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**ON OBTAINING ESTIMATES OF PARENT-OF-ORIGIN EFFECTS
EFFECTIVELY AND THEIR EXPLOITATION IN ASSOCIATION
GENETIC MAPPING**

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„Das Schönste, was wir erleben können, ist das Geheimnisvolle. ...“ ALBERT EINSTEIN

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GENERAL INTRODUCTION

Motivation

Genomic imprinting is a phenomenon where the expression of genes is limited to one of the two inherited gametes (e.g., reviewed in O'Doherty *et al.*, 2015). This limitation can either be full or partial. The latter occurs when the imprinting status changes either over time or between tissues (Gould and Pfeifer, 1998; Yu *et al.*, 1998). Usually, a gene is assumed to be paternally or maternally imprinted when the paternal or maternal allele, respectively, is silenced. The effects of imprinted genes belong to the parent-of-origin effect (POE) family, which is a comprehensive term for effects appearing as phenotypic differences between heterozygotes, depending on their parental origin (Lawson *et al.*, 2013). According to Tier and Meyer (2012), the neglect of genomic imprinting in animal breeding programs could bias, e.g., breeding values and estimated genetic parameters. As described in detail later, statistical methods are available to incorporate genomic imprinting in breeding value prediction programs (e.g., Neugebauer *et al.*, 2010a;b). However, these methods still need to be validated (e.g., by appropriate simulation studies) and modifications are necessary to avoid time-consuming calculations of the POE's prediction error variances. Furthermore, the available statistical models have to be processed to address existing computational challenges due to the increasing amount of data. In addition, new approaches to map imprinted loci cost-efficiently using the given genomic information are to be provided. In the present thesis, statistical methods are developed and refined to resolve the existing inconveniences and are validated using simulated and practical data. Comprehensive data sets offer accurate estimates of genetic parameters and extend the knowledge with regard to the relevance of genomic imprinting for the livestock industry.

Biological background

As imprinting relates to the field of epigenetics, it is not a result of DNA-sequence alterations but of chemical DNA modifications. Investigations reveal complex mechanisms such as DNA methylation (Li *et al.*, 1993), histone modifications (Weaver and Bartolomei, 2014), varying replication timing (Kitsberg *et al.*, 1993; Simon *et al.*, 1999) and RNA-mediated effects (O'Doherty *et al.*, 2015). Approximately 80% of all imprinted genes (including paternally and maternally imprinted genes) are physically organized in megabase-sized chromosomal clusters

(Reik and Walter, 2001; Wan and Bartolomei, 2008). Depending on their epigenetic condition, imprinting control elements, which are occasionally located a long distance away from their site of action, regulate the expression of imprinted genes within these clusters (Barlow, 2011; Barlow and Bartolomei, 2014).

The imprinting status of a gene has a life cycle as it is repetitively renewed from generation to generation: the cycle is initiated when the parental imprints are erased in an organism's primordial germ cells (Reik and Walter, 2001; Messerschmidt *et al.*, 2014). Then imprinting marks, such as DNA methylations, are set during gametogenesis depending on the organism's sex (Kafri *et al.*, 1992). At the time of fertilization paternal and maternal genomes possess different methylation patterns, which are passed to the organism's offspring (Kafri *et al.*, 1992). In the offspring these imprinting marks remain so that they are maintained throughout development and later life (Abramowitz and Bartolomei, 2012). The imprinting life cycle closes when the parental imprints are newly erased within the offspring's primordial germ cells and starts again when the offspring's epigenetic signatures are newly set depending on its own sex (Reik and Walter, 2001).

The occurrence of imprinting

The existence of imprinted genes is established in plants (Feil and Berger, 2007) and in a number of mammalian species (genomic imprinting databases are, e.g., available under <http://www.geneimprint.org/>¹ or www.otago.ac.nz/IGC (Morison *et al.*, 2005)). Although imprinting has been confirmed in less than one percent of all genes in mammalian species (Morison *et al.*, 2005; Lawson *et al.*, 2013), it is a known cause for a wide range of human maladies such as Prader-Willi syndrome (e.g., reviewed in Angulo *et al.*, 2015), Angelman syndrome (e.g., reviewed in Margolis *et al.*, 2015) or Beckwith-Wiedemann syndrome (e.g., reviewed in Maher and Reik, 2000). This suggests that imprinting is involved in many biological processes. In fact, imprinted genes have not only been revealed to affect fetal development and behavior, but also have crucial functions in stem cells, neuronal differentiation and growth (Plasschaert and Bartolomei, 2014).

With regard to livestock relevant traits, O'Doherty *et al.* (2015) reviewed contributions to the variation in body weight, metabolism, muscle traits and resistance to diseases. A pioneering discovery was the Callipyge phenotype, which is responsible for an enormous accretion of

¹ Jirtle, R.L. Geneimprint.

muscle mass in the hindquarters of sheep (Cockett *et al.*, 1994; 1999). A similarly relevant finding was a purely paternally expressed polymorphism within the insulin like growth factor 2 gene (*IGF2*) on chromosome 2 in pig, which explained up to 30% of the variance in lean meat content, lean meat mass and back fat thickness (Jeon *et al.*, 1999; Nezer *et al.*, 1999). Further investigations in pig revealed imprinted quantitative trait loci (QTLs) affecting meat quality, reproduction and growth traits (de Koning *et al.*, 2001a;b). Moreover, a genome scan uncovered imprinted QTLs, which influenced back fat thickness, muscle depth and intramuscular fat content within the same species (de Koning *et al.*, 2000). With respect to cattle, Imumorin *et al.* (2011) recently detected 24 QTLs with POEs on growth and carcass traits in a genome scan; six QTLs showed a paternal expression pattern, 15 a maternal expression pattern and three were partially imprinted. Following Imumorin *et al.* (2011), the guanine nucleotide binding protein, alpha stimulating gene (*GNAS*) and the paternally expressed 3 gene (*PEG3*), which were previously experimentally validated to be imprinted in cattle, map to the discovered QTLs. This finding may be relevant for animal breeding programs as single nucleotide polymorphism (SNPs) within the maternally imprinted *PEG3* gene cluster are associated with fertility traits (Magee *et al.*, 2010), whereas SNPs within the *GNAS* domain show significant associations with traits such as exterior, calving difficulty and milk yield (Sikora *et al.*, 2011). Evidence for the existence of genomic imprinting in avian species is limited. However, Tuiskula-Haavisto *et al.* (2004) identified imprinted QTLs in egg layer lines for age at first egg, egg weight, number of eggs, feed intake, body weight and egg quality. Lopes Pinto *et al.* (2014) recently found between 500 and 650 imprinted SNPs, predominantly paternally expressed, in liver, hypothalamus and breast muscle in high and low body weight chicken lines.

Methods to investigate imprinting

To investigate the impact of imprinted genes on important agricultural traits, diverse methods have been introduced. These can broadly be categorized as mapping experiments, expression studies and variance component analyses.

Mapping experiments are usually based on F_2 designs (line cross designs) where homozygous inbred lines are crossed to eventually yield a F_2 generation with the genotypes QQ , Qq , qQ and qq at a QTL. Thereby, as well summarized in Sandor and Georges (2008), inbred individuals (P_0 generation), assumed to be fixed for Q and q , respectively, are crossed creating a F_1 generation of individuals being heterozygote at the QTL. In accordance to the third Mendelian law,

intercrossing the F_1 individuals generates a F_2 population with a genotyping ratio of 1:2:1 at the QTL (QQ , Qq , qq). In awareness of the parental origin of the alleles (phasing information), a statistically tested phenotypic difference between Qq and qQ indicates imprinting while no difference suggests the opposite. Following this approach, Nezer *et al.* (1999) and Jeon *et al.* (1999) detected the polymorphism causing the paternally expressed QTL in the *IGF2* region in pig (see previous section).

Expression studies are increasingly used due to the recent progress in next generation sequencing technologies. Using phasing information the parental allele expression levels are investigated and tested for a preferential expression. For instance, Lopes Pinto *et al.* (2014) examined allele-specific RNA expression levels in three different tissues of F_1 progeny of a reciprocal cross between a high and low body weight line in chicken.

Variance component analyses make use of large pedigrees and phenotypic data to assess paternal and maternal gametic variations and their contributions to the genetic or phenotypic variance. Due to software limitations, first variance component analyses based on a proposal from Tier and Sölkner (1993), which allowed the investigation of gametic variation using standard genetic evaluation techniques. For instance, de Vries *et al.* (1994) and Engellandt and Tier (2002) used an animal model adding either a random maternal or a random paternal gametic effect. To model the covariance structure, they treated the gametes as homozygous diploid individuals allowing their incorporation into the additive genetic relationship matrix. In contrast, Essl and Voith (2002) estimated the paternal and maternal gametic variances using a sire model and a dam model for the same trait and concluded the existence of genomic imprinting in case the gametic variances differed significantly.

However, the models used so far are only able to find either complete maternal or complete paternal imprinting. They are unable to detect partially imprinted genes, which lead to covariances (σ_{bp} , σ_{bm} and σ_{pm}) between biparental (σ_b^2), pure paternal (σ_p^2) and pure maternal (σ_m^2) contributions to the genetic variance (Hill and Keightley, 1988). Thus, to consider partial imprinting, six gametic covariance components have to be estimated. However, their simultaneous estimation is not possible as stated by Reinsch and Guiard (FBN, unpublished data). Therefore, Neugebauer *et al.* (2010a;b) introduced a model (thereafter referred to as *Neugebauer model*) that includes two random gametic effects per animal. In their studies the authors applied the model in a reduced version using the additive genetic relationship matrix A

instead of a gametic relationship matrix \mathbf{G} (Gibson *et al.*, 1988; Schaeffer *et al.*, 1989). This version can be written as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_s\mathbf{a}_s + \mathbf{Z}_d\mathbf{a}_d + \mathbf{e},$$

where \mathbf{y} corresponds to the vector of observations; \mathbf{a}_s and \mathbf{a}_d constitute the genetic effects of an animal contributed to its offspring under a paternal expression pattern and under a maternal expression pattern (both provided as transmitting abilities); $\boldsymbol{\beta}$ is a vector of fixed effects; \mathbf{Z}_s , \mathbf{Z}_d and \mathbf{X} are the corresponding incidence matrices connecting the effects with their observations; and \mathbf{e} is a vector of random residuals. In terms of gametic variances the covariances of the random effects can be written as:

$$\text{Var} \begin{bmatrix} \mathbf{a}_s \\ \mathbf{a}_d \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} 1/2\mathbf{A}\sigma_s^2 & 1/2\mathbf{A}\sigma_{sd} & 0 \\ 1/2\mathbf{A}\sigma_{sd} & 1/2\mathbf{A}\sigma_d^2 & 0 \\ 0 & 0 & \mathbf{W} \end{bmatrix},$$

where the paternal variance σ_s^2 is a result of $\sigma_b^2 + \sigma_p^2 + 2\sigma_{bp}$ and the maternal variance σ_d^2 is a composition of $\sigma_b^2 + \sigma_m^2 + 2\sigma_{bm}$. This clarifies the fact that σ_s^2 and σ_d^2 summarize the parental contributions to the gametic variations, respectively. The difference between σ_s^2 and σ_d^2 can be expressed as the imprinting variance $\sigma_i^2 = \sigma_s^2 + \sigma_d^2 - 2\sigma_{sd}$, which is the variation of the estimated imprinting effect i ($a_s - a_d$) and a result of all maternal ($\sigma_d^2 - \sigma_{sd}$) and paternal ($\sigma_s^2 - \sigma_{sd}$) variance contributions. The covariance σ_{sd} summarizes all covariances and is a result of $\sigma_b^2 + \sigma_{bp} + \sigma_{bm} + \sigma_{pm}$. The total additive genetic variance is given by $\sigma_a^2 = \sigma_s^2 + \sigma_d^2$ and is a composition of an imprinted (σ_i^2) and an unimprinted part of inheritance (Neugebauer *et al.*, 2010a; b). The mixed model equations are:

$$\begin{bmatrix} \mathbf{X}'\mathbf{W}^{-1}\mathbf{X} & \mathbf{X}'\mathbf{W}^{-1}\mathbf{Z}_s & \mathbf{X}'\mathbf{W}^{-1}\mathbf{Z}_d \\ \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{X} & \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{Z}_s + \mathbf{A}^{-1}\lambda_1 & \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{Z}_d + \mathbf{A}^{-1}\lambda_2 \\ \mathbf{Z}_d'\mathbf{W}^{-1}\mathbf{X} & \mathbf{Z}_d'\mathbf{W}^{-1}\mathbf{Z}_s + \mathbf{A}^{-1}\lambda_2 & \mathbf{Z}_d'\mathbf{W}^{-1}\mathbf{Z}_d + \mathbf{A}^{-1}\lambda_3 \end{bmatrix} \begin{bmatrix} \boldsymbol{\beta} \\ \mathbf{a}_s \\ \mathbf{a}_d \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{W}^{-1}\mathbf{y} \\ \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{y} \\ \mathbf{Z}_d'\mathbf{W}^{-1}\mathbf{y} \end{bmatrix},$$

where $\boldsymbol{\lambda}$ is equivalent to:

$$\begin{bmatrix} \lambda_1 & \lambda_2 \\ \lambda_2 & \lambda_3 \end{bmatrix}^{-1} = \frac{1}{2\sigma_e^2} \begin{bmatrix} \sigma_s^2 & \sigma_{sd} \\ \sigma_{sd} & \sigma_d^2 \end{bmatrix}$$

and the diagonal matrix \mathbf{W} has the elements:

$$w_{ii} = \left[\frac{1/2\sigma_s^2(1-F_{s_i}) + 1/2\sigma_d^2(1-F_{d_i}) + \sigma_e^2}{1/2\sigma_s^2 + 1/2\sigma_d^2 + \sigma_e^2} \right]^{-1}$$

and corrects the error variance of each observation due to the Mendelian sampling component with regard to the respective inbreeding coefficient of the parents (F_{s_i}, F_{d_i}).

In case genomic imprinting exists, the parental breeding values are either of different magnitude or not fully correlated or both. With respect to the variance components the parental alleles do not contribute the same amount to the total genetic variance so that σ_s^2 , σ_d^2 and σ_{sd} are different.

Content of the thesis

Chapter 1 provides an introduction to the investigation of genomic imprinting using variance component analyses and examines the impact of partial imprinting on the genetic covariance in theory and by simulation. The total imprinting variance is theoretically derived and estimated in simulated data by employing the *Neugebauer model*. The validity of the underlying theory and the broad generality of the applied model are demonstrated.

Chapter 2 concerns the practical problem of the *Neugebauer model*, which is to receive the prediction error variance of the imprinting effects that is defined as:

$$\text{Var}(\hat{i} - i) = [\text{Var}(\hat{a}_d - a_d) + \text{Var}(\hat{a}_s - a_s) - 2\text{Cov}(\hat{a}_d - a_d, \hat{a}_s - a_s)].$$

Whereas the prediction error variance can be easily derived from the diagonal elements of the inverted left-hand side of the mixed model equations, the prediction error covariance is computationally hard to achieve as the off-diagonal elements are needed. Therefore, a model equivalent to the *Neugebauer model* is developed that tackles the mentioned issue by incorporating a random imprinting effect. Furthermore, the application of this model to slaughter data in a linear and generalized linear manner delivers results about the impact of genomic imprinting on continuous and categorical beef performance traits in Brown Swiss.

Chapter 3 refers to the problem that today large data sets are available for genetic evaluation purposes and that the dimension of the mixed model equations of the *Neugebauer model* becomes too large to estimate the imprinting variance. Referring to this difficulty a new imprinting model solely based on male ancestors is introduced. Applying this model in a linear and generalized linear form to slaughter data provided by 1.36 million Fleckvieh fattening bulls

accompanied by a pedigree of more than 2.5 million sires and dams offers estimates for the imprinting variance in Austrian and German Simmental.

Chapter 4 demonstrates that de-regressed POEs can be used as dependent variables in association studies by regressing them on the marker allele count of parents, rather than on the phased genotypes of offspring. This approach provides a viable and cost-efficient alternative to imprinting effect mapping analyses (e.g., line cross designs), which depend on the availability of phased marker information.

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CHAPTER 1

Genetic variance components when fluctuating imprinting patterns are present

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Genetic variance components when fluctuating imprinting patterns are present

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ABSTRACT

Genomic imprinting may be unstable over time (developmentally regulated imprinting) or between tissues (tissue specific imprinting). How this phenomena affect genetic covariance was investigated both in theory and by simulation. Thereby the first aim was to theoretically derive the total imprinting variance, which has contributions from all imprinted loci with different kinds of stable (paternal, maternal) or fluctuating (changing from either paternal or maternal to unimprinted) expression patterns. Second, the total imprinting variance was estimated in simulated data by employing a recently proposed model with two additive genetic effects per individual. REML-estimates of variance components were in excellent agreement with simulated parameters. Therefrom validity of the underlying theory can be concluded as well as the broad generality of the applied imprinting BLUP-model.

Keywords: genomic imprinting, epigenetics, variance components

INTRODUCTION

Genomic imprinting arises when the expression of genes depends on the parental origin of their alleles. Complete imprinting occurs, where e.g., the paternal (maternal) allele at an imprinted locus is fully inactivated while the maternal (paternal) one shows active expression. A scenario in which the lack of expression is not complete, is called partial imprinting. Further, loci may be imprinted in certain tissues or organs, while they are not in others (tissue specific imprinting, e.g., Yu *et al.*, 1998). Likewise, the imprinting status of a locus may vary over time during successive developmental stages (developmentally regulated imprinting, e.g., Gould and Pfeifer, 1998).

Recently, in analyses of pig (Neugebauer *et al.*, 2010a) and beef cattle (Neugebauer *et al.*, 2010b) data, a BLUP model has been proposed with two additive effects (as sire and as dam) per animal in order to account for all variants of genomic imprinting. The imprinting variance σ_i^2 summarizes contributions from all imprinted loci, no matter to which of the before mentioned kinds of imprinting they belong. The aim of this investigation was to demonstrate the generality of the model by explicitly simulating loci with fluctuating patterns of imprinted action on the phenotype. Generality and suitability of the model, which is subsequent called the “compact model”, is demonstrated by comparing simulated parameters with estimates obtained with this model. For the purpose of deriving the theoretical outcomes, however another covariance model with six parameters is employed.

