Effects of cytoplasmic inheritance on preweaning traits of Hereford cattle

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Abstract

The influence of cytoplasmic inheritance on birth and weaning weight was evaluated in an experimental Hereford herd. Data on 1,720 records for birth and weaning weights from calves born between 1963 and 2002 were studied. Variance components were estimated using MTDFREML procedures and an animal model was fitted for each trait. Direct and maternal additive effects and permanent environment and maternal lineage effects were treated as random, while year and month of birth, age of dam and sex of the calf were treated as fixed. Identification of maternal lineages was based on pedigree information. The contribution to phenotypic variance of cytoplasmic lineages defined by pedigree information was negligible for both traits. Mitochondrial genotypes of cows present in the herd in 2002 were analyzed by single strand conformation polymorphism (SSCP) analysis. Only five different genotypes were identified among 23 maternal lineages. All the animals with records were assigned to maternal genotypes based on pedigree information. The statistical analysis was repeated, removing maternal lineage from the model and including mitochondrial genotype as a fixed effect. No evidence of genotype effects was detected. These results suggest a negligible effect of the mitochondrial genome on the preweaning traits of this Hereford herd.

Key words: beef cattle, Hereford, birth weight, weaning weight, cytoplasmic inheritance, SSCP.

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Introduction

The Hereford herd at the National Institute of Agricultural Technology (Instituto Nacional de Tecnología Agropecuaria, INTA) Experimental Station at Balcarce, Argentina was created in 1959 with cows bought from different private ranches and has been under selection for preweaning traits since 1986. Selection was originally based on a phenotypic index aimed at increasing relative preweaning growth rate while keeping birth weight constant (Melucci et al., 1983). More recently, the index was reformulated in order to include additive direct effects for growth rate and birth weight estimated by an animal model instead of the corresponding phenotypic values (Melucci, 1995).

Response to selection and changes in genetic parameters in this population have been monitored (Melucci and Mezzadra, 2003) but all the studies were focused on the effects of the nuclear genome and the influence of the mitochondrial genome, another potential source of genetic variation, has never been evaluated in this herd.

The mitochondrial genome has much fewer genes than the nuclear genome but a high proportion of mitochondrial genes are involved in the regulation of cellular energy metabolism (Anderson et al., 1982) and given the high number of mitochondria per cell (Alberts et al., 1994), a slight difference in the effects of these genes could have an impact in cell metabolism and consequently, could also influence the performance of economically important traits. In short, there could exist a link between economically important cattle traits and genetic variation at the level of the mitochondrial genome.

It has been assumed that the mitochondrial genome is transmitted almost exclusively through the maternal line. In most South American countries, new breeds and biotypes within breeds have been introduced through semen importation in order to upgrade the local breeds (Mirol et al., 2003). In this situation, the cytoplasmic genetic background remains fairly constant over time and it would be
worthwhile to assess the existence of more productive mitochondrial genotypes.

Several studies have addressed the influence of cytoplasmic inheritance in dairy and beef cattle, either by estimation of the contribution of maternal ancestry to total variability or by direct association between mitochondrial DNA polymorphisms and productive traits. The results of these experiments are controversial and do not confirm any advantage of one given approach (i.e. biometric or molecular) over the other. While some workers have reported a significant influence of mitochondrial genes on performance (Schutz et al., 1994; Mannen et al., 1998; Roughsedge et al., 1999; Schnitzenlehner and Essl, 1999) others have concluded that the effect of such genes is negligible (Rohrer et al., 1994; Albuquerque et al., 1998; Rorato et al., 1999) and it therefore appears that the importance of mitochondrial genome on cattle performance has not yet been fully elucidated.

Because there is some evidence suggesting a relevant role of the mitochondrial genome on cattle performance, and in order to complete the genetic characterization of the INTA Hereford herd we evaluated the influence of cytoplasmic inheritance on preweaning traits using several different strategies. In one approach we estimated the contribution of maternal lineage effects to total variance using an animal model. In another approach the identity of maternal lineages was confirmed by identifying mitochondrial DNA (mtDNA) genotypes using single strand conformation polymorphism (SSCP) analysis and correlated these genotypes with preweaning traits.

