	0	BSE
1314	8161	9027	9050		?	0	BSE
1315	8256	9111	9156		?	0	BSE

MME = Multiple Markers Exclusions, SME = Single Marker Exclusion, BSE = Both Sires Excluded, A = Analyzed, NA = Not Analyzed

Figure 1: Test of paternity examples.

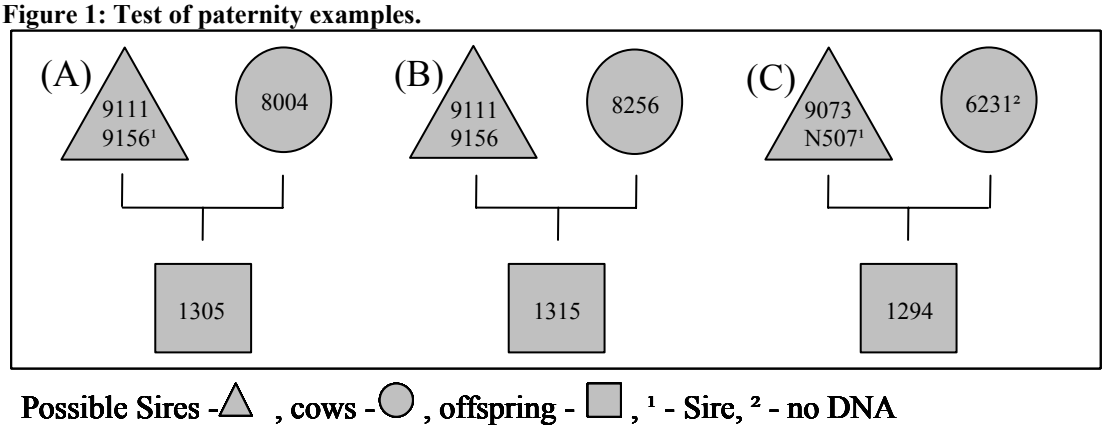

Table 3: Paternity exclusions.

Family	Marker / Animal ID	BMS2142		BMS434		BM6507		BM2809		HUJ246		BM757		CSSM22	
A	**1305**	93	107	120	120*	156	156	160	164	260	260	191*	193*	217	221
	8004	107	107	120	120	156	158	160	164	258	260	191	193	217	221
	9111	93	107	110*	114*	156	158	164	170	260	260	195*	205*	221	227
	9156	93	107	114	120	156	156	164	164	254	260	191	193	221	223
B	**1315**	93	93	114	120	156	158*	160*	170	254	260	191*	191	221	227*
	8256	85	93	114	120	156	156	164	170	254	260	191	191	217	221
	9111	93	107	110	114	156	158	164*	170*	260	260	195*	205*	221	227
	9156	93	107	114	120	156*	156*	164*	164*	254	260	191	193	221*	223*
C	**1294**	85	91	110*	120*	156	158	164	164	254	260	191	205	221	227
	9073	85	85	114*	114*	156	158	164	170	254	260	191	221	221	227
	N507	85	93	110	114	156	160	164	170	254	260	205	221	221	221

***- Exclusion allele**