MATERIALS AND METHODS

Theory. Considering a genome with several unlinked loci and purely additive gene action Hill and Keightley (1988) assumed all loci to be classified into one of three different expression patterns if genomic imprinting is involved. The first one includes Mendelian loci, which are not imprinted. The second (third) class relates to paternal (maternal) expressed loci whose maternal (paternal) allele is fully suppressed. Under these assumptions three uncorrelated random effects and their corresponding variance components can be estimated. An adequate linear mixed model is:

$$Y = X\beta + Z_b g_b + Z_p g_p + Z_m g_m + e,$$

where Y is a vector of observations; β is a vector of fixed effects; g_b , g_p and g_m are gametic effects summarizing the impact of Mendelian (b), paternally (p) and maternally (m) expressed alleles; and e is the vector of random residuals. The design matrices X , Z_b , Z_p and Z_m link observations to fixed and random effects. The covariance of random genetic effects can be written as:

$$\text{Var} \begin{bmatrix} g_b \\ g_p \\ g_m \end{bmatrix} = \mathbf{G} \otimes \mathbf{S}_E = \mathbf{G} \otimes \begin{bmatrix} \sigma_b^2 & 0 & 0 \\ 0 & \sigma_p^2 & 0 \\ 0 & 0 & \sigma_m^2 \end{bmatrix},$$

where σ_b^2 , σ_p^2 and σ_m^2 are defined as the variances of the gametic effects, \mathbf{G} is the gametic relationship matrix (Gibson *et al.*, 1988; Schaeffer *et al.*, 1989) and \otimes denotes the Kronecker product.

Fluctuating expression patterns require an extension of this model by somehow incorporating expression patterns with, e.g., imprinted loci, which are reactivated in later development stages. In principle this can be accommodated by introducing non-zero covariances into the matrix \mathbf{S}_E , which leads to:

$$\text{Var} \begin{bmatrix} \mathbf{g}_b \\ \mathbf{g}_p \\ \mathbf{g}_m \end{bmatrix} = \mathbf{G} \otimes \mathbf{S}_D = \mathbf{G} \otimes \begin{bmatrix} \sigma_b^2 & \sigma_{bp} & \sigma_{bm} \\ \sigma_{bp} & \sigma_p^2 & \sigma_{pm} \\ \sigma_{bm} & \sigma_{pm} & \sigma_m^2 \end{bmatrix},$$

where σ_{bp} , σ_{bm} and σ_{pm} are the covariances between the three gametic effects in the model and all other symbols are as defined before. The reason is that a locus may, e.g., first contribute to σ_p^2 when its maternal allele is fully suppressed in an early stage, and later to σ_b^2 when the imprint has been removed and both parental alleles are equally active. The total contribution of such a locus to the gametic variance is then $\sigma_b^2 + \sigma_p^2 + 2\sigma_{bp}$. Hereby we assume that the trait (e.g., growth) is the sum of contributions from each stage.

The compact model. As outlined in Reinsch and Guiard (FBN, unpublished data) the estimation of covariances is not possible with the explicit model. For this reason the so-called compact model was proposed, which divides breeding values into paternal and maternal gametic effects:

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_s\mathbf{g}_s + \mathbf{Z}_d\mathbf{g}_d + \mathbf{e},$$

where \mathbf{g}_s is a vector of random gametic effects under paternal expression patterns ($\mathbf{g}_b + \mathbf{g}_p$ in terms of the explicit model); \mathbf{g}_d is a vector of random gametic effects under maternal expression patterns ($\mathbf{g}_b + \mathbf{g}_m$); \mathbf{X} , \mathbf{Z}_s and \mathbf{Z}_d are the corresponding design matrices; and \mathbf{e} is the vector of random residuals. The covariance of gametic effects can be written as $\mathbf{G} \otimes \mathbf{S}_C$:

$$\text{Var} \begin{bmatrix} \mathbf{g}_s \\ \mathbf{g}_d \end{bmatrix} = \mathbf{G} \otimes \mathbf{S}_C = \mathbf{G} \otimes \begin{bmatrix} \sigma_s^2 & \sigma_{sd} \\ \sigma_{sd} & \sigma_d^2 \end{bmatrix},$$

where σ_s^2 (σ_d^2) is the gametic variance as sire (dam), corresponding to a paternal (maternal) expression pattern and σ_{sd} is their covariance. In terms of the explicit model $\sigma_s^2 = \sigma_b^2 + \sigma_p^2 + 2\sigma_{bp}$,

$\sigma_d^2 = \sigma_b^2 + \sigma_m^2 + 2\sigma_{bm}$ and $\sigma_{sd} = \sigma_b^2 + \sigma_{bp} + \sigma_{bm} + \sigma_{pm}$. The imprinting variance σ_i^2 is a function of these three variance components (Neugebauer *et al.*, 2010a;b):

$$\sigma_i^2 = \sigma_s^2 + \sigma_d^2 - 2\sigma_{sd},$$

and the total additive genetic variance is given by:

$$\sigma_a^2 = \sigma_s^2 + \sigma_d^2.$$

Data simulation. A two generation pedigree with 100 unrelated sires and 100 unrelated dams was simulated. These founders were intermated in a cross-classified manner, giving rise to 10,000 equally sized full-sib families with 10 descendants per family. In each of 300 replications, 100,000 observations were simulated, one for each progeny. The reduced version of the compact model (Neugebauer *et al.*, 2010a;b) could be used for variance component estimation. Five unlinked loci were simulated, four of them imprinted according to different patterns. Locus four and five were assumed to be imprinted during a first developmental stage (imprinting effect different from zero), but not in the second stage (additive effect a_2 different from zero). Developmentally unstable loci had different additive effects a_1 and a_2 in both stages, while only a single additive effect a_1 was assigned to developmentally stable loci ($a_2 = 0$). All relevant parameters are summarized in Table 1. Phenotypes were created by adding additive effects of both stages (a_1 and a_2) and the imprinting effect i for each locus according to the simulated genotype. Residuals were simulated with a variance of $\sigma_e^2 = 2$. REML-estimates of variance components were obtained via the ASReml-package (Gilmour *et al.*, 2006).

Table 1. Additive genetic (a) and imprinting (i) effects and gene frequencies (p) of loci 1 to 5 for both developmental stages (1 and 2).

Locus	a_1	i_1	a_2	p
1	0.6	0.0	0.0	0.6
2	0.5	0.5	0.0	0.6
3	0.3	-0.3	0.0	0.5
4	0.6	0.6	0.3	0.4
5	0.5	-0.5	0.5	0.7

RESULTS AND DISCUSSION

Within the first stage the simulated explicit variance-covariance-matrix can be written as:

$$\mathbf{S}_E = \begin{bmatrix} 0.086 & 0.000 & 0.000 \\ 0.000 & 0.586 & 0.000 \\ 0.000 & 0.000 & 0.3000 \end{bmatrix}.$$

Since there are no contributions of completely imprinted loci to the biparental variance, there are no covariances between genetic effects. All covariance-components within the first stage can be seen separately for each locus in Table 2. Table 3 shows the same components for each locus but for the second stage.

Table 2. Simulated biparental (σ_b^2), paternal (σ_p^2) and maternal (σ_m^2) variances and their covariances (σ_{bp} ; σ_{bm}) in terms of the explicit model for all loci within the first stage.

Locus	σ_b^2	σ_p^2	σ_m^2	σ_{bp}	σ_{bm}
1	0.086	0.000	0.000	0.000	0.000
2	0.000	0.240	0.000	0.000	0.000
3	0.000	0.000	0.090	0.000	0.000
4	0.000	0.346	0.000	0.000	0.000
5	0.000	0.000	0.210	0.000	0.000
Σ	0.086	0.586	0.300	0.000	0.000

σ_{bp} : Covariance between biparental and paternal effects; σ_{bm} : Covariance between biparental and maternal effects.

Table 3. Simulated biparental (σ_b^2), paternal (σ_p^2) and maternal (σ_m^2) variances and their covariances (σ_{bp} ; σ_{bm}) in terms of the explicit model for all loci within the second stage.

Locus	σ_b^2	σ_p^2	σ_m^2	σ_{bp}	σ_{bm}
1	0.000	0.000	0.000	0.000	0.000
2	0.000	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.000	0.000
4	0.021	0.000	0.000	0.000	0.000
5	0.053	0.000	0.000	0.000	0.000
Σ	0.074	0.000	0.000	0.000	0.000

σ_{bp} : Covariance between biparental and paternal effects; σ_{bm} : Covariance between biparental and maternal effects.

At the end of the first stage the previously fully suppressed alleles of locus four and five are expressed. Therefore, they also contribute to the biparental variance and covariances arise. Thus, at the end of the second stage the aggregated covariance matrix S_D becomes:

$$S_D = \begin{bmatrix} 0.161 & 0.084 & 0.105 \\ 0.084 & 0.586 & 0.000 \\ 0.105 & 0.000 & 0.300 \end{bmatrix}.$$

This can be transformed into the covariance matrix S_C for the compact model. The result is:

$$S_C = \begin{bmatrix} 0.919 & 0.352 \\ 0.352 & 0.671 \end{bmatrix}.$$

All covariance-components of the explicit and the compact model can be seen separately for each locus in Table 4 and 5.

Table 4. Simulated biparental (σ_b^2), paternal (σ_p^2) and maternal (σ_m^2) variances and their covariances (σ_{bp} ; σ_{bm}) in terms of the explicit model for all loci after both stages.

Locus	σ_b^2	σ_p^2	σ_m^2	σ_{bp}	σ_{bm}
1	0.086	0.000	0.000	0.000	0.000
2	0.000	0.240	0.000	0.000	0.000
3	0.000	0.000	0.090	0.000	0.000
4	0.021	0.346	0.000	0.084	0.000
5	0.053	0.000	0.210	0.000	0.105
Σ	0.161	0.586	0.300	0.084	0.105

σ_{bp} : Covariance between biparental and paternal effects; σ_{bm} : Covariance between biparental and maternal effects.

Table 5. Simulated gametic variances as sire (σ_s^2) and as dam (σ_d^2) and their covariances (σ_{sd}) in terms of the compact model for all loci after both development stages. σ_i^2 and σ_a^2 describe the imprinting and the additive genetic variances.

Locus	σ_s^2	σ_d^2	σ_{sd}	σ_i^2	σ_a^2
1	0.086	0.086	0.086	0.000	0.172
2	0.240	0.000	0.000	0.240	0.240
3	0.000	0.090	0.000	0.090	0.090
4	0.540	0.022	0.108	0.346	0.562
5	0.052	0.473	0.157	0.210	0.525
Σ	0.919	0.671	0.352	0.886	1.589

σ_s^2 : Paternal variance of paternal effects (compact model). $\sigma_s^2 = \sigma_b^2 + \sigma_p^2 + 2\sigma_{bp}$; σ_d^2 : Maternal variance of maternal effects (compact model). $\sigma_d^2 = \sigma_b^2 + \sigma_m^2 + 2\sigma_{bm}$; σ_{sd} : Covariance of paternal and maternal effects (compact model).

$\sigma_{sd} = \sigma_b^2 + \sigma_{bp} + \sigma_{bm}$; σ_i^2 : Imprinting variance. $\sigma_i^2 = \sigma_s^2 + \sigma_d^2 - 2\sigma_{sd}$; σ_a^2 : Additive genetic variance. $\sigma_a^2 = \sigma_s^2 + \sigma_d^2$.

The results of analyzing the simulated data using ASReml are shown in Table 6. Comparing these estimated covariance components with their simulated counterparts in Table 5 it can be seen that the estimated values coincide nearly perfectly with the theoretical ones.

Table 6. Estimated gametic variances as sire (σ_s^2) and as dam (σ_d^2), their covariance (σ_{sd}), the imprinting (σ_i^2) and additive genetic variance (σ_a^2) and their standard errors (SE).

	$\hat{\sigma}_s^2$	$\hat{\sigma}_d^2$	$\hat{\sigma}_{sd}$	$\hat{\sigma}_i^2$	$\hat{\sigma}_a^2$
$\hat{\sigma}^2$	0.915	0.664	0.353	0.872	1.597
SE	± 0.012	± 0.009	± 0.008	± 0.025	± 0.031

CONCLUSION

It could be shown that fluctuating imprinting patterns due to variations of successive development stages may lead to additional covariances between gametic effects. The compact

model suggested by Neugebauer *et al.* (2010a;b) is well suited to take every variant of genomic imprinting into account. It thus represents a universal tool in quantitative genetic livestock studies.

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CHAPTER 2

A new model for parent-of-origin effect analyses applied to Brown Swiss cattle slaughterhouse data

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A new model for parent-of-origin effect analyses applied to Brown Swiss cattle slaughterhouse data

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ABSTRACT

Genomic imprinting is a phenomenon that arises when the expression of genes depends on the parental origin of alleles. Epigenetic mechanisms may induce the full or partial suppression of maternal or paternal alleles, thereby leading to different types of imprinting. However, imprinting effects have received little consideration in animal breeding programmes, although their relevance to some agricultural important traits has been demonstrated. A recently proposed model (imprinting model) with two path-of-transmission (male and female) specific breeding values for each animal accounts for all types of imprinting simultaneously (paternal, maternal, full and partial). Imprinting effects (or more generally: parent-of-origin effects (POEs)) are determined by taking the difference between the two genetic effects in each animal. However, the computation of their prediction error variance (PEV) is laborious; thus, we propose a new model that is equivalent to the aforementioned imprinting model, which facilitates the direct estimation of imprinting effects instead of taking the differences and the PEV is readily obtained. We applied the new model to slaughterhouse data for Brown Swiss cattle, among which imprinting has never been investigated previously. Data were available for up to 173,051 fattening bulls, where the pedigrees contained up to 428,710 animals representing the entire Brown Swiss population of Austria and Germany. The traits analysed comprised the net BW gain, fat score, EUROP class and killing out percentage. The analysis demonstrated that the net BW gain, fat score and EUROP class were influenced significantly by POEs. After estimating the POEs, the new model yielded estimates with reliabilities ranging between zero and 0.9. On average, the imprinting variances accounted for 9.6% of the total genetic variance, where the maternal gamete was the main contributor. Moreover, our results agreed well with those obtained using linear models when the EUROP class and fat score were treated as categorical traits by applying a generalised linear model with a logit link function.

Keywords: beef trait, Brown Swiss cattle, epigenetics, imprinting variance, parent-of-origin effect.

IMPLICATIONS

Genomic imprinting is an epigenetic phenomenon where the expression of genes depends on the parental origin of their alleles. Imprinting is known to affect a variety of value-determining traits in agricultural species, and thus it should be considered in animal breeding. However, the existing methods are still difficult in practice using standard statistical software. Therefore, we propose a new statistical model that allows the direct estimation of imprinting effects and their prediction error variances (PEVs). We applied this model to slaughterhouse data for Brown Swiss cattle, a breed in which imprinting has never been investigated previously.

INTRODUCTION

Genomic imprinting is known to be caused by allele-specific DNA methylation and histone modifications during gametogenesis, which depend on the sex of an animal (for a review, see Reik and Walter, 2001). Thus, imprinting is an epigenetic phenomenon that alters the expression of genes according to the parental origin of their alleles. Therefore, imprinting effects often are referred to as parent-of-origin effects (POEs), which are, however, not synonymous, as the latter include parent-of-origin dependent effects which do not, by definition, constitute imprinting effects (e.g., maternal genetic effects as emphasized by Hager *et al.*, 2008). A well-known example for genomic imprinting is the callipyge mutation in sheep, which causes extreme muscle hypertrophy that only becomes evident when the offspring inherit the mutation from their sire (Cockett *et al.*, 1999). A scenario where the paternal allele of an imprinted locus is fully inactivated but the maternal allele exhibits active expression is referred to as complete paternal imprinting. The opposite scenario is defined as complete maternal imprinting. An incomplete lack of allele expression known as partial imprinting is caused by unstable imprinting patterns over time or between tissues (e.g., Gould and Pfeifer, 1998).

A series of mapping experiments based on quantitative trait loci (QTL) led to the identification of a polymorphism that causes a paternally expressed QTL in the *IGF2* region of the pig (e.g., Nezer *et al.*, 1999; Van Laere *et al.*, 2003). This polymorphism explained 15%–30% of the

phenotypic variation in muscle mass (Van Laere *et al.*, 2003). More recently, Lopes Pinto *et al.* (2014) performed an expression study and detected parental single nucleotide polymorphisms at up to 650 loci in three different chicken tissues that indicated predominant paternal imprinting.

The first study of this type was reported by de Vries *et al.* (1994) and the analysis of variance components is now a *sui generis* approach in livestock genetics for investigating the importance of imprinting effects for genetic variation. The first versions of this approach used an animal model with an additional random parental effect (e.g., Engellandt and Tier, 2002) to account for either full paternal or full maternal imprinting. In 2010, Neugebauer *et al.* (2010a;b) introduced a model with two additive effects (one “as sire” and one “as dam”) per animal to account for all variants of imprinting: paternal, maternal, full and partial. Based on analyses of slaughter data, they found 19 traits in pigs (Large White) and 10 traits in cattle (German Simmental) with significant influences of POEs. The imprinting variance accounted for 5% to 19% and 8% to 25% of the total genetic variance, respectively. Recently, equivalent gametic models were applied by Tier and Meyer (2012) to analyse ultrasonic measures of body composition in cattle, thereby determining an average relative imprinting variance of 28%.

To consider genomic imprinting in animal breeding programmes, Nishio and Satoh (2014) proposed a new genomic BLUP model, where they used a genomic imprinting relationship matrix constructed from paternal and maternal marker alleles to indicate an improvement in the genetic prediction reliability in a simulation study.

Nevertheless, imprinting effects are still not considered routinely during genetic evaluation. To promote the integration process, further investigations are necessary to determine the effects of imprinting on important agricultural traits. However, none of the previously proposed models used for analysing the variance components can estimate these imprinting effects directly. In addition, the computation of their PEVs requires laborious procedures to evaluate their reliability (Neugebauer *et al.*, 2010a;b).