Materials and Methods

Experimental design

At the National Institute of Agricultural Technology (Instituto Nacional de Tecnología Agropecuaria, INTA) Experimental Station at Balcarce, Argentina, a herd of about 120 Hereford cows is kept on pasture throughout the year. Calving season normally runs from August to October, although in some years cows have calved in November. Calves do not have access to creep feed. Some Hereford cows are included in crossbreeding experiments each year, so in our analysis records relating to calves not sired by Hereford bulls were removed. Although most male calves are left intact, some castrated animals produced information and were included in the data set.

Maternal lineages were identified by tracing female paths to the last female ancestor in the herd. In order to provide a better estimation of variance components only those maternal lines with at least five records were considered in our analysis (Roughsedge et al., 1999). The final data set consisted of 1,720 records of birth and weaning weight linearly adjusted to 180 days of age, from calves born between 1963 and 2002 (one to nine records per cow). A total of 2,389 animals were in the pedigree file.

Molecular analysis

Blood samples were collected from most of the cows present in the herd in 2002. The DNA was extracted from whole blood according to standard procedures (Maniatis et al., 1982). Two to four cows from each of twenty-three maternal lineages were picked at random for mitochondrial DNA (mtDNA) analysis. Two different regions of the hypervariable mitochondrial displacement loop (D-Loop) were amplified by PCR. Fragment 1 corresponded to a 489 bp fragment spanning nucleotides 16,184 to 340 of the mitochondrial genome (Anderson et al., 1982) and was amplified using primers designed by Eledath and Hines (1996). Fragment 2 corresponded to the region between nucleotides 15,960 and 16,334 (375 bp) and was amplified according to Cymbron et al. (1999). The 25 µL PCR reaction mix contained 2 µL of total DNA, 0.4 µL of each primer, 0.1 mM of dNTPs and 0.8 U of Taq polymerase (Invitrogen, Carlsbad, CA, USA) in 20 mM Tris-HCl (pH 8.4), 50 mM KCl and 4 mM MgCl2, under mineral oil. The PCR protocol consisted of an initial step of 2 min at 94 °C followed by 30 cycles of 45 s at 94 °C, 60 s at 50 °C and 60 s at 72 °C, with a final elongation step of 5 min at 72 °C. Genotypes at each site were distinguished by single-strand conformation polymorphism analysis (SSCP) (Orita et al., 1989). Six microliters of each PCR productwere added to 12 µL of LIS loading dye (10% sucrose, 0.01% bromophenol blue, 0.01% xylene cyanol) and 10 µL of water. The samples were then heated at 96 °C for 10 min, cooled on ice for at least 5 min, loaded onto a 10% polyacrylamide gel (381 acrylamide/bisacrylamide) and subjected to electrophoreses at 4 °C, 200 V, in 0.5X TBE buffer for 16 h. The gels were subsequently fixed in 5% ethanol, stained with 0.2% AgNO3, and revealed with 2% CaCO3.

Statistical analyses

Maternal lineage effects can be properly accounted for with a full animal model that includes separate estimates of these effects and maternal random additive effects (Gibson et al., 1997). The data were analyzed using the MTDFREML algorithm (Boldman et al., 1995) and the animal model was used:

\[ Y = \beta + Z_{1} u_{D} + Z_{2} u_{M} + Z_{3} u_{P} + Z_{4} u_{I} + e \]

where \( Y \) is the observation vector, \( \beta \) the fixed effect vector (year and month of birth, sex of calf, age of dam), \( u_{D} \) the additive direct genetic effect vector, \( u_{M} \) the additive maternal genetic effect vectors, \( u_{P} \) the permanent environment effect vector, \( u_{I} \) the maternal line effect vector, \( e \) the residual effect vector and, \( X, Z_{1}, Z_{2}, Z_{3} \) and \( Z_{4} \) the incidence matrices that associate the appropriate effects to \( Y \). It was assumed that nuclear and cytoplasmic genetic effects were uncorrelated. Due to the size of the data set and the large number of parameters to be estimated, multivariate analysis
including birth and weaning weights was not feasible so these traits were analyzed separately.