Therefore, in this study, we propose a new model that is equivalent to the model of Neugebauer *et al.* (2010a;b), in which the direct estimation of imprinting effects is facilitated, and their PEVs can be obtained easily using existing software. To demonstrate its practical use, we applied this model to Brown Swiss cattle slaughter data. Four value-determining slaughter traits were available for Austrian and German fattening bulls. Two traits were analysed a second time by applying a generalised linear mixed model (GLMM) with a logit link function.

MATERIALS AND METHODS

Beef trait data

A data set comprising 247,883 Brown Swiss fattening bulls slaughtered between 1994 and 2013 was provided by the genetic evaluation centre of the Landesamt für Geoinformation und Landentwicklung in Baden-Württemberg, Germany. This is a known dairy breed, but Brown Swiss bulls are fattened up to an end weight of approximately 600 kg. Data from Austria and Germany are used at regular intervals to predict breeding values for Brown Swiss and German Simmental within a joint genetic evaluation procedure for both breeds. The sires are evaluated using their progeny performance, which is routinely recorded at slaughterhouses. Thus, we used the net BW gain (carcass weight divided by age (g/days)), carcass conformation, carcass fatness and killing out percentage (carcass weight divided by life weight (%)).

The carcass conformation was defined according to the European muscle conformation system EUROP (E = excellent to P = poor). However, these EUROP grades were replaced by five monetary values (670, 655, 635, 585, 525), which reflect the fact that although prices differ over time, the price differences between classes remain stable (Engelland *et al.*, 1999a). The majority of the fattening bulls were categorised into classes O (19.54%) and R (78.45%).

Carcass fatness was available as scores ranging from 1 (lean) to 5 (very fat) where most of the animals were classified with scores of 2 (23.27%) and 3 (71.9%).

All of the fattening bulls with missing sires and/or dams, as well as all bulls belonging to a comparison group with less than five animals per group (four animals per group for the killing out percentage) were eliminated from the data set, which led to varying numbers of observations. The highest number was available for net BW gain (173,051) and the smallest for the killing out percentage (3,226). A summary of the data is given in Table 1.

The number of pedigrees was 428,710 for net BW gain (up to 21 generations), 420,626 for carcass conformation class and fat score (up to 21 generations), and 24,329 for the killing out percentage (up to 20 generations). The pedigree for net BW gain was pruned using the SECATEURS program (Meyer, 2003). This procedure considerably reduced the number of animals due to the elimination of uninformative parents for the estimation of genetic parameters. As suggested by Westell and Van Vleck (1987), unknown parents (phantom parents) were assigned to genetic groups based on their expected year of birth. The birth years of unidentified animals were assigned according to the average generation intervals, which were estimated for

four paths of selection using the software package pedig (Boichard, 2002). Those groups represented the average genetic merit of animals selected as parents on a contemporary basis. All of the phantoms that were likely to have been born before 1974 were assigned to the first group. All other groups were specified according to three-year periods until 1996. Missing animals likely to have been born after 1996 were assigned to the last group. Furthermore, two parallel sets of groups were specified to characterize phantom sires and dams because the male and female paths of selection are assumed to differ in terms of genetic merit (Westell and Van Vleck, 1987). Thus, 18 genetic groups in total were assigned to unknown animals in each pedigree as groups based on a combination of time and sex.

Table 1. Descriptive statistics, number of fattening bulls (n), pedigree sizes and heritabilities for the traits analysed in this study. The standard errors are given in brackets.

Trait	mean	SD	n	pedigree	h_{LMM}^2	h_{GLMM}^2
Net BW gain (g/days)	646.72	74.24	173,051	428,710	0.26 (0.01)	-
Fat score	2.81	0.50	133,671	420,626	0.22 (0.01)	0.47 (0.02)
Conformation class	625.20	20.98	133,671	420,626	0.15 (0.01)	0.43 (0.02)
Killing out percentage (%)	56.63	1.17	3,226	24,329	0.52 (0.08)	-

h_{LMM}^2 = heritability estimated using a linear animal model.

h_{GLMM}^2 = heritability estimated using a generalised linear animal model.

Models for analysis

Imprinting model. To investigate the role of imprinting effects, Neugebauer *et al.* (2010a;b) developed a model with two additive genetic effects per animal, which are only estimated for the parents. This model accounts for all variants of genomic imprinting and it is known as the *imprinting model*. In matrix notation, the model is:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_s\mathbf{a}_s + \mathbf{Z}_d\mathbf{a}_d + \mathbf{e},$$

where \mathbf{y} is a vector of observations; $\boldsymbol{\beta}$ is a vector of fixed effects; \mathbf{a}_s (\mathbf{a}_d) is a vector of random genetic effects under a paternal (maternal) expression pattern, which corresponds to the vector of the transmitting ability (TA) for the sire (dam); \mathbf{X} , \mathbf{Z}_s and \mathbf{Z}_d are the corresponding incidence matrices; and \mathbf{e} is a vector of random residuals. In terms of gametic variances, the variance-covariance components of random effects can be written as:

$$\text{Var} \begin{bmatrix} \mathbf{a}_s \\ \mathbf{a}_d \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} 1/2\mathbf{A}\sigma_s^2 & 1/2\mathbf{A}\sigma_{sd} & 0 \\ 1/2\mathbf{A}\sigma_{sd} & 1/2\mathbf{A}\sigma_d^2 & 0 \\ 0 & 0 & \mathbf{W} \end{bmatrix}.$$

The mixed model equations are:

$$\begin{bmatrix} \mathbf{X}'\mathbf{W}^{-1}\mathbf{X} & \mathbf{X}'\mathbf{W}^{-1}\mathbf{Z}_s & \mathbf{X}'\mathbf{W}^{-1}\mathbf{Z}_d \\ \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{X} & \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{Z}_s + \mathbf{A}^{-1}\lambda_1 & \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{Z}_d + \mathbf{A}^{-1}\lambda_2 \\ \mathbf{Z}_d'\mathbf{W}^{-1}\mathbf{X} & \mathbf{Z}_d'\mathbf{W}^{-1}\mathbf{Z}_s + \mathbf{A}^{-1}\lambda_2 & \mathbf{Z}_d'\mathbf{W}^{-1}\mathbf{Z}_d + \mathbf{A}^{-1}\lambda_3 \end{bmatrix} \begin{bmatrix} \boldsymbol{\beta} \\ \mathbf{a}_s \\ \mathbf{a}_d \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{W}^{-1}\mathbf{y} \\ \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{y} \\ \mathbf{Z}_d'\mathbf{W}^{-1}\mathbf{y} \end{bmatrix},$$

where the matrix of λ -coefficients is equivalent to:

$$\begin{bmatrix} \lambda_1 & \lambda_2 \\ \lambda_2 & \lambda_3 \end{bmatrix}^{-1} = \frac{1}{2\sigma_e^2} \begin{bmatrix} \sigma_s^2 & \sigma_{sd} \\ \sigma_{sd} & \sigma_d^2 \end{bmatrix},$$

\mathbf{A} is the numerator relationship matrix and the diagonal matrix \mathbf{W} has the elements:

$$w_{ii} = \left[\frac{1/2\sigma_s^2(1-F_{s_i}) + 1/2\sigma_d^2(1-F_{d_i}) + \sigma_e^2}{1/2\sigma_s^2 + 1/2\sigma_d^2 + \sigma_e^2} \right]^{-1}$$

and it corrects the error variance of each observation due to the Mendelian sampling component with regard to the respective inbreeding coefficient (F_{s_i} , F_{d_i}) of the parents. The difference between both parental genetic effects is referred to as the imprinting effect ($i = \mathbf{a}_s - \mathbf{a}_d$) and its variance defines the imprinting variance $\sigma_i^2 = \sigma_s^2 + \sigma_d^2 - 2\sigma_{sd}$. The total additive genetic variance is given by $\sigma_a^2 = \sigma_s^2 + \sigma_d^2$, which comprises the imprinted (σ_i^2) and Mendelian ($\sigma_M^2 = \sigma_a^2 - \sigma_i^2$) parts of inheritance.

The equivalent model. As mentioned earlier, the imprinting effect i can be derived easily as the difference between both parental TAs using the *imprinting model*. However, determining their PEVs is demanding because the off-diagonal elements of the inverted coefficient matrices of the mixed model equations are necessary (Neugebauer *et al.*, 2010a;b). Therefore, we propose an equivalent imprinting model that allows the direct estimation of imprinting effects as well as their PEVs. As stated by Henderson (1985), alternative models can generate a class of variance-covariance estimates that are identical to those generated by the original model after linear transformation. Our new model is equivalent to the *imprinting model* according to Henderson (1985), so we refer to it as the *equivalent model*. This model can be written as:

$$y_{ijk} = \mu + a_{s_i} + a_{s_j} + (a_{d_j} - a_{s_j}) + e_{ijk},$$

where y_{ijk} is the observation of the k^{th} progeny of sire i and dam j and μ is the overall mean. The effect a_{s_i} corresponds to the TA of sire i as sire and a_{s_j} is the TA of dam j as sire. However, the dam's influence comprises her TA as dam, so her imprinting effect $(a_{d_j} - a_{s_j})$ needs to be added. Thus, the dam's TA corresponds to her TA as sire plus her imprinting effect. The effect e_{ijk} is the random residual. In terms of gametic variances, the corresponding variance-covariance components are:

$$\text{Var} \begin{bmatrix} \mathbf{a}_s \\ (\mathbf{a}_d - \mathbf{a}_s) \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} 1/2 \mathbf{A} \sigma_s^2 & 1/2 \mathbf{A} (\sigma_{sd} - \sigma_s^2) & 0 \\ 1/2 \mathbf{A} (\sigma_{sd} - \sigma_s^2) & 1/2 \mathbf{A} \sigma_i^2 & 0 \\ 0 & 0 & \mathbf{W} \end{bmatrix},$$

where $(\sigma_{sd} - \sigma_s^2)$ is the covariance between the TA as sire and the imprinting effect. To satisfy Henderson's condition of equivalence, the variance-covariance components estimated using the *equivalent model* can be converted in a linear manner into those estimated using the *imprinting model*. The mixed model equations of the *equivalent model* can be written as:

$$\begin{bmatrix} \mathbf{X}'\mathbf{W}^{-1}\mathbf{X} & \mathbf{X}'\mathbf{W}^{-1}\mathbf{Z}_s & \mathbf{X}'\mathbf{W}^{-1}\mathbf{Z}_i \\ \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{X} & \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{Z}_s + \mathbf{A}^{-1}\lambda_1 & \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{Z}_i + \mathbf{A}^{-1}\lambda_2 \\ \mathbf{Z}_i'\mathbf{W}^{-1}\mathbf{X} & \mathbf{Z}_i'\mathbf{W}^{-1}\mathbf{Z}_s + \mathbf{A}^{-1}\lambda_2 & \mathbf{Z}_i'\mathbf{W}^{-1}\mathbf{Z}_i + \mathbf{A}^{-1}\lambda_3 \end{bmatrix} \begin{bmatrix} \boldsymbol{\beta} \\ \mathbf{a}_s \\ (\mathbf{a}_d - \mathbf{a}_s) \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{W}^{-1}\mathbf{y} \\ \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{y} \\ \mathbf{Z}_i'\mathbf{W}^{-1}\mathbf{y} \end{bmatrix}.$$

In contrast to the *imprinting model*, where the incidence matrix \mathbf{Z}_s comprises only one non-zero element per row, each row of \mathbf{Z}_s in the *equivalent model* contains two ones. The first links observations to the TAs of sires as sires. The second connects observations to the TAs of dams as sires. Incidence matrix \mathbf{Z}_i is identical to incidence matrix \mathbf{Z}_d in the *imprinting model* but in this case, it links observations to imprinting effects. All of the other quantities are the same as those defined earlier and the λ -values correspond to:

$$\begin{bmatrix} \lambda_1 & \lambda_2 \\ \lambda_2 & \lambda_3 \end{bmatrix}^{-1} = \frac{1}{2\sigma_e^2} \begin{bmatrix} \sigma_s^2 & (\sigma_{sd} - \sigma_s^2) \\ (\sigma_{sd} - \sigma_s^2) & \sigma_i^2 \end{bmatrix}.$$

In this study, we used the estimated variance-covariance components of the *imprinting model* to predict the imprinting effects and their PEVs using the *equivalent model* in a single iteration. The PEV was then used to calculate the reliability (r^2) of the imprinting effects, as follows:

$$r^2 = 1 - \frac{PEV}{1/2\sigma_i^2(1+F_i)}.$$

Effects in the model

The model includes the following effects:

$$y_{ijklmn} = SD_i + PN_j + BT_k + b_1x + b_2x^2 + b_3x^3 + a_{s_l} + a_{d_m} + e_{ijklmn},$$

where y_{ijklmn} is a beef trait record; SD_i the fixed effect of the i^{th} comparison group (combination of fattening farm and date of slaughter); PN_j the fixed effect of the j^{th} parity number (first, second and more calvings); BT_k the fixed effect of the k^{th} birth type (singleton or twin); b the linear (b_1), quadratic (b_2) and cubic (b_3) regression on slaughter age x ; a_{s_l} the random additive genetic effect as sire l ; a_{d_m} the random additive genetic effect as dam m ; and e_{ijklmn} the random residual. It should be noted that carcass fatness is used as a fixed effect in the routine genetic evaluation. However, we treated it as a trait because it is known to be genetically influenced. Modified equations for an animal model were used to consider missing parents within genetic groups, as described by Quaas and Pollak (1981) and Westell *et al.* (1988). Y-chromosomal and mitochondrial effects were not considered because neither were found to be of significant importance for beef traits (Reinsch *et al.*, 1999; Neugebauer *et al.*, 2010a;b). All of the variance-covariance components were estimated via the ASReml-package Version 3.0 (Gilmour *et al.*, 2009).

Most fattening bulls were assigned to conformation classes O and R (98%) and fat scores 2 and 3 (95%), so both traits were also treated as ordered categorical traits with binomial distributions. Thus, fattening bulls were classified either to class zero (conformation classes E, U and R; fat scores 1 and 2) or to class one (conformation classes O and P; fat scores 3, 4 and 5). A logit link was chosen for the GLMM because there is an anecdotal evidence that logit GLMM converges better than probit GLMM when the variance components are estimated using the pseudo-likelihood approach of Gilmour *et al.* (2009). The probability that an observation with index k belongs to class zero is:

$$\pi(\eta_k) = \exp(\eta_k) / [1 + \exp(\eta_k)],$$

where the linear predictor is:

$$\eta_k = x_k \boldsymbol{\beta} + z_{s,k} \mathbf{a}_s + z_{i,k} (\mathbf{a}_d - \mathbf{a}_s)$$

and x_k , $z_{s,k}$ and $z_{i,k}$ are the k^{th} rows of the aforementioned incidence matrices \mathbf{X} , \mathbf{Z}_s and \mathbf{Z}_i , respectively. The vectors $\boldsymbol{\beta}$, \mathbf{a}_s and $(\mathbf{a}_d - \mathbf{a}_s)$ are defined as described in the corresponding linear models.

Test of hypotheses

Tests for significant imprinting variance were performed as described by Neugebauer *et al.* (2010a;b). The null hypothesis assumed no imprinting effects, whereas the alternative hypothesis implied their existence. Two models were fitted per trait. The first corresponds to the *imprinting model* and the second to an animal model. We determined the model with the best fit to the data, thereby testing for the existence of significant imprinting effects, by comparing the REML log-likelihoods of both models using a REML likelihood ratio test (RLRT). The RLRT is asymptotically distributed as a mixture of two χ^2 -distributions with one and two df (Self and Liang, 1987). The mixture proportions deviate from 1:1 with correlated observations and they are difficult to determine, so we applied a conservative test with a χ^2 -distribution with two df (Neugebauer *et al.*, 2010a;b). This testing technique is only valid for linear mixed models (LMM). Using the GLMM, the ASReML-package employs an approximate likelihood (penalised quasi-likelihood) that cannot be used to test differences (Gilmour *et al.*, 2009).

RESULTS AND DISCUSSION

The equivalent model

According to Henderson's (1985) condition of equivalence, the *imprinting model* and *equivalent model* were assumed to yield the same results after linear transformation. The satisfaction of this condition was formally proved (Supplementary Material S1) and verified using simulated data sets from a previous study (Blunk and Reinsch, 2014). Moreover, we applied the *equivalent model* to Brown Swiss data in case the imprinting variance was significant. The *equivalent model* was found to require more iterations to converge in likelihood, which may differ when the variance components change. However, given the corresponding variance components, the major

advantage of this method is that the desired effects and their PEVs can be achieved within a single iteration using software packages such as ASReml. Further computations are not necessary because only the diagonal elements of the inverted coefficient matrix are required. For the equivalent model as a GLMM, a single iteration may not be sufficient because logit analyses are performed on an underlying scale using a working variable, which takes several iterations to stabilise. After the variable stabilised, the genetic parameters estimated using the *equivalent model* as a GLMM agreed completely with the genetic parameters estimated using the *imprinting model* as a GLMM.

In large routine applications inverting the coefficient matrix and the exact calculation of PEVs may become infeasible for either model. Consequently, approximations would be useful in such situations, as they already have been developed for different kinds of models (see e.g., Tier and Meyer, 2004 and the references herein). Such approximations, however, need to be evaluated if they work satisfactorily for the data structure of a certain breed or trait and the *equivalent model* may be a useful tool for that purpose.

It should be noted that the *equivalent model* relates three genetic effects to each observation: the TA of sire i as dam (a_{d_i}) and dam j as dam (a_{d_j}) plus the imprinting effect ($a_{s_i} - a_{d_i}$). Alternatively, the imprinting effect could be defined with an opposite sign as ($a_{d_j} - a_{s_j}$), which clearly leaves the imprinting variance unaffected. Then the three genetic effects were as follows: the TA of sire i as sire (a_{s_i}) and dam j as sire (a_{s_j}) plus the imprinting effect ($a_{d_j} - a_{s_j}$). The covariance between the imprinting effect and the two possible types of TAs in the model is either $(\sigma_{sd} - \sigma_d^2)$ or $(\sigma_{sd} - \sigma_s^2)$. Both covariances represent negative parental contributions to the imprinting variance (Neugebauer *et al.*, 2010a;b) and, when signs are reversed, add up to $\sigma_i^2 = (\sigma_d^2 - \sigma_{sd}) + (\sigma_s^2 - \sigma_{sd})$. Hence, their sum must be positive although a single covariance may become negative.

Reliability of parent-of-origin effects and genetic trends

The ease of PEV computation using the *equivalent model* facilitated a closer inspection of the reliability of the predicted POEs despite the huge number of animals included. For the net BW gain, the reliability of the POEs ranged from 0.09 to 0.82 for sires and from zero to 0.91 for dams, with an average of 0.27 for sires and 0.29 for dams. The reliability of the POEs on the fat scores ranged from zero to 0.84 for sires and zero to 0.42 for dams, with an average of 0.11 for

sires and 0.10 for dams. For the conformation class, the reliability of the POEs ranged from zero to 0.79 for sires and zero to 0.39 for dams, with an average of 0.09 for sires and 0.08 for dams. The reliability of the POEs generated using the GLMM had a slightly wider range from zero to 0.88 for both traits.

In general, the reliability of genetic estimates depends mainly on the availability of data such as individual records and kinship information (Mrode, 2014). The amount of kinship information depends mainly on the number of progeny. In the present study, a high number of male progeny was needed per animal, but a high number of daughters and maternal grandsons was also a necessary prerequisite because the *imprinting model* includes the genetic effect as sire as well as the genetic effect as dam. This was highlighted by our analysis of given family structures. For example, sires with differences in the average reliability for the estimated POEs on the net BW gain had different average numbers of sons, daughters and maternal grandsons, as follows:

$r^2 < 0.30$	0.74	11.31	4.86
$0.30 \leq r^2 \leq 0.40$	0.96	17.47	9.37
$r^2 > 0.40$	3.57	37.67	7.87

The left-hand side of Fig. 1 shows the POEs estimated for each individual (horizontal axes) using the *equivalent model* as LMM and GLMM relative to its reliability. The regression of less reliable POEs to their expected mean of zero was observed for all traits. By contrast, more reliable effects exhibited increasing variation, most of which could be assigned to male animals because males are biologically capable of having more kinship information than females. An exception was the net BW gain, where the most reliable POEs could be assigned to females. These animals were mostly bull dams with sons, which were also sires of many sons and daughters. This yielded an informative family structure, which was facilitated by the large amount of pedigree available for net BW gain. The panels on the right in Fig. 1 illustrate the changes in the reliability of the POEs for animals born from 1940 to 2010. An increase, especially for males, was followed by a drop in the presence of the top reliabilities from about 2000. This increase was related to the growing amount of available data collected from 1994. However, younger animals had less opportunity to accumulate information from grandsons, which explains the lack of top reliabilities (> 0.40) in the more recent birth cohorts.

Overall, the genetic trends in the TAs and POEs appeared to be fairly constant, with the exception of a clear undesired trend in the conformation class, which was almost identical in the LMM and GLMM (Fig. 2). This trend is attributable to a correlated response to intense selection for milk performance in Brown Swiss, as well as the slightly positive genetic trend in the net BW gain.

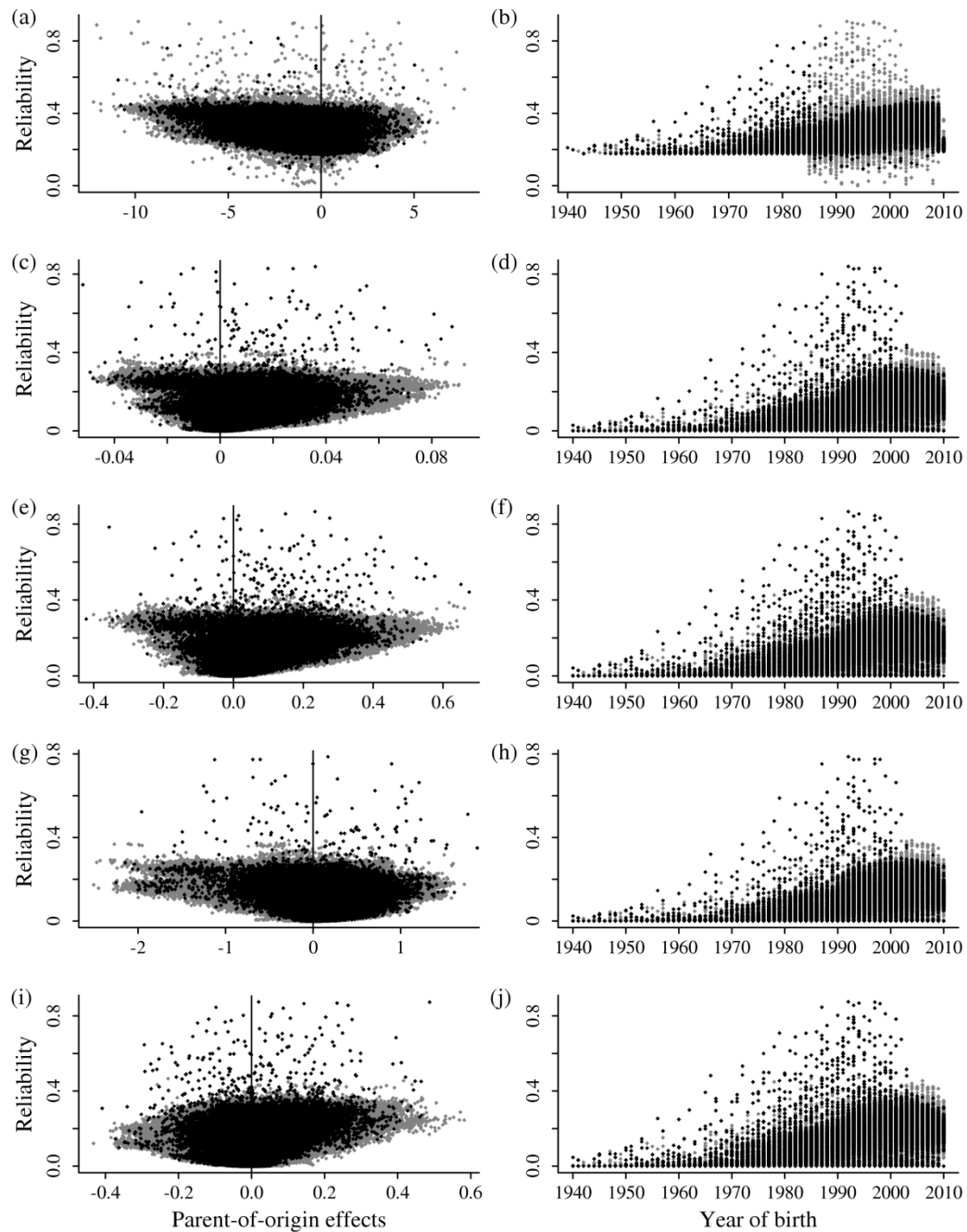


Figure 1. Parent-of-origin effects for sires (black) and dams (grey) relative to their reliability (left side), as well as their reliability relative to the year of birth (right side). Parent-of-origin effects were estimated using a linear mixed model for the net BW gain in (g/days) (a, b), fat score (c, d) and conformation class (g, h). Parent-of-origin effects were estimated using a generalised linear mixed model for the fat score (e, f) and conformation class (i, j).

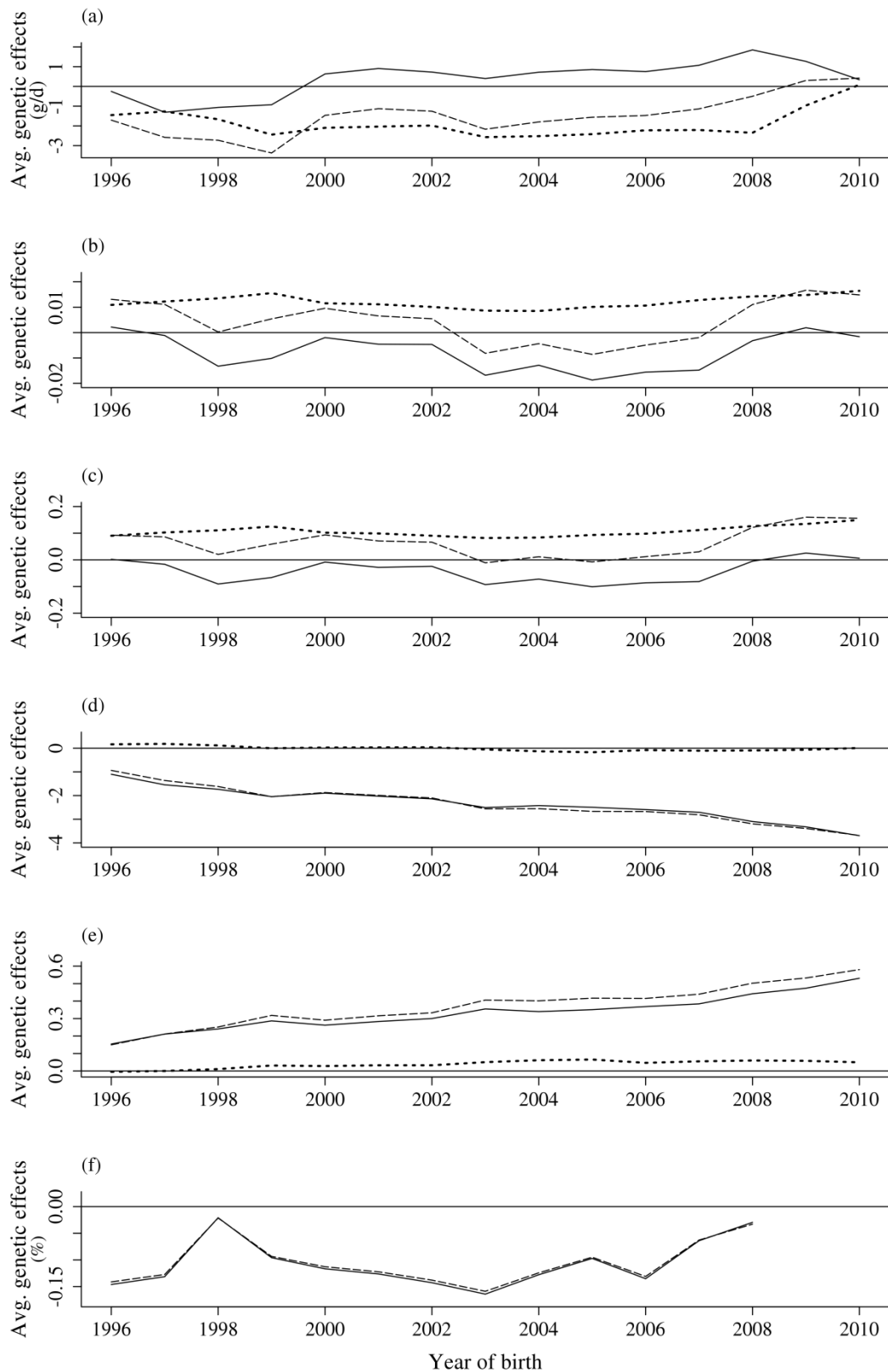


Figure 2. Average parent-of-origin effects (dotted line) and transmitting abilities for animals as sire (solid line) and as dam (dashed line) relative to the year of birth. The genetic effects were estimated using a linear mixed model for the net BW gain (a), fat score (b), conformation class (d) and killing out percentage (f). A generalised linear mixed model was used for the fat score (c) and conformation class (e).

Significance of imprinting variances

Significant imprinting variances were found for the net BW gain, fat score and conformation class (Table 2), where the error probabilities were all < 0.001 . The estimated imprinting variance accounted for 10.58% of the total additive genetic variance in the net BW gain, as well as 9.17% for the fat score and 9.12% for the conformation class. The imprinting variances were driven by both deviating parental variances and imperfect correlations of about 0.9 between parental effects. It should be mentioned that this correlation was not constrained to one (Tier and Meyer, 2012) to ensure that the test for the existence of the imprinting variance remained as general as possible.

The significances could not be tested formally, but the imprinting variances obtained from the GLMM accounted for slightly higher proportions of the total genetic variance. The ratios of 11.31% and 13.95% were in good agreement with the proportions obtained from the LMM (Table 2), although the absolute values are not directly comparable because the GLMM operates on an unobservable logit scale (Dempster and Lerner, 1950).

The analysis yielded no significant results for the killing out percentage ($P = 0.704$). The incorporation of genetic groups had no noticeable impact on the estimates of genetic parameters for all of the traits analysed.

There are no comparable studies with respect to POEs in Brown Swiss cattle, so comparisons were made with the POE analysis conducted in German Simmental by Neugebauer *et al.* (2010b). In contrast to our findings, they found that the killing out percentage was significantly affected by POEs, where the imprinting variance accounted for 24% of the total genetic variance. However, we only had 3,226 observations, so our study was clearly underpowered for this trait. Moreover, there were no significant imprinting variances for the net BW gain, which agreed with the findings of Engellandt and Tier (2002) in German Gelbvieh. However, comparisons with different breeds should be treated with caution because there is a great emphasis on milk performance in Brown Swiss, whereas German Simmental and German Gelbvieh are dual-purpose breeds, with some focus on the beef performance.

The relative imprinting variance determined by Neugebauer *et al.* (2010b) for the conformation class was similar to our results, but their estimated relative imprinting variance for the fat score (24.77%) was more than double the proportion estimated for the fat score in our study. In addition to differences in the breed backgrounds, this may be explained by the fact that different

recording techniques were used. Thus, instead of using five visually observed scores, Neugebauer *et al.* (2010b) employed 15 automatically video-recorded categories, which probably captured the actual degree of phenotypic variation with much greater precision.

Allelic contributions to the imprinting variance

The parental contributions of gametes to the imprinting variance can be calculated as $(\sigma_s^2 - \sigma_{sd})/\sigma_i^2$ for the paternal contribution and $(\sigma_d^2 - \sigma_{sd})/\sigma_i^2$ for the maternal contribution. For the net BW gain, the relative contribution of maternal alleles to the imprinting variance was almost exactly 100% (Table 2).

For the carcass quality traits, the maternal gamete contributed 87.7% to the imprinting variance in the fat score and 92.7% to the imprinting variance in the conformation class (Table 2). The standard errors of these contributions were larger (22.2% and 28.3%) than the respective paternal contributions of 12.3% and 7.3%. However, the results obtained by GLMM were different, where the covariance between paternal and maternal effects was larger than the paternal variance, thereby resulting in negative paternal contributions of -35.7% for the fat score and -37.9% for the carcass conformation. Both values exceeded their standard errors (20.7% and 20.0%, respectively) in magnitude by about one-third or more. Thus, the maternal contributions for both traits were almost exactly four-thirds.

Our findings differed from those obtained by Neugebauer *et al.* (2010b), who attributed most of the imprinting variance in the fat score and conformation class to paternal gametes in dual-purpose German Simmental. However, our results obtained in Brown Swiss agreed with an analysis based on ultrasonic measures of body composition in Australian beef cattle (Tier and Meyer, 2012). In principle, maternal genetic effects can lead to overestimates of the imprinting variance (Hager *et al.*, 2008), and thus the estimated maternal contributions. On the other hand, unaccounted paternally inherited effects may lead to biased estimates for variance components in models with maternal genetic and direct effects (Varona *et al.*, 2015). We cannot rule out the existence of maternal genetic effects in the Brown Swiss data set by own investigations. They are, however, generally considered unimportant and they are not included in the models used for routine genetic evaluations for that breed, mainly on the practical grounds that the separation of calves from their dams shortly after birth is a common practice and they are raised with a formula diet. It should also be noted that Tier and Meyer (2012) attributed the occurrence of

Table 2. Genetic parameters, correlation coefficient, test statistic and variance component ratios estimated using linear and generalised linear mixed models with two additive genetic effects per animal for all traits. The standard errors are given in brackets.

Trait	\hat{h}^2	$\hat{\sigma}_a^2$	$\hat{\sigma}_s^2$	$\hat{\sigma}_d^2$	$\hat{\sigma}_{sd}$	$\hat{\sigma}_e^2$	\hat{r}	RLRT ¹	$\frac{\hat{\sigma}_i^2}{\hat{\sigma}_a^2}$	$\frac{(\hat{\sigma}_s^2 - \hat{\sigma}_{sd})}{\hat{\sigma}_i^2}$	$\frac{(\hat{\sigma}_d^2 - \hat{\sigma}_{sd})}{\hat{\sigma}_i^2}$
Net BW gain (g/days)	0.28 (0.01)	804.960 (34.870)	359.660 (18.48)	445.300 (25.468)	359.890 (18.074)	2071.200 (27.362)	0.899 (0.020)	122.8***	10.58 (2.10)	-0.3 (16.8)	100.3 (16.8)
Fat score	0.23 (0.01)	0.044 (0.002)	0.021 (0.001)	0.024 (0.002)	0.020 (0.001)	0.149 (0.002)	0.910 (0.022)	69.2***	9.17 (2.20)	12.3 (22.2)	87.7 (22.2)
Fat score ²	0.46 (0.02)	1.960 (0.085)	0.790 (0.052)	1.170 (0.061)	0.869 (0.048)	3.290	0.904 (0.025)	-	11.31 (2.58)	-35.7 (20.7)	135.7 (20.7)
Conformation class	0.16 (0.01)	58.614 (3.900)	27.027 (1.980)	31.587 (2.850)	26.634 (1.980)	320.080 (3.289)	0.912 (0.029)	27.6***	9.12 (2.80)	7.3 (28.3)	92.7 (28.3)
Conformation class ²	0.43 (0.02)	1.796 (0.083)	0.678 (0.049)	1.119 (0.062)	0.773 (0.048)	3.290	0.888 (0.031)	-	13.95 (3.26)	-37.9 (20.0)	137.9 (20.0)
Killing out percentage (%)	0.52 (0.09)	1.348 (0.267)	0.701 (0.138)	0.648 (0.201)	0.672 (0.135)	1.262 (0.221)	0.998 (0.096)	0.7	-	-	-

\hat{h}^2 = heritability; $\hat{\sigma}_a^2$ = additive genetic variance; $\hat{\sigma}_s^2$ = additive genetic variance as sire; $\hat{\sigma}_d^2$ = additive genetic variance as dam; $\hat{\sigma}_{sd}$ = covariance; $\hat{\sigma}_e^2$ = residual variance; \hat{r} = correlation between parental effects; $\hat{\sigma}_i^2$ = imprinting variance; $\hat{\sigma}_i^2/\hat{\sigma}_a^2$ = relative imprinting variance (%); $(\hat{\sigma}_s^2 - \hat{\sigma}_{sd})/\hat{\sigma}_i^2$ = paternal contribution to the imprinting variance (%); $(\hat{\sigma}_d^2 - \hat{\sigma}_{sd})/\hat{\sigma}_i^2$ = maternal contribution to the imprinting variance (%).