In order to take into account molecular information relating to the mtDNA genotypes the statistical analysis described above was repeated, removing maternal lineage from the model and including mitochondrial genotype as a fixed effect. A total of 824 calves were allocated to each genotypic class based on pedigree information. Heritabilities and genetic correlations were not estimated in this second analysis.

Results

The mean birth weight was 32.3 ± 5.0 kg and the adjusted weaning weight was 151.4 ± 24.7 kg. It is worth noting that since its creation this Hereford herd had never been subjected to selection for postweaning growth rate or size, which means that adult body size and correlated growth measurements are lower than those of many commercial herds where selection has been practiced.

A total of 223 maternal lines were identified by pedigree analysis, with the largest maternal families spanning eight generations. On average there were nine records per line (range: 1 to 76 records/line) but because only those maternal lines with five or more records per line were included in the analysis the number of maternal lineages in the final data set was 89, of which only 29 were present in the herd in 2002 when the DNA samples were collected. Direct effect heritability estimates were 0.55 ± 0.10 for birth weight and 0.04 ± 0.04 for weaning weight (Table 1), agreeing with previous estimates of the genetic parameters of this herd (Melucci and Mezzadra, 2003). Estimates of maternal effect heritability were 0.28 ± 0.07 for birth weight and 0.16 ± 0.07 for weaning weight (Table 1). Genetic correlations between direct and maternal effects were negative for birth weight and very close to zero for weaning weight. Maternal lineages accounted for a minimal proportion of the phenotypic variance both for birth and weaning weight. Estimates of variance were consistent between the analyses that included maternal lineage (Table 1) or mitochondrial genotypes (Table 2), with the exception of direct effects for weaning weight.

Surprisingly, when fragment 1 was analyzed using mtDNA SSCP analysis only two genotypes were identified, one of which appeared in just five of the maternal lines sampled in 2002. To find a more informative D-Loop site a second DNA fragment upstream of the first was subjected to SSCP analysis and at this site five different genotypes were identified which we named A to E (Table 3). Seventeen out of 23 maternal lines for which we had DNA samples shared the same genotype. Combining the genotypes at both sites into haplotypes did not produce additional information due to the strong imbalance of genotype frequencies at both sites, this being the case we only considered fragment 2 genotypes in the statistical analysis.

Conflicting results were obtained for three maternal lineages in which two cows with different fragment 2 genotypes were identified, this being resolved for two of the lineages by typing at least three cows but because the third lineage was represented by only two cows it was not possible to assign it unequivocally to any genotypic class. The four cows with discrepant genotypes were not considered in the statistical analysis.

Statistical comparisons of estimated effects failed to detect any significant difference between mitochondrial genotypes for either birth or weaning weight (Table 3).

Discussion

Although our study focused on the influence of cytoplasmic inheritance on birth and weaning weights,

| Table 1 - Estimated (co)variance components and heritabilities for the analysis that included maternal lineage as a random effect for Hereford cattle birth and weaning weight. |
| --- | --- | --- |
| Effect | Birth weight (kg²) | Adjusted weaning weight (kg²) |
| Variance/covariance | | |
| Additive direct | 10.92 | 14.70 |
| Additive maternal | 5.51 | 61.68 |
| Direct-maternal | -2.29 | 0.08 |
| Maternal permanent environment | 0.00 | 78.15 |
| Maternal lineage | 0.22 | 0.00 |
| Residual | 5.59 | 222.85 |
| Phenotypic | 19.95 | 377.46 |
| Heritability/correlation | | |
| Additive direct | 0.55 ± 0.10 | 0.04 ± 0.04 |
| Additive maternal | 0.28 ± 0.07 | 0.16 ± 0.07 |
| Direct-maternal | -0.30 ± 0.12 | 0.00 ± 0.48 |
| Maternal permanent environment | 0.00 ± 0.03 | 0.21 ± 0.05 |
| Maternal lineage | 0.01 ± 0.03 | 0.00 ± 0.03 |