¹REML likelihood ratio test; RLRT = 2(log likelihood_{Imprinting model} - log likelihood_{Animal model}).

²Treated as ordered categorical traits using a generalised linear mixed model.

*** $P < 0.001$.

negative contributions (the covariance exceeds one of both variances) to the effects of partially imprinted loci because fully imprinted loci only contribute to the variances.

Heritability

To estimate the heritability (h^2) for all of the given slaughter traits, we first used linear animal models. The estimated values of h^2 obtained from animal models are summarized in Table 1. The results obtained from the LMM were 0.52 for the killing out percentage, 0.26 for net BW gain, 0.22 for fat score and 0.15 for conformation class. GLMM obviously captured a larger proportion of the genetic variability with estimates of 0.46 for the fat score and 0.43 for conformation class. The standard errors were not > 0.02 , with the exception of the killing out percentage with 0.08. The resulting estimates were quite similar (Table 2) when imprinting was part of the model, with a small increase of about 1% compared with the results obtained by the LMM. The standard errors of the heritabilities also remained about the same (Table 2).

To the best of our knowledge, the genetic parameters of beef traits have not been reported previously for Brown Swiss cattle. In German Simmental, Neugebauer *et al.* (2010b) estimated an h^2 value of 0.22 for the killing out percentage using the same imprinting model. A considerably higher value of 0.50 was found for the dressing percentage in German Gelbvieh fattening bulls (Engellandt *et al.*, 1999b), which are raised under comparable production circumstances. The latter estimate agrees well with our result of slightly $> 50\%$ for Brown Swiss. Neugebauer *et al.* (2010b) estimated h^2 values of 0.28 for the net BW gain and 0.25 for the fat score in German Simmental. These results agree almost perfectly with our results. However, for the conformation class, their estimate of 0.31 was double our estimate of about 0.15. As mentioned earlier, Neugebauer *et al.* (2010b) automatically video-recorded the carcass quality traits according to a scale with 15 different categories, whereas our data comprised a coarse subjective categorization with only five categories. Despite this difference, the GLMM picked up a high h^2 value of 0.43, which was similar to the estimate obtained by Neugebauer *et al.* (2010b). In contrast, lower h^2 values of 0.22 and 0.12 were obtained for the conformation class and fat score in a Bayesian analysis of German Simmental data (Reinsch *et al.*, 1999), where both traits were treated as dichotomous binary traits.

Generalized linear mixed model v. linear mixed model

We determined high correlations between the POEs predicted using the *equivalent model* as LMM and using the *equivalent model* as GLMM, with values of 0.95 for the fat score and -0.90 for the conformation class (the negative sign is due to the reversed order of categories in the GLMM). High correlations were also obtained for the reliability of the POEs, with values of 0.98 and 0.97. The clear linear relationships between the TAs from the GLMM and the TAs from the LMM can be seen in Fig. 3 for the fat score (a) and conformation class (b). The residual variation in the TAs obtained from the GLMM regressed on the TAs from LMM was fairly constant over the entire range for the conformation class (Fig. 3d), whereas the variation increased slightly more for the fat score for larger TAs (Fig. 3b). Overall, these comparisons demonstrate that both models accounted for a large proportion of the same type of variation, although there was no full agreement in terms of the respective estimates of genetic effects.

The LMM yields estimates that can be interpreted directly in terms of monetary differences but this is not the case with the GLMM. Kempster *et al.* (1982) reported that the conformation class only explained approximately 30% of the variation in meat content. Therefore, from a biological perspective, the nature of the underlying continuity (Falconer, 1960) is not clear for the conformation class. In contrast, Drennan *et al.* (2008) reported a high positive correlation ($r = 0.83$) between the carcass fat score and carcass fat proportion in bulls, and thus it is plausible that the underlying continuous variable for the fat class is generally identical to the carcass fatness. However, the approximate monetary value of a one unit change in the TA on the underlying scale for the carcass conformation can be derived by assessing the associated changes in the average frequencies for all categories and in the average value of a carcass.

CONCLUSION

In this study, we developed a new model that facilitates the direct estimation of imprinting effects and PEVs for a large number of animals using existing software. Furthermore, we determined significant imprinting variances for three of four beef traits analysed in Brown Swiss fattening bulls using LMM and GLMM. The imprinting variances accounted for approximately 10% of the total genetic variance, where the maternal gametes provided the largest contributions. These findings highlight the importance of POE and support the need to incorporate them into selection decisions.

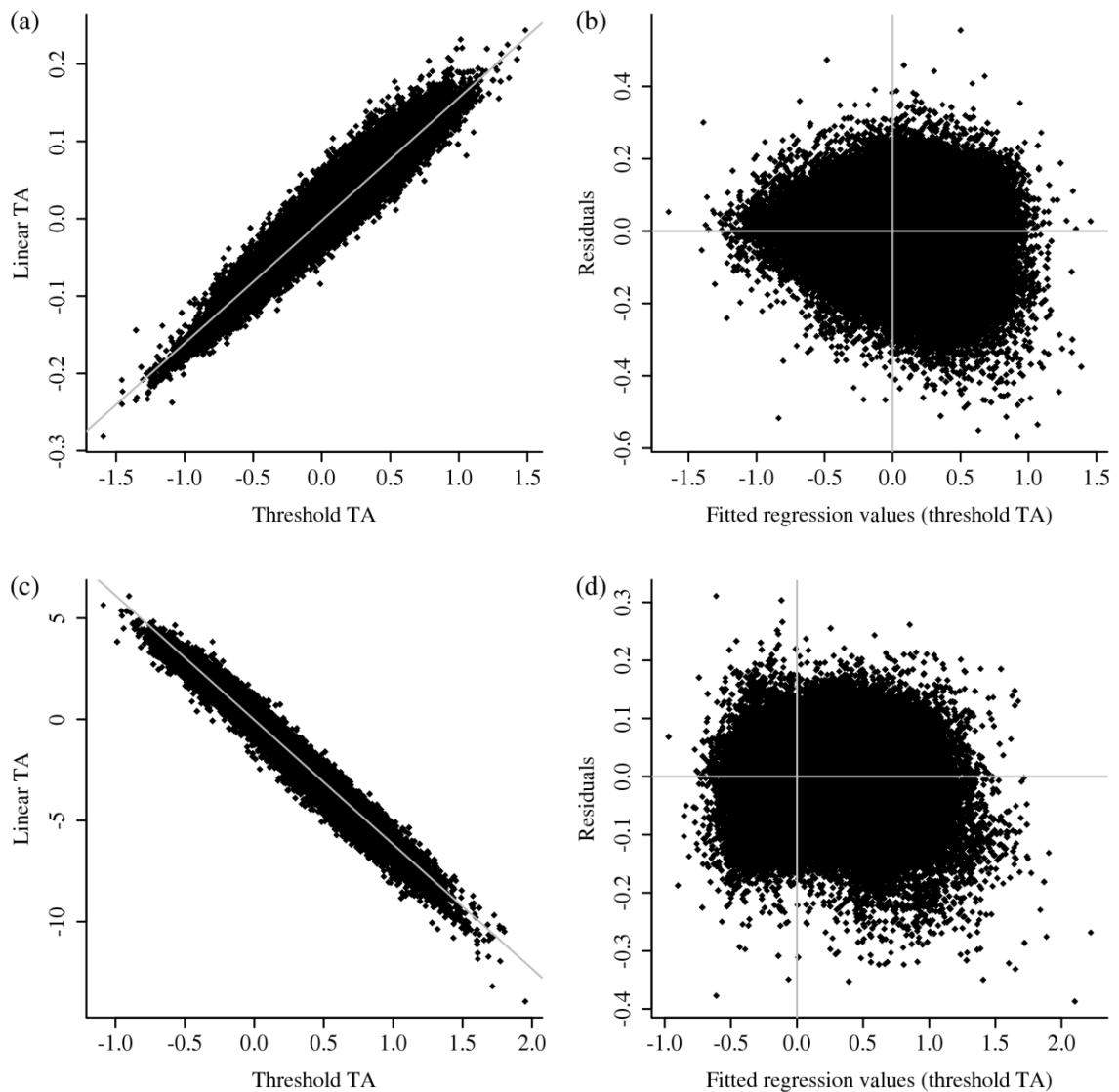


Figure 3. Correlations between transmitting abilities (TA, left side) estimated using a linear (linear TA) and a generalised linear mixed model (threshold TA). The threshold TA was fitted using the linear TAs as independent variables with respect to their residuals (right side) for the fat score (a, b) and conformation class (c, d).

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SUPPLEMENTARY MATERIAL

Proof of model equivalence

Model equivalence in Henderson's sense (1985) requires the expectation $E(\mathbf{y})$ and variance $Var(\mathbf{y})$ of the observation vector to be identical under the specifications of both models. Both models do not differ in their fixed effects parts and all random variables have zero expectations. Therefore, $E(\mathbf{y})$ is obviously equal for the imprinting model and the equivalent model with:

$$E(\mathbf{y}) = \mathbf{X}\boldsymbol{\beta}.$$

The variance of the observations $Var(\mathbf{y})$ in the imprinting model can be written as:

$$\begin{bmatrix} \mathbf{Z}_s & \mathbf{Z}_d \end{bmatrix} \left[1/2 \mathbf{A} \otimes \begin{pmatrix} \sigma_s^2 & \sigma_{sd} \\ \sigma_{sd} & \sigma_d^2 \end{pmatrix} \right] \begin{bmatrix} \mathbf{Z}'_s \\ \mathbf{Z}'_d \end{bmatrix} + \mathbf{W}\sigma_e^2.$$

The second part of the latter sum reflects the residual variation and is identical in both models, as already explained. The first part is associated with the numerator relationship matrix \mathbf{A} and depicts the covariance of observations due to the resemblance of relatives. After multiplication this covariance becomes:

$$\mathbf{Z}_s 1/2 \mathbf{A} \sigma_s^2 \mathbf{Z}'_s + \mathbf{Z}_d 1/2 \mathbf{A} \sigma_{sd} \mathbf{Z}'_s + \mathbf{Z}_s 1/2 \mathbf{A} \sigma_{sd} \mathbf{Z}'_d + \mathbf{Z}_d 1/2 \mathbf{A} \sigma_d^2 \mathbf{Z}'_d. \quad (1)$$

The variance of the observations $Var(\mathbf{y})$ in the equivalent model can be expressed in terms of the incidence matrices and variance components from the imprinting model. In particular, the incidence matrix for the TA of sires as sires in the equivalent model is the sum of \mathbf{Z}_s and \mathbf{Z}_d in the imprinting model, and the incidence matrix \mathbf{Z}_i for imprinting effects in the equivalent model equals \mathbf{Z}_d from the imprinting model, as explained before. Therefore, $Var(\mathbf{y})$ in the equivalent model becomes:

$$\begin{bmatrix} (\mathbf{Z}_s + \mathbf{Z}_d) & \mathbf{Z}_d \end{bmatrix} \left[1/2 \mathbf{A} \otimes \begin{pmatrix} \sigma_s^2 & \sigma_{sd} - \sigma_s^2 \\ \sigma_{sd} - \sigma_s^2 & \sigma_s^2 + \sigma_d^2 - 2\sigma_{sd} \end{pmatrix} \right] \begin{bmatrix} (\mathbf{Z}_s + \mathbf{Z}_d)' \\ \mathbf{Z}'_d \end{bmatrix} + \mathbf{W}\sigma_e^2.$$

Again we consider only the first part of this sum, which is associated with \mathbf{A} , in detail. Multiplication gives:

$$\begin{aligned}
& (\mathbf{Z}_s + \mathbf{Z}_d)1/2 \mathbf{A} \sigma_s^2 (\mathbf{Z}_s + \mathbf{Z}_d)' + (\mathbf{Z}_s + \mathbf{Z}_d)1/2 \mathbf{A} (\sigma_{sd} - \sigma_s^2) \mathbf{Z}_d' + \mathbf{Z}_d 1/2 \mathbf{A} (\sigma_{sd} - \sigma_s^2) (\mathbf{Z}_s + \mathbf{Z}_d)' \\
& + \mathbf{Z}_d 1/2 \mathbf{A} (\sigma_s^2 + \sigma_d^2 - 2\sigma_{sd}) \mathbf{Z}_d' \\
& = \mathbf{Z}_s 1/2 \mathbf{A} \sigma_s^2 \mathbf{Z}_s' + \mathbf{Z}_s 1/2 \mathbf{A} \sigma_s^2 \mathbf{Z}_d' + \mathbf{Z}_d 1/2 \mathbf{A} \sigma_s^2 \mathbf{Z}_s' + \mathbf{Z}_d 1/2 \mathbf{A} \sigma_s^2 \mathbf{Z}_d' \\
& + \mathbf{Z}_s 1/2 \mathbf{A} \sigma_{sd} \mathbf{Z}_d' - \mathbf{Z}_s 1/2 \mathbf{A} \sigma_s^2 \mathbf{Z}_d' + \mathbf{Z}_d 1/2 \mathbf{A} \sigma_{sd} \mathbf{Z}_d' - \mathbf{Z}_d 1/2 \mathbf{A} \sigma_s^2 \mathbf{Z}_d' \\
& + \mathbf{Z}_d 1/2 \mathbf{A} \sigma_{sd} \mathbf{Z}_s' - \mathbf{Z}_d 1/2 \mathbf{A} \sigma_s^2 \mathbf{Z}_s' + \mathbf{Z}_d 1/2 \mathbf{A} \sigma_{sd} \mathbf{Z}_d' - \mathbf{Z}_d 1/2 \mathbf{A} \sigma_s^2 \mathbf{Z}_d' \\
& + \mathbf{Z}_d 1/2 \mathbf{A} \sigma_s^2 \mathbf{Z}_d' + \mathbf{Z}_d 1/2 \mathbf{A} \sigma_d^2 \mathbf{Z}_d' - 2\mathbf{Z}_d 1/2 \mathbf{A} \sigma_{sd} \mathbf{Z}_d' \\
& = \mathbf{Z}_s 1/2 \mathbf{A} \sigma_s^2 \mathbf{Z}_s' + \mathbf{Z}_d 1/2 \mathbf{A} \sigma_{sd} \mathbf{Z}_s' + \mathbf{Z}_s 1/2 \mathbf{A} \sigma_{sd} \mathbf{Z}_d' + \mathbf{Z}_d 1/2 \mathbf{A} \sigma_d^2 \mathbf{Z}_d'. \quad q.e.d.
\end{aligned}$$

The latter is identical to term 1. In conclusion both conditions from Henderson (1985) are fulfilled and model equivalence has been formally proofed.

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CHAPTER 3

Parsimonious model for analyzing parent-of-origin effects related to beef traits in dual-purpose Simmental

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Parsimonious model for analyzing parent-of-origin effects related to beef traits in dual-purpose Simmental

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ABSTRACT

Genomic imprinting occurs when allelic effects depend on their parental origin. These parent-of-origin effects (POEs) occur due to epigenetic DNA modifications during gametogenesis according to the sex of an animal. Animal breeding programs give little consideration to imprinting, although its relationship to important traits has been shown in different agricultural species. To incorporate imprinting, a previously proposed model (imprinting model) contains the genetic effects of the sire and dam, and it provides an estimate of the variance component due to POEs, which is referred to as imprinting variance. Large volumes of data are sometimes available for commercial populations, so the dimension of mixed model equations can become very large or even excessively large when estimating imprinting variances and other genetic parameters. To address this issue, we replaced the genetic effect as dam with the effect of the maternal grandsire in the imprinting model. When combined with appropriate weightings of the observations, this replacement yields an imprinting model with a parsimonious number of genetic effects for male parents and ancestors of slaughter animals, and it enables the inclusion of large volumes of data. In addition, we derived an equivalent model to facilitate the direct estimation of POEs and their prediction error variances. We applied the parsimonious model to 1,366,160 fattening bulls as well as a pedigree of 2,637,761 ancestors to investigate the relevance of POEs for beef performance in dual-purpose Simmental. We analyzed the killing out percentage, net body weight gain, carcass muscularity, and fat score as slaughter traits. The parsimonious model was applied as both linear and generalized linear versions with a logit link function. The proportions of the total genetic variance attributable to POEs ranged between 8.6% and 17.1%. For three of the four traits, the maternal gamete accounted for a greater proportion of the imprinting variance. The effects of POEs and their reliabilities were estimated for up to

27,567 bulls and all traits, where the reliabilities ranged between zero and 0.98. Thus, our new parsimonious model is appropriate for estimating the imprinting variance using large pedigree data sets. Our results highlight the need to consider POEs in genetic evaluations.