| Table 2 - Estimated (co) variance components for the analysis that included mitochondrial genotype as a fixed effect for Hereford cattle birth and weaning weight. |
| --- | --- | --- |
| Effect | Birth weight (kg²) | Adjusted weaning weight (kg²) |
| Additive direct | 7.84 | 0.13 |
| Additive maternal | 4.85 | 51.57 |
| Direct-maternal | -1.63 | 2.6 |
| Maternal permanent environment | 0.0002 | 53.24 |
| Residual | 6.49 | 205.02 |
| Phenotypic | 17.62 | 312.57 |
heritability was also estimated in order to assess the genetic variability linked to two traits of major interest, especially in the maternal component. Estimated heritability (direct and maternal) for weaning weight was unexpectedly low, however, a previous study based on the same population (Melucci and Mezzadra, 2002) demonstrated that the genetic trend for direct weaning weight was positive as a consequence of selection for that trait.

Many studies have attempted to quantify the contribution of the mitochondrial genome to performance in cattle. In an experiment involving beef and dairy cattle, Brown et al. (1988) studied variables related to mitochondria metabolism (i.e., oxygen consumption, ADP/O; ratio and ATP synthesis) but found very little association between mitochondrial metabolism and growth traits in beef cattle although in dairy cattle there tended to be a positive correlation between mitochondrial-related variables and performance, principally in respect to milk yield. In a similar experiment with mice, Brown et al. (1989) found no differences in growth traits related to cytoplasmic genetic effects between inbred lines, even though there were differences in mitochondrial metabolic activity. Lindberg et al. (1989) were able to detect a higher production efficiency of ATP in mammary gland mitochondria derived from a mouse line selected for high milk production as compared to a line selected for low milk production. Taken together, these results and those from Brown et al. (1988) suggest that differences in mitochondrial metabolism due to genetic variation will be detected mainly in highly energy demanding processes, such as milk production, where the ability of the mitochondria to provide ATP is challenged.

Despite the evidence provided by metabolic studies, experimental results from dairy cattle are not conclusive. Roughsdale et al. (1999) confirmed a significant effect of maternal lineage on fat yield (5% of the phenotypic variance) in a Holstein Friesian herd. Interestingly, Schnitzenlehner and Essl (1999) detected a significant maternal line contribution to variance in fitness-related traits (persistency, days open and herd life) in Austrian Simmental cattle. Analysis of a Holstein cattle data set by Albuquerque et al. (1998) concluded that the contribution of the cytoplasmic line to the total variance of milk yield was only 1.1%, to fat yield 0.8% and to percentage fat 0.9% and were too small to be relevant to genetic evaluations, a conclusion subsequently supported by the work of Rorato et al. (1999) who found that in the same data set the maternal lineage only accounted for 1.1% of the phenotypic variance of milk yield.

These significant differences in performance detected between maternal lineages have been supported by association studies between mtDNA polymorphisms and production traits (Schutz et al., 1993; Schutz et al., 1994; Boettcher et al., 1996); however, it has been pointed out that in many cases associations detected as significant corresponded to rare and probably detrimental mutations (Gibson et al., 1997).

Tess et al. (1987) reported significant cytoplasmic effects (accounting for 1% to 5% of the phenotypic variance) for preweaning traits in beef cattle in a Hereford herd but the least-squares methodology used by Tess et al. (1987) proved later not to be appropriate for that kind of analysis, in fact, reanalysis of the same data set using mixed model methodologies showed no significant maternal lineage effects (Tess and Robinson, 1990). Similar results were obtained in another population of the same breed by Tess and McNeil (1994) and also in our present survey. Rohrer et al. (1994) also failed to detect any significant maternal lineage effects on birth or weaning weights of Brangus cattle. Interestingly, a very significant mitochondrial haplotype effect on body composition and beef quality has been reported in Japanese Black cattle (Mammen et al., 1998).