Keywords: carcass trait, epigenetics, genomic imprinting, maternal grandsire, parent-of-origin effect, Simmental

INTRODUCTION

Genomic imprinting is related to the field of epigenetics where parental alleles exhibit different degrees of expression. For example, the *Igf2* gene in mice is purely paternally expressed and thus fully maternally imprinted (DeChiara *et al.*, 1991). In addition, incomplete lack of expression is known as partial imprinting. The strategies employed for investigating imprinting effects, which are included under the more general term of parent-of-origin effects (POEs), comprise QTL mapping experiments (e.g., Van Laere *et al.*, 2003), expression studies (e.g., Lopes Pinto *et al.*, 2014), and variance components analyses. For the latter, Neugebauer *et al.* (2010a;b) proposed a specialized reduced model with two correlated genetic effects, where the first mirrors a gene's action under a paternal expression pattern, whereas the second is its maternal counterpart. This model simultaneously accommodates imprinted loci of all types, i.e., fully, partially, paternally, or maternally imprinted. This model estimates the transmitting abilities (TAs) of only parents, but not the genetic effects of slaughter animals without own progenies. However, the number of equations required with large volumes of data may be a limiting factor during the estimation of genetic parameters. Moreover, although the imprinting effect can be derived easily as the difference between the two parental effects, the derivation of its reliability is computationally demanding.

In the present study, we aimed to analyze four slaughter traits comprising 1,366,160 Simmental fattening bulls as well as pedigrees of up to 2,637,761 animals to investigate the existence of variance components due to POEs and to obtain estimates of their size when significant. Therefore, we propose a model that contains the paternal effects of sires but the effect as maternal grandsire (MGS) replaces the effect as dam, which further reduces the number of equations required. An equivalent model facilitates the direct estimation of POEs and it is easier to calculate their reliabilities.

MATERIALS AND METHODS

Slaughter house and pedigree data

A comprehensive set of slaughter data for Austrian and German Fleckvieh fattening bulls was collected within the scope of progeny field tests between 1995 and 2014. Data for four value-determining slaughter traits, which were routinely recorded by all 62 abattoirs, were provided by the genetic evaluation center of Landesamt für Geoinformation und Landentwicklung in Baden-Württemberg, Germany. These data were obtained routinely so Institutional Animal Care and Use Committee approval was not obtained. The traits analyzed comprised the killing out percentage (carcass weight divided by live weight, %), net BW gain (carcass weight divided by age, g/d), carcass fatness, and carcass muscularity. The last three traits were available for 1,366,160 fattening bulls after editing. For 358,830 animals, considerably less data were available for the killing out percentage because its recording is not mandatory in abattoirs. The carcass fatness was defined using classes of 1 (very lean) to 5 (very fat), which were basically assigned according to the external fat cover on the carcasses. Carcass muscularity was assessed according to the European muscle conformation classification of E-U-R-O-P (E = excellent to P = poor), but it was replaced by monetary values (670-655-635-585-525) to reflect the fact that price differences between grades remain stable, whereas prices in general may differ over time (e.g., Engellandt *et al.*, 1999). A summary of all the data sets is given in Table 1. The pedigrees comprised 1,172,530 for the killing out percentage and 2,637,761 for the net BW gain, carcass fatness, and carcass muscularity.

Table 1. Number of fattening bulls (*n*), mean, SD, additive genetic variance, heritability, size of pedigree, and number of male ancestors for all traits. SE are shown in parentheses.

Trait	<i>n</i>	Mean	SD	Additive genetic variance ¹	h^2_1	h^2_2	Size of pedigree	Number of male ancestors
Killing out percentage, %	358,830	57.83	2.08	0.97 (0.029)	0.326 (0.008)	-	1,172,530	20,844
Net BW gain, g/d	1,366,160	671.44	88.22	649.70 (15.661)	0.207 (0.005)	-	2,637,761	27,567
Carcass fatness	1,366,160	2.59	0.53	0.05 (0.001)	0.198 (0.004)	0.321 (0.007)	2,637,761	27,567
Carcass muscularity	1,366,160	648.05	13.38	31.00 (0.767)	0.199 (0.004)	0.311 (0.007)	2,637,761	27,567

¹Estimated using a linear model with a sire effect assuming no imprinting.

²Estimated using a generalized linear model with a sire effect assuming no imprinting.

Imprinting model based on paternal genetic effects

To investigate the effects of genomic imprinting, Neugebauer *et al.* (2010a;b) introduced a model (imprinting model) that accounts for imprinting by fitting two correlated genetic effects per animal: one as sire (reflecting the paternal expression pattern) and one as dam (reflecting the maternal expression pattern). If genomic imprinting is present, these effects are different, and the variation in this difference is referred to as the imprinting variance. All genes affected by imprinting contribute to this variance, regardless of whether the imprinting is full or partial. Neugebauer *et al.* (2010a;b) used a special type of reduced model in their study (Quaas and Pollak, 1980). This model is particularly suitable for genetic evaluations of beef cattle because records originate only from non-parents (fattening bulls), and the effects are fitted only for their ancestors, which reduces the number of equations considerably. For Simmental, large data sets are available because of the extensive collection of data for genetic evaluation purposes. However, when using this information, the dimensionality of mixed model equations becomes excessively large during the estimation of variance components, even when a reduced model is used. To address this problem, we replaced the genetic effect as dam with the genetic effect as MGS in the *imprinting model*. Thus, according to the *imprinting model*, the new model (MGS imprinting model) contains two genetic effects per animal: one as sire (reflecting the paternal expression pattern) and one as MGS (reflecting the maternal expression pattern). Hence, the number of equations is reduced further because genetic effects are predicted only for male ancestors. As a consequence, the pedigrees were decreased to 20,844 ancestors for the killing out percentage data set (with up to 10 generations) and to 27,567 ancestors for the net BW gain, carcass fatness, and carcass muscularity data sets (with up to 10 generations). The model can be written as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_s\mathbf{a}_s + \mathbf{Z}_{mgs}\mathbf{a}_{mgs} + \mathbf{e}, \quad [1]$$

where \mathbf{y} is equal to the phenotype, $\boldsymbol{\beta}$ describes a vector of fixed effects, and \mathbf{a}_s and \mathbf{a}_{mgs} correspond to the vectors of TAs as sire and as MGS, respectively. In this case, $2\mathbf{a}_{mgs}$ is equivalent to \mathbf{a}_d , which corresponds to the TA as dam in the *imprinting model*, \mathbf{e} describes a random residual, and \mathbf{X} , \mathbf{Z}_s , and \mathbf{Z}_{mgs} are incidence matrices that connect the corresponding

effects to their respective observations. The covariance components of the genetic effects in terms of gametic variances (Neugebauer *et al.*, 2010a;b) are assumed to be:

$$\text{Var} \begin{bmatrix} \mathbf{a}_s \\ \mathbf{a}_{mgs} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} 1/2\mathbf{A}\sigma_s^2 & 1/2(1/2\mathbf{A}\sigma_{s,d}) & 0 \\ 1/2(1/2\mathbf{A}\sigma_{s,d}) & 1/4(1/2\mathbf{A}\sigma_d^2) & 0 \\ 0 & 0 & \mathbf{W} \end{bmatrix}. \quad [2]$$

Note that the *MGS imprinting model* determines the variance in the TAs as MGS ($1/2\sigma_{mgs}^2$) and the covariance between \mathbf{a}_s and \mathbf{a}_{mgs} ($1/2\sigma_{s,mgs}$). A conversion factor of four is required to convert the former to half of the gametic variance as dam ($1/2\sigma_d^2$), and a conversion factor of two is required to convert the latter to $1/2\sigma_{s,d}$, that is, the covariance between $1/2\sigma_s^2$ and $1/2\sigma_d^2$. The mixed model equations can be written as:

$$\begin{bmatrix} \mathbf{X}'\mathbf{W}^{-1}\mathbf{X} & \mathbf{X}'\mathbf{W}^{-1}\mathbf{Z}_s & \mathbf{X}'\mathbf{W}^{-1}\mathbf{Z}_{mgs} \\ \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{X} & \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{Z}_s + \mathbf{A}^{-1}\lambda_1 & \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{Z}_{mgs} + \mathbf{A}^{-1}\lambda_2 \\ \mathbf{Z}_{mgs}'\mathbf{W}^{-1}\mathbf{X} & \mathbf{Z}_{mgs}'\mathbf{W}^{-1}\mathbf{Z}_s + \mathbf{A}^{-1}\lambda_2 & \mathbf{Z}_{mgs}'\mathbf{W}^{-1}\mathbf{Z}_{mgs} + \mathbf{A}^{-1}\lambda_3 \end{bmatrix} \begin{bmatrix} \boldsymbol{\beta} \\ \mathbf{a}_s \\ \mathbf{a}_{mgs} \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{W}^{-1}\mathbf{y} \\ \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{y} \\ \mathbf{Z}_{mgs}'\mathbf{W}^{-1}\mathbf{y} \end{bmatrix}, \quad [3]$$

where \mathbf{A} is the additive genetic relationship matrix containing the relationships between all sires and MGS of fattening bulls and $\boldsymbol{\lambda}$ is the ratio of the covariance components, which corresponds to:

$$\begin{bmatrix} \lambda_1 & \lambda_2 \\ \lambda_2 & \lambda_3 \end{bmatrix}^{-1} = \frac{1}{2\sigma_R^2} \begin{bmatrix} \sigma_s^2 & 1/2\sigma_{s,d} \\ 1/2\sigma_{s,d} & 1/4\sigma_d^2 \end{bmatrix}, \quad [4]$$

where σ_R^2 is the residual variance in a reduced model. \mathbf{W} is a diagonal matrix for adjusting the variation in the residuals relative to the variation in Mendelian sampling due to the parental inbreeding coefficients, which has the diagonal elements w_{ii} :

$$w_{ii} = \left[\frac{\sigma_R^2 - 1/2 F_{s_i} \sigma_s^2 - 1/8 F_{mgs_i} \sigma_d^2}{\sigma_R^2} \right]^{-1}, \quad [5]$$

where F_s and F_{mgs} are the inbreeding coefficients of sires and MGS, respectively, derived from a pedigree containing sires and dams. The imprinting variance defined in Neugebauer *et al.* (2010a;b) can be expressed as:

$$\sigma_i^2 = \sigma_s^2 + \sigma_d^2 - 2\sigma_{s,d}. \quad [6]$$

The additive genetic variance can be estimated as:

$$\sigma_a^2 = \sigma_s^2 + \sigma_d^2. \quad [7]$$

In general, the additive genetic variance is the sum of the imprinting variance and the genetic part of the variance that is not affected by imprinting. The definition of h^2 can be written as:

$$h^2 = \frac{\sigma_s^2 + \sigma_d^2}{1/2\sigma_s^2 + 1/8\sigma_d^2 + \sigma_R^2}. \quad [8]$$

Model effects

According to the standard genetic evaluation, the genetic parameters are estimated based on the following model effects:

$$y_{ijklmn} = BS_i + KN_j + GT_k + b_1x + b_2x^2 + b_3x^3 + a_{s_l} + a_{mgs_m} + e_{ijklmn}, \quad [9]$$

where all of the beef trait records are described using y_{ijklmn} ; BS_i corresponds to the fixed effect of the interaction between farm and slaughter date summarized for i comparison groups (with no less than five animals per group); KN_j is the fixed effect of parity number j (first, second, and more calvings); GT_k is the fixed effect of birth type k (k has two levels [singleton or twin] for the killing out percentage trait and three levels [singleton, twin, or more calves] for all other traits); b corresponds to the linear (b_1), quadratic (b_2), and cubic (b_3) regression on slaughter age x ; a_{s_l} is the random additive genetic effect of sire l ; a_{mgs_m} is the random additive genetic effect of MGS m ; and e_{ijklmn} corresponds to the random residual. In contrast to the standard genetic evaluation, carcass fatness was treated as a trait rather than a fixed effect because it is known to be genetically influenced. Many studies have noted that the Y-chromosome and mitochondria do not significantly affect beef traits (Reinsch *et al.*, 1999; Neugebauer *et al.*, 2010a;b). Thus, these effects were not considered in the model. All covariance components and genetic effects were obtained using the ASReml-package (version 3.0; Gilmour *et al.*, 2009).

In total, 98% of all fattening bulls were classified into fat classes 2 and 3, and 93% of all animals were classified into carcass muscularity scores U and R, so these traits could also be treated as ordered categorical traits. Therefore, we used a threshold model where we assumed that y was binomially distributed. Fat classes 1 and 2 and muscularity scores E and U were converted to zero, whereas fat classes 3, 4, and 5 and muscularity scores R, O, and P were converted to one. To associate the expected value of y with the linear explanatory variables in the model, we chose

the canonical link function logit because there is anecdotal evidence that logit threshold models converge better than probit threshold models when the pseudo-likelihood approach is used. The link function converts y , which is either zero or one, into a whole real line ranging from minus to plus infinity (McCullagh and Nelder, 1989), and this can be written as:

$$\log \left[\frac{\pi}{(1-\pi)} \right] = \eta, \quad [10]$$

where π is the probability of y being one and η is the linear predictor, which in our model can be written as $\eta_j = x_j \beta + z_{s,j} \mathbf{a}_s + z_{mgs,j} \mathbf{a}_{mgs}$, where j denotes the index of observation y_j ; x_j , $z_{s,j}$, and $z_{mgs,j}$ are the rows in the incidence matrices corresponding to observation j ; and β , \mathbf{a}_s , and \mathbf{a}_{mgs} are the vectors of the fixed and genetic effects.

Testing the significance of the imprinting variance

The existence of significant imprinting variances must be verified statistically. In this study, the alternative hypothesis corresponded to the existence of imprinting, and it was determined by fitting the *MGS imprinting model*. The null hypothesis implied the non-existence of imprinting, and it was obtained by fitting an equivalent model with one genetic effect as sire. To statistically verify the model with the best fit to the data and thus whether POEs significantly affected the considered trait, a REML likelihood ratio test (RLRT) with two df was used, which is given by:

$$RLRT = 2(l_{R2} - l_{R1}), \quad [11]$$

where l_{R2} is the REML log-likelihood of the *MGS imprinting model* and l_{R1} is the REML log-likelihood of the model representing the null hypothesis. Under the null hypothesis the test statistic is asymptotically distributed as a 1:1 mixture of two χ^2 distributions with one and two df (Self and Liang, 1987). This, however, requires between-subject independence, a condition that is not satisfied because of the relationships between the animals. Following Neugebauer *et al.* (2010a;b), we therefore applied a χ^2 distribution with two df to achieve a more conservative test. Note that this test procedure is not valid for generalized linear models (GLMM) because the ASReml-package uses a penalized quasi-likelihood, which cannot be used to test differences (Gilmour *et al.*, 2009).

Direct estimation of imprinting effects

The imprinting effects were investigated further if the statistical test detected significant imprinting variances, which comprised a difference between parental effects:

$$\hat{\mathbf{i}} = 2\hat{\mathbf{a}}_{mgs} - \hat{\mathbf{a}}_s, \quad [12]$$

where $\hat{\mathbf{i}}$ corresponds to the imprinting effect, which can be estimated using a model equivalent to the *MGS imprinting model* (Blunk *et al.*, 2016). A formal proof for equivalence is given in the appendix. The model can be written as:

$$y_{ijk} = \mu + a_{s_i} + a_{s_j} + (a_{mgs_j} - a_{s_j}) + e_{ijk}. \quad [13]$$

In this equation, y_{ijk} corresponds to the phenotype of fattening bull k , and μ is the overall mean. The effect a_{s_i} is equal to the TA as sire i , and a_{s_j} is equal to $\frac{1}{2}$ TA of the MGS j as sire. However, it is conceivable that the MGS's TA might not correspond fully to the sire's TA, where this difference $(a_{mgs_j} - a_{s_j})$ corresponds to the imprinting effect of the MGS j and it needs to be added to the model. The effect e_{ijk} is a random residual. The mixed model equations can be written as:

$$\begin{bmatrix} \mathbf{X}'\mathbf{W}^{-1}\mathbf{X} & \mathbf{X}'\mathbf{W}^{-1}\mathbf{Z}_s & \mathbf{X}'\mathbf{W}^{-1}\mathbf{Z}_i \\ \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{X} & \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{Z}_s + \mathbf{A}^{-1}\lambda_1 & \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{Z}_i + \mathbf{A}^{-1}\lambda_2 \\ \mathbf{Z}_i'\mathbf{W}^{-1}\mathbf{X} & \mathbf{Z}_i'\mathbf{W}^{-1}\mathbf{Z}_s + \mathbf{A}^{-1}\lambda_2 & \mathbf{Z}_i'\mathbf{W}^{-1}\mathbf{Z}_i + \mathbf{A}^{-1}\lambda_3 \end{bmatrix} \begin{bmatrix} \boldsymbol{\beta} \\ \mathbf{a}_s \\ (\mathbf{a}_{mgs} - \mathbf{a}_s) \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{W}^{-1}\mathbf{y} \\ \mathbf{Z}_s'\mathbf{W}^{-1}\mathbf{y} \\ \mathbf{Z}_i'\mathbf{W}^{-1}\mathbf{y} \end{bmatrix}, \quad [14]$$

where incidence matrix \mathbf{Z}_s contains a one per row connecting observations with the TAs of sires as sire. In addition, the same row contains a half connecting observations with the TAs of the MGS as sire. A half is needed because the TA of grandsires is equivalent to only half of the TA as sires. Incidence matrix \mathbf{Z}_i is equivalent to incidence matrix \mathbf{Z}_{mgs} in Eq. [3] and it links the observations to the imprinting effects. The covariance components of the effects in Eq. [13] are:

$$\text{Var} \begin{bmatrix} \mathbf{a}_s \\ (\mathbf{a}_{mgs} - \mathbf{a}_s) \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} 1/2\mathbf{A}\sigma_s^2 & 1/4\mathbf{A}(\sigma_{s,d} - \sigma_s^2) & 0 \\ 1/4\mathbf{A}(\sigma_{s,d} - \sigma_s^2) & 1/8\mathbf{A}\sigma_i^2 & 0 \\ 0 & 0 & \mathbf{W} \end{bmatrix}, \quad [15]$$

where all of the symbols are defined as before and $(\sigma_{s,d} - \sigma_s^2)$ is the covariance between the TA as sire and the imprinting effect of the MGS. This covariance and its maternal counterpart

$(\sigma_{s,d} - \sigma_d^2)$ can become negative, whereas their sum comprising the imprinting variance must always be positive. The covariance components according to Eq. [15] are included in Eq. [14] as λ , i.e.:

$$\begin{bmatrix} \lambda_1 & \lambda_2 \\ \lambda_2 & \lambda_3 \end{bmatrix}^{-1} = \frac{1}{2\sigma_R^2} \begin{bmatrix} \sigma_s^2 & 1/2(\sigma_{s,d} - \sigma_s^2) \\ 1/2(\sigma_{s,d} - \sigma_s^2) & 1/4\sigma_i^2 \end{bmatrix}. \quad [16]$$

The weights w_{ii} in diagonal matrix \mathbf{W} remain unchanged and can be calculated using Eq. [5]. In summary, the breeding value as MGS is replaced by the breeding value as sire plus its own imprinting effect, and this corresponds to half of the total imprinting effect. We used this model, called the *equivalent model*, within a single run by applying the previously estimated covariance components using the *MGS imprinting model*. The achieved prediction error variances (PEVs) were then employed to calculate the reliabilities of the predicted imprinting effects using:

$$r^2 = 1 - \frac{PEV}{1/8\sigma_i^2(1+F_i)}. \quad [17]$$

RESULTS AND DISCUSSION

Significance of parent-of-origin effects

The analysis using the *MGS imprinting model* yielded significant imprinting variances for all of the traits analyzed with p -values below 0.001. The estimated relative imprinting variance (fraction of the total additive genetic variances) was lowest for carcass muscularity (8.56%) and highest for carcass fatness (16.64%). The generalized *MGS imprinting model* obtained slightly higher proportions (Table 2). However, the estimates were not directly comparable because proportions estimated using the GLMM were measured on an unobservable logit scale (Dempster and Lerner, 1950).