Leaving aside the real influence of the mitochondrial genome on performance, the lack of significance of maternal line effects manifested in many studies could be attributed to weaknesses within the design of the studies (Gibson et al., 1997; Roughsdale et al., 2001). Although mixed model methodologies allow for an adequate partition of variance and the unbiased estimation of maternal lineage effects, such methodologies rely on pedigree records for the definition of maternal lines. Two factors could affect the

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of maternal lines</th>
<th>Number of calves</th>
<th>Genotype effects</th>
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<tr>
<td></td>
<td></td>
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<td>Birth weight</td>
</tr>
<tr>
<td>A</td>
<td>17</td>
<td>582</td>
<td>2.77</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
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<td>2.68</td>
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<td>43</td>
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<td>D</td>
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<td>2.85</td>
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<tr>
<td>E</td>
<td>1</td>
<td>68</td>
<td>2.40</td>
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1SSCP of mtDNA, positions 15,960 to 16,334.
2Maternal lines present in the herd in 2002 when DNA samples were collected.
3Number of calves allocated to each genotypic class based on pedigree information.
4Phenotypic means were 31.1 ± 4.7 kg for birth weight and 146.6 ± 23.8 kg for weaning weight.
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al statistical power of designs based on pedigree information, namely pedigree errors and incomplete pedigree information (Roughsedge et al., 2001). Simulation studies have demonstrated that pedigree errors could lead to severe underestimation of true line effects (Roughsedge et al., 2001). Misidentification rates as high as 23% have been reported for commercial populations (Ron et al., 1996). In our case, the data corresponded to an experimental herd and therefore we expected a minimal contribution of that source of error. However, the design of our study was sensitive to errors due to the possible existence of maternal subfamilies that are actually part of larger families sharing a common mitochondrial genome.

In order to evaluate the possibility of incorrect assignment of cows to mitochondrial lineages that could mask putative maternal line effects, we attempted to identify mitochondrial lineages based on direct mtDNA analysis. The first of the sites analyzed by SSCP identified only two genotypes in Hereford cows. When the same site was studied on 16 maternal lineages of the Holstein breed, six different genotypes were identified (Eledath and Hines, 1996). These results suggest that in order to identify with confidence different mitochondrial genotypes, the most informative sites should be defined on a per-breed basis. The analysis of a second site in the D-loop produced five different genotypes in the Hereford herd. Interestingly, of 23 lineages sampled in 2002, 17 shared the same genotype. This strong imbalance between genotype frequencies persisted when the original data was redistributed to these cytoplasmic lineages, supporting the hypothesis that the lack of cytoplasmic effect could be in part attributed to a much lower variability than expected, based on the number of maternal lines.

In our study we identified maternal genotypes using SSCP analysis, a very sensitive technique able to detect even single nucleotide polymorphisms. However, we could have missed some polymorphisms, because SSCP conditions were not optimized for detection of individual polymorphisms known a priori. Sequencing the entire D-Loop region would allow for a better definition of mitochondrial haplotypes and provide more statistical power to the tests (Mammen et al., 1998) but it is more expensive and time consuming.

On the other hand, it is important to consider that the mitochondrial D-Loop is not a coding sequence. Because mitochondria are inherited asexually through the maternal line, it is accepted that polymorphisms in the D-Loop tag haplotypes over the entire genome. However, polymorphism analysis at the level of the D-Loop could probably identify haplotypes that are actually monomorphic at the gene level, because the D-Loop has a much higher mutation rate than the sequence of mitochondrial genes. Therefore, grouping of maternal lines based on D-loop polymorphisms probably establishes an upper limit for the actual number of mitochondrial variants.

Using the information of mitochondrial genotypes, we grouped the maternal families into larger mitochondrial lineages under the assumption that mtDNA is strictly maternally inherited and its sequence is conserved through a maternal lineage. However, the existence of different mitochondrial genotypes within maternal lines has been previously reported (Ashley et al., 1989; Koehler et al., 1991). In fact, we found three cases of cows from the same maternal line that differed in their mitochondrial genotypes. It remains to be proved whether these were real cases of heteroplasmy or pedigree errors.

In summary, neither maternal lineage nor mitochondrial genotypes showed any significant effect on the traits analyzed in this experiment. These results suggest that in this Hereford herd the effect of the mitochondrial genome on birth and weaning weights can be considered negligible.

References


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