For the killing out percentage, the significant contributions of the imprinting variance to the additive genetic variance confirmed the findings of Neugebauer *et al.* (2010b), who reported a relative contribution of 24.56% in German Simmental. By contrast, they could not detect significant imprinting variances in the net BW gain, which was confirmed by an imprinting analysis of the net BW gain in German Gelbvieh (Engellandt and Tier, 2002). However, this

comparison should be treated with caution because Gelbvieh and Simmental are breeds with different selection histories. For the carcass fatness, many studies agree with our detection of a significant imprinting variance. Engellandt and Tier (2002) found that the imprinting variance could account for 11% and 22% of the phenotypic variance in pelvic and kidney fat, respectively. Using their *imprinting model*, Neugebauer *et al.* (2010a;b) detected relative imprinting variances of 24.77% in the fat scores. For the carcass muscularity, our findings agree with those obtained by Neugebauer *et al.* (2010a;b), who estimated a relative imprinting variance of 10.75%. Tier and Meyer (2010) confirmed the presence of imprinting effects on the eye muscle area, which also supports the influence of imprinting on carcass conformation traits.

In the light of the significant imprinting variances, considering POEs in routine genetic evaluations is a logical consequence. For a comparison of predicted transmitting abilities (PTAs) from the *MGS imprinting model* and the MGS Mendel model, see the additional information in the Supplementary Material.

Table 2. Variance ratios, correlation coefficients, and statistical test results obtained using a model with an effect as sire and maternal grandsire in a linear and generalized linear form for all traits. Standard errors are shown in parentheses.

Trait	Relative imprinting variance ¹ %	Maternal contribution ¹ %	Paternal contribution ¹ %	Correlation coefficient ²	RLRT ³
Killing out percentage, %	14.15 (1.42)	70.07 (8.27)	29.93 (8.27)	0.86 (0.014)	534***
Net BW gain, g/d	12.41 (1.03)	82.20 (7.24)	17.80 (7.24)	0.88 (0.010)	1138***
Carcass fatness	16.64 (1.29)	49.59 (5.86)	50.41 (5.86)	0.83 (0.013)	1372***
Carcass muscularity	8.56 (0.87)	62.94 (10.14)	37.06 (10.14)	0.92 (0.009)	636***
Carcass fatness ⁴	17.13 (1.33)	55.88 (5.81)	44.12 (5.81)	0.83 (0.013)	-
Carcass muscularity ⁴	10.31 (1.00)	82.36 (8.74)	17.64 (8.74)	0.90 (0.010)	-

¹Variance ratios based on additive genetic variances of sires and dams.

²Correlation between genetic effects of sires and maternal grandsires.

³Test statistic for the REML likelihood ratio test.

⁴Treated as binomial traits by applying a generalized linear model.

*** $P < 0.001$.

Parental contributions and allelic correlations

The parental contributions to the imprinting variance were calculated as $(\sigma_s^2 - \sigma_{s,d}) / \sigma_i^2$ for paternal contributions and as $(\sigma_d^2 - \sigma_{s,d}) / \sigma_i^2$ for maternal contributions. The imprinting variances in the killing out percentage, net BW gain and carcass muscularity were mainly contributed by maternal gametes. The imprinting variance in carcass fatness consisted almost equally of both parental variances, which was due mainly to an imperfect correlation between gametes.

Applying the GLMM resulted in an increase of the maternal contributions to the imprinting variances for carcass muscularity and carcass fatness (Table 2).

For the killing out percentage, Neugebauer *et al.* (2010b) obtained the opposite result where the paternal gamete contributed 70% to the imprinting variance. Different results were also obtained for the carcass fatness and carcass muscularity, where Neugebauer *et al.* (2010b) found that the fat score was fully maternally imprinted, and they suggested that the paternal allele was the main contributor to carcass muscularity (~70%). These differences may be attributable to discrepancies in the trait definitions, where Neugebauer *et al.* (2010b) used 15 automatically video-recorded categories to assess the carcass conformation quality, whereas we used five visually observed categories as well as defining the carcass muscularity as monetary scores. Thus, there were large disparities between our results and those obtained by Neugebauer *et al.* (2010b), but our findings agreed well with those obtained by Tier and Meyer (2012) in Australian beef cattle, for which their estimated variances due to maternal alleles were constantly higher than those due to paternal alleles. Only analyses of traits observed in Angus heifers demonstrated the opposite.

In addition to differences in the parental variances, the genomic imprinting variance was influenced by the imperfect correlations between parental gametic effects. In the present study, the correlation between the genetic effect as sire and the genetic effect as MGS was not constrained to one (Tier and Meyer, 2012) because we were only interested in investigating whether the imprinting variance deviated significantly from zero and identifying the model that was most suitable for use in genetic evaluations. As shown in Table 2, the estimated correlations ranged between 0.83 (carcass fatness) and 0.92 (carcass muscularity). In agreement with our findings, Neugebauer *et al.* (2010b) obtained the lowest correlations for the killing out percentage ($r_{s,d} = 0.78$) and fat score ($r_{s,d} = 0.76$). The result they obtained for carcass muscularity ($r_{s,d} = 0.91$) was also in good agreement, but their correlations refer to the correlation between the genetic effects as sire and dam.

Heritabilities

First, we applied the model that assumed an imprinting variance of zero to estimate h^2 for all of the given traits. The estimates ranged between 0.198 (fat score) and 0.326 (killing out percentage). Applying the model in a generalized linear form yielded consistently greater estimates (Table 1).

The estimate for the killing out percentage tended to be within a moderate range compared with reported estimates, and it was higher than that reported by Neugebauer *et al.* (2010b), who estimated $h^2 = 0.22$ in German Simmental using an animal model without POE. For the net BW gain, Engellandt *et al.* (1999) estimated h^2 values ranging between 0.123 and 0.161 in German Simmental. By contrast, Neugebauer *et al.* (2010b) obtained an estimate of 0.28 after applying their animal model to the same breed. Thus, our h^2 value (0.207) can be considered as an intermediate estimate. By using a GLMM including a random sire and an uncorrelated Y-chromosomal effect, Reinsch *et al.* (1999) evaluated h^2 for carcass muscularity and carcass fatness in Simmental. Our finding for carcass muscularity agreed with their result ($h^2 = 0.202$), but that for carcass fatness was about 0.08 higher than their result ($h^2 = 0.122$). Neugebauer *et al.* (2010b) obtained higher estimates for both traits, where their analysis yielded a h^2 of 0.25 for carcass fatness and 0.31 for carcass muscularity. The differences in these results may be attributable to the specific carcass quality trait definitions employed because their 15 video-recorded categories may have been better at capturing the phenotypic variance.

Compared with the h^2 estimated using the model that assumed the nonexistence of POEs, the inclusion of POEs did not alter the magnitude of h^2 . However, there was a very slight increase, which applied to both the linear and generalized linear models (Table 3). This increase differs from the findings of Neugebauer *et al.* (2010b) and Engellandt and Tier (2002), where both investigated the existence of POEs using models with two additive genetic effects per animal or with an additional uncorrelated gametic effect as sire. They detected a reduction in h^2 and concluded that the additive genetic variance was overestimated when POEs were not included. Tier and Meyer (2012) reached the same conclusion on the basis of a model with two gametic effects equivalent to the *imprinting model* of Neugebauer *et al.* (2010a;b). The increase observed in our study might be explained by the additional consideration of genetic information due to the incorporation of the MGS effect in the model. When the maternal side of inheritance was not considered, the estimated value of h^2 depended on the genetic information due to the resemblance between paternal half-sibs and the variance in the paternal gametes. However, as mentioned earlier, the maternal gamete makes greater contributions to the genetic variance, which was not considered when using the model containing only one genetic effect as sire (i.e., without POEs). Thus, by incorporating the MGS effect, the variance due to maternal gametes could be considered adequately, thereby increasing the total genetic variance and yielding higher estimates of h^2 .

Table 3. Genetic parameters estimated using a model with an effect as sire and maternal grandsire in a linear and generalized linear form for all traits. Standard errors are shown in parentheses.

Trait	Residual variance	Additive genetic variance	Gametic variance as sire	Gametic variance as dam	Covariance	h^2
Killing out percentage, %	2.468 (0.006)	1.035 (0.033)	0.488 (0.015)	0.547 (0.025)	0.444 (0.016)	0.372 (0.011)
Net BW gain, g/d	2808.890 (3.529)	690.730 (17.289)	317.760 (8.188)	372.970 (12.775)	302.490 (8.736)	0.229 (0.005)
Carcass fatness	0.207 (0.0003)	0.046 (0.001)	0.023 (0.001)	0.023 (0.001)	0.019 (0.001)	0.208 (0.005)
Carcass muscularity	140.471 (0.176)	31.607 (0.813)	15.453 (0.405)	16.154 (0.566)	14.450 (0.413)	0.210 (0.005)
Carcass fatness ¹	3.300	1.294 (0.033)	0.634 (0.017)	0.660 (0.025)	0.536 (0.017)	0.351 (0.008)
Carcass muscularity ¹	3.300	1.268 (0.033)	0.592 (0.016)	0.676 (0.024)	0.569 (0.017)	0.345 (0.008)

¹Treated as binomial traits by applying a generalized linear model.

Estimated POE and their reliabilities using the equivalent model

To avoid the laborious procedure required to generate the PEV of the imprinting effect, we used an equivalent model to obtain the imprinting effects and their PEVs directly. As explained in the following, it is useful to interpret the imprinting effects as POEs because the possibility of nuisance factors inflating the imprinting variance cannot be excluded. As expected for the equivalent models, the predicted POEs agreed fully with those calculated using the *MGS imprinting model* with Eq. [12] (the correlation coefficient equaled one).

In standard genetic evaluations, it is common to assess the prediction of breeding values on the basis of the prediction accuracies, which are usually expressed in terms of reliabilities (Mrode, 2014). The same method can be used to evaluate the prediction of imprinting effects. Thus, we used the directly obtained PEVs to calculate the reliabilities with Eq. [17]. The average reliabilities were 0.20 for the killing out percentage and 0.18 for net BW gain. For the carcass fatness, the linear and generalized linear models yielded identical results of 0.19. For the carcass muscularity, the linear model yielded an average reliability of 0.16 and the generalized linear model an average reliability of 0.17.

The left-hand side of Fig. 1 shows the individual POEs for all the traits relative to their reliabilities, which demonstrates that unreliable POEs regressed to their general mean of zero, whereas the more reliable effects were increasingly distributed around zero. The right-hand side of Fig. 1 shows the reliabilities of the POEs relative to the year of birth. There has been a

considerable increase in the reliabilities since the mid-1960s, where the maximum was reached for animals born approximately between 1980 and 1995. This increase may be attributable to enhanced data collection processes during these years because the reliabilities of genetic effects depend mainly on the availability of data, which usually comprise individual records and kinship information (Mrode, 2014). The model used in this study included two rather than only one genetic effect, so many records for sons and maternal grandsons were needed to predict reliable effects as sires and MGS. An examination of the available data confirmed these conditions, where animals that possessed POEs with reliabilities < 0.4 had an average of 15 sons and 16 maternal grandsons with records, whereas animals that possessed POEs with reliabilities > 0.4 had an average number of 993 sons and 978 maternal grandsons with records. Since 1995, the decreasing reliabilities of POEs have become evident, which might be because younger bulls have not been sufficiently proven as MGS yet.

Genetic trends in parental effects

Figure 2A shows the trend in the average PTAs as sires and dams for the killing out percentage, where the trend in the average POEs reflects the trend in their relationship. After 1993, the trends of the average PTAs declined until 1996, and because of the growing divergence between them, the trend in POEs deviated from zero. In 1997, the average PTAs increased and the trend in POEs almost reached zero. Subsequently, the parental trends declined until they reached their lowest points in 2000. Despite slight drops in 2006 and 2008, they have subsequently started rising again ever since. Due to the growing gap in the average PTAs, the trend in the average POEs deviated from zero after 2004.

Figure 2B shows the trend in the parental effects for net BW gain. Because of a lack of data in later years, the trends can only be shown up to 2003. The maternal trend slightly exceeded the paternal trend, so the trend in the average POEs exhibited a slight deviation from zero. However, this deviation was very small, and it vanished in 2003. Overall, there was an increasing trend in both parental PTAs, which is expected because Simmentals are known to be a dual-purpose breed in which an expected genetic improvement in milk production is combined with an increasing genetic trend in beef performance (BW gain).

No particular genetic trend was obtained for the carcass fatness and the average PTAs as sires and dams were almost identical until 2001 (Fig. 2C). However, when both of the genetic trends decreased in 2002, the difference between the parental effects widened because the PTAs as sire

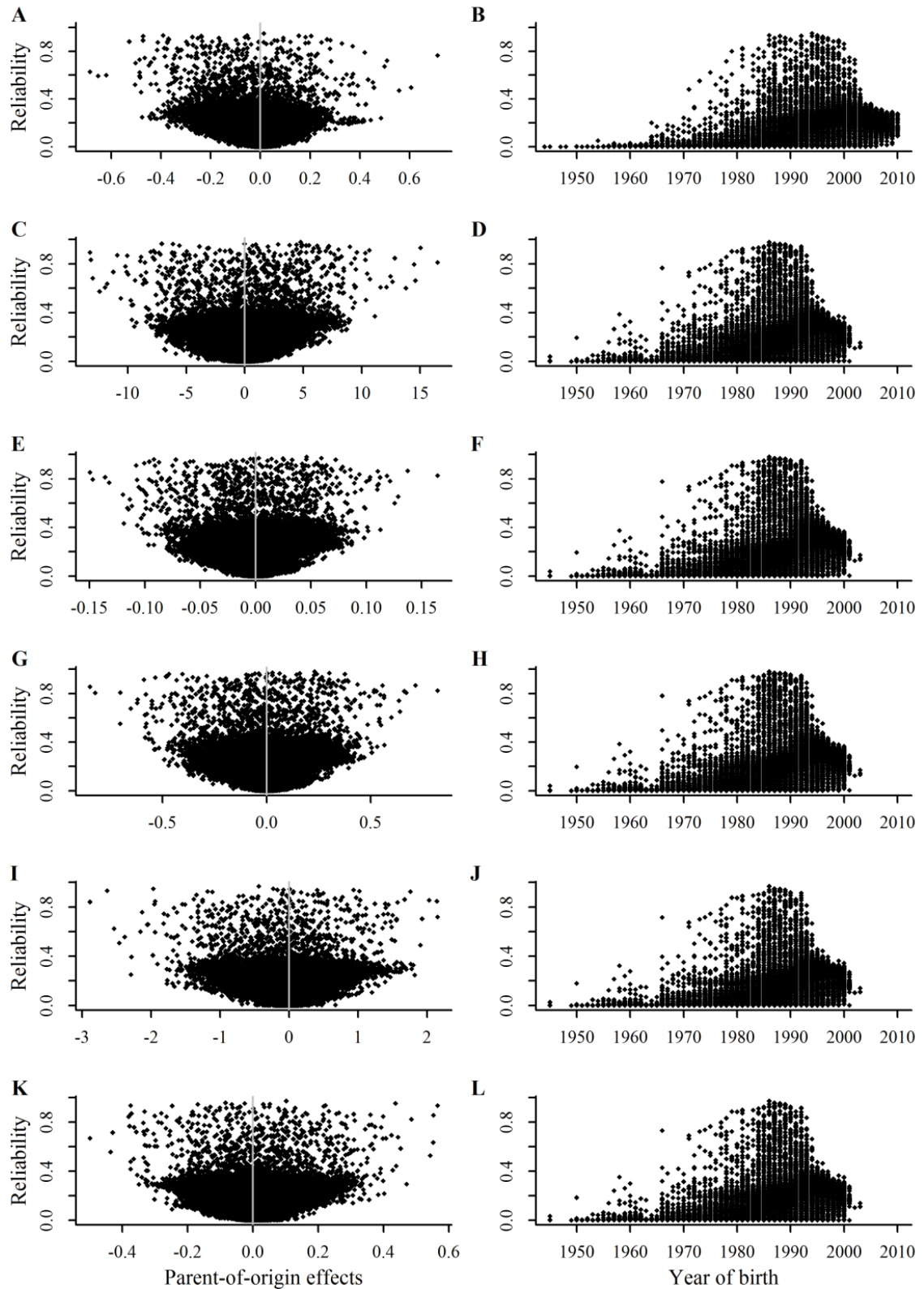


Figure 1. Parent-of-origin effects by reliabilities (left-hand side) and reliabilities by year of birth (right-hand side). The parent-of-origin effects were predicted using a linear model for (A) and (B) the killing out percentage, (C) and (D) net BW gain (in g/d), (E) and (F) carcass fatness, and (I) and (J) carcass muscularity. A generalized linear model was used for (G) and (H) carcass fatness and (K) and (L) carcass muscularity.

exhibited a stronger decrease. Subsequently, both parental effects increased, and an approximation was obtained. For the trend in the parental PTAs predicted using the GLMM (Fig. 2D), there was almost no difference from Fig. 2C. Figure 2E shows the trend in the parental effects for carcass muscularity. No particular difference was detected, and the trend in the average POEs remained at a level of zero. After declining to their minimum in 1997, the trends in the average PTAs increased until 1999 and then dropped in 2001. Subsequently, the genetic trends increased until 2002, and they have remained constant ever since. For the parental effects predicted using the GLMM (Fig. 2F), the genetic trends fully agreed with the genetic trends shown in Fig. 2E. However, the genetic trends were in the opposite direction because of the reverse coding of the carcass muscularity classes. Overall, genetic improvements in beef performance traits were observed that were due mainly to a constant increase in the net BW gain. The large contribution of the net BW gain may be explained by the fact that this trait has the greatest economic weight in the beef index, and thus, it is the main focus of selection.

Suitability of the GLMM

Fattening bulls were predominantly graded with fat scores of 2 and 3 and carcass muscularity classes U and R. Hence, we assumed that there was a lack of information for fat scores 1, 4, and 5 and carcass muscularity classes E, O, and P. Thus, it was necessary to identify the model that captures the actual degree of genetic variation best, that is, the linear model based on five category traits or the GLMM based on two category traits.

Both models produced comparable POEs, with correlations of 0.98 for carcass fatness and -0.91 for carcass muscularity. The correlations between their reliabilities reached almost one for both traits. The comparison of the PTAs obtained by the linear model (linear PTAs) with the PTAs produced by the GLMM (threshold PTAs) yielded a high correlation of 0.99 for carcass fatness (Fig. 3A). There was a correlation of -0.94 for carcass muscularity (Fig. 3C), where a nonlinear relationship was increasingly evident between the PTAs for lower ranks (and upper ranks for the threshold PTAs due to reverse coding).

The regression of the threshold PTAs on the linear PTAs demonstrated that there was sufficient independence between the residuals for carcass fatness (Fig. 3B). Thus, it may be concluded that both models captured similar parts of the variation in this trait. This conclusion agrees with Drennan *et al.* (2008), who reported high positive correlations ($r = 0.83$) between the fat scores

and carcass fat proportions in bulls, which suggests that the underlying continuous variables may be identical.

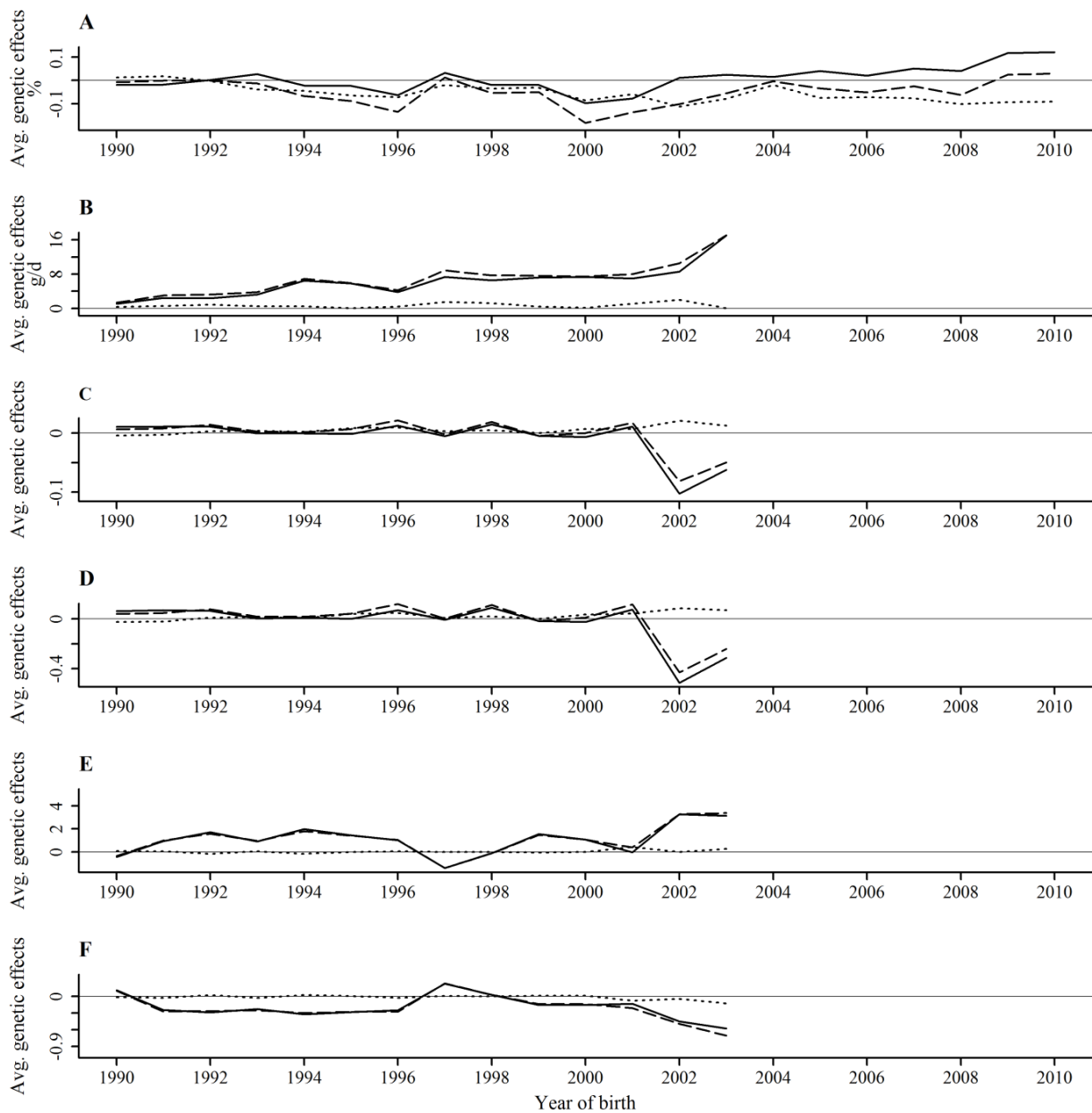


Figure 2. Average parent-of-origin effects (dotted line) and average transmitting abilities for animals as sire (solid line) and as dam (dashed line) by year of birth. The genetic effects were predicted using a linear model for (A) the killing out percentage, (B) net BW gain, (C) carcass fatness, and (E) carcass muscularity. A generalized linear model was used for (D) carcass fatness and (F) carcass muscularity.

The residual variation was discontinuous for carcass muscularity, especially for the threshold PTAs of the upper ranks, which were related to animals with progeny categorized in lower carcass muscularity classes (Fig. 3D). Kempster *et al.* (1982) stated that conformation classes

account for only roughly 30% of the variation in the meat content. Thus, from a biological viewpoint, the source of the underlying continuity is uncertain (Falconer, 1960), especially in the lower conformation classes.

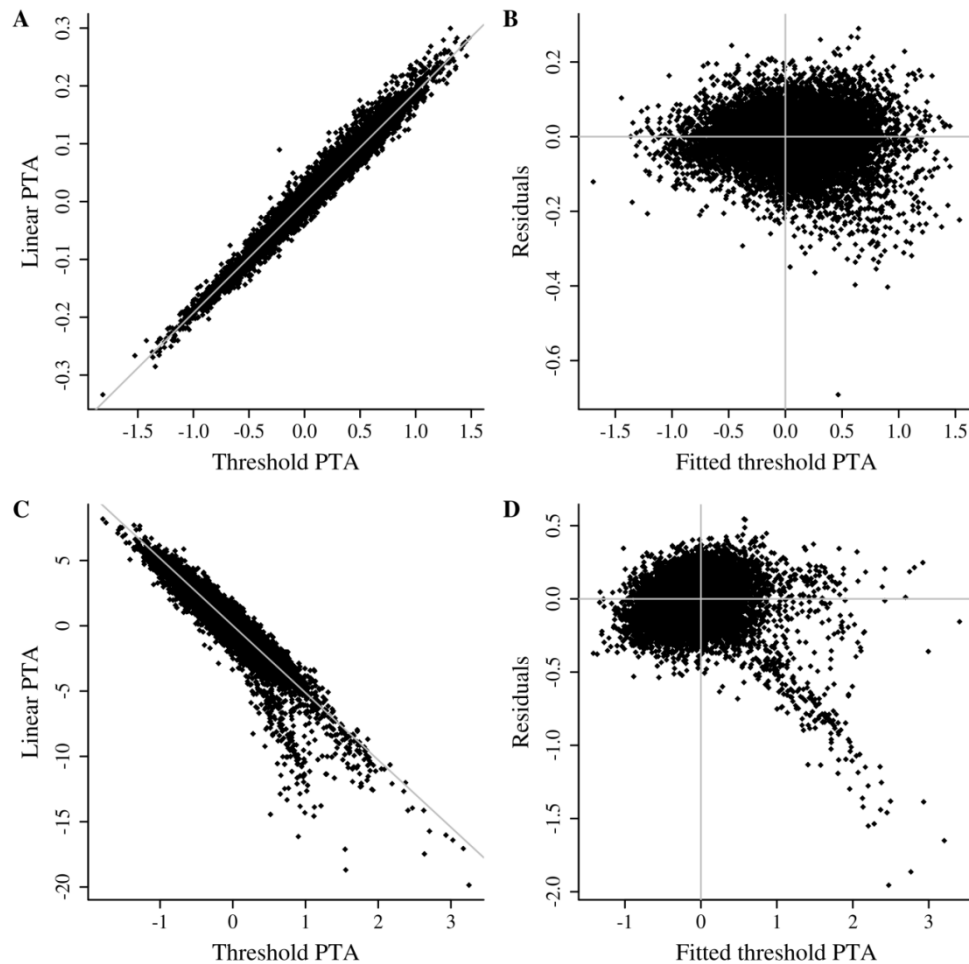


Figure 3. Correlations between the transmitting abilities (PTA) predicted using a linear (linear PTA) and generalized linear (threshold PTA) model (left-hand side) and the threshold PTA fitted using a linear regression with the linear PTA as the independent variable relative to their residuals (right-hand side) for (A) and (B) carcass fatness and (C) and (D) carcass muscularity.

Maternal side of inheritance

Overall, it must be considered that effects excluded from the analysis may have inflated the imprinting variance. Confounding factors may include maternal genetic effects and Y-chromosomal effects. The variance in maternal genetic effects cannot be distinguished from the imprinting variance because they generate the same phenotypic variation patterns (Hager *et al.*, 2008; Neugebauer *et al.*, 2010a;b). Simmental calves are generally separated from their dams

shortly after birth and raised with a milk replacer. Therefore, maternal effects may be assumed to be of minor importance. However, their existence cannot be fully excluded because of prenatal influences: A dam's uterine environment has been reported to affect not only the offspring's birth weight but also its performance from weaning until slaughter (e.g., reported in Larson *et al.* (2009) for steer progenies of dams held under different feeding conditions). With regard to the Y-chromosomal effects, a previous study demonstrated that they are of almost no importance for beef traits (Reinsch *et al.*, 1999).

In addition to the factors mentioned above, other factors cannot be considered by applying the *MGS imprinting model* per se because they are inherited only by females. The MGS is expected to provide a good approximation of the variation due to the daughter's gamete, but it cannot approximate her Mendelian sampling component, which is thus part of the residual (Jenko *et al.*, 2013). This also applies to mitochondrial effects because they are derived only from females; however, they were shown to be negligible in a previous study (Neugebauer *et al.*, 2010a,b). In contrast to mitochondria, the X-chromosome cannot be regarded as negligible, but it has attracted little attention in previous quantitative imprinting analyses, and its role as a critical factor that contributes to maternal gametic variation still needs to be addressed. For example, VanRaden (1987) considered that 5% to 10% of the genetic variation in milk production can be explained by X-chromosomal gene effects. By contrast, although they found some variation in North American Holsteins, Boettcher *et al.* (2001) suggested that the X-chromosome was not a major causal factor. Nevertheless, it is not possible to exclude the possibility of inflated imprinting variances. Therefore, the predicted effects should be interpreted as POEs, and the imprinting variances may be regarded as only the upper limits of parentally induced variances.

CONCLUSION

In this study, significant imprinting variances were obtained for all of the evaluated beef performance traits. A parsimonious *imprinting model* that reflects both parental expression patterns by incorporating sire and MGS effects allowed us to exploit a large volume of pedigree data. On average, the imprinting variance accounted for 13% of the total additive genetic variance when a linear model was applied, and the use of a GLMM confirmed these findings. This new model facilitates the direct prediction of POEs and their evaluation using PEVs. Thus, highly reliable POEs could be predicted.

ACKNOWLEDGEMENT

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APPENDIX: FORMAL PROOF OF EQUIVALENCE

According to Henderson (1985), the condition of equivalence is fulfilled when the expectation $E(\mathbf{y})$ and the variance $Var(\mathbf{y})$ of the observation vector are identical under both model specifications. For their fixed effect parts, the models do not differ, and all the random variables have an expectation of zero. Hence, $E(\mathbf{y})$ is equal for the *MGS imprinting model* and the *equivalent model*, with:

$$E(\mathbf{y}) = \mathbf{X}\boldsymbol{\beta}.$$

The variance in the observations $Var(\mathbf{y})$ in the *MGS imprinting model* can be written as:

$$\begin{bmatrix} \mathbf{Z}_s & \mathbf{Z}_{ms} \end{bmatrix} \left[1/2 \mathbf{A} \begin{pmatrix} \sigma_s^2 & \sigma_{s,ms} \\ \sigma_{s,ms} & \sigma_{ms}^2 \end{pmatrix} \right] \begin{bmatrix} \mathbf{Z}'_s \\ \mathbf{Z}'_{ms} \end{bmatrix} + \mathbf{W} \sigma_e^2,$$

where the second summand reflects the residual variation and it is identical in both models. The first summand is associated with the numerator relationship matrix \mathbf{A} , and it reflects the covariance of observations due to the resemblance of relatives. After multiplication, this covariance is:

$$\mathbf{Z}_s 1/2 \mathbf{A} \sigma_s^2 \mathbf{Z}'_s + \mathbf{Z}_{ms} 1/2 \mathbf{A} \sigma_{s,ms} \mathbf{Z}'_s + \mathbf{Z}_s 1/2 \mathbf{A} \sigma_{s,ms} \mathbf{Z}'_{ms} + \mathbf{Z}_{ms} 1/2 \mathbf{A} \sigma_{ms}^2 \mathbf{Z}'_{ms}. \quad [18]$$

The variance in the observations $Var(\mathbf{y})$ in the *equivalent model* can be expressed in terms of the incidence matrices and variance components obtained from the *MGS imprinting model*. The sum of \mathbf{Z}_s and $1/2 \mathbf{Z}_{ms}$ in the *MGS imprinting model* corresponds to the incidence matrix \mathbf{Z}_s in the *equivalent model*. The incidence matrix \mathbf{Z}_i for the imprinting effects in the *equivalent model*

equals \mathbf{Z}_{ms} from the *MGS imprinting model*. Therefore, in the *equivalent model*, $\text{Var}(\mathbf{y})$ can be written as:

$$\left[(\mathbf{Z}_s + 1/2 \mathbf{Z}_{ms}) \mathbf{Z}_{ms}' \right] \left[1/2 \mathbf{A} \otimes \begin{pmatrix} \sigma_s^2 & 1/2(2\sigma_{s,ms} - \sigma_s^2) \\ 1/2(2\sigma_{s,ms} - \sigma_s^2) & 1/4(\sigma_s^2 + 4\sigma_{ms}^2 - 2(2\sigma_{s,ms})) \end{pmatrix} \right] \begin{bmatrix} (\mathbf{Z}_s + 1/2 \mathbf{Z}_{ms})' \\ \mathbf{Z}_{ms}' \end{bmatrix} + \mathbf{W} \sigma_e^2.$$

Again, we only consider the first summand in detail. Multiplication gives:

$$\begin{aligned} & (\mathbf{Z}_s + 1/2 \mathbf{Z}_{ms}) 1/2 \mathbf{A} \sigma_s^2 (\mathbf{Z}_s + 1/2 \mathbf{Z}_{ms})' + (\mathbf{Z}_s + 1/2 \mathbf{Z}_{ms}) 1/4 \mathbf{A} (2\sigma_{s,ms} - \sigma_s^2) \mathbf{Z}_{ms}' \\ & + \mathbf{Z}_{ms} 1/4 \mathbf{A} (2\sigma_{s,ms} - \sigma_s^2) (\mathbf{Z}_s + 1/2 \mathbf{Z}_{ms})' + \mathbf{Z}_{ms} 1/8 \mathbf{A} (\sigma_s^2 + 4\sigma_{ms}^2 - 2(2\sigma_{s,ms})) \mathbf{Z}_{ms}' \\ = & \mathbf{Z}_s 1/2 \mathbf{A} \sigma_s^2 \mathbf{Z}_s' + \mathbf{Z}_s 1/2 \mathbf{A} \sigma_s^2 1/2 \mathbf{Z}_{ms}' + 1/2 \mathbf{Z}_{ms} 1/2 \mathbf{A} \sigma_s^2 \mathbf{Z}_s' + 1/2 \mathbf{Z}_{ms} 1/2 \mathbf{A} \sigma_s^2 1/2 \mathbf{Z}_{ms}' \\ & + \mathbf{Z}_s 1/4 \mathbf{A} 2\sigma_{s,ms} \mathbf{Z}_{ms}' - \mathbf{Z}_s 1/4 \mathbf{A} \sigma_s^2 \mathbf{Z}_{ms}' + 1/2 \mathbf{Z}_{ms} 1/4 \mathbf{A} 2\sigma_{s,ms} \mathbf{Z}_{ms}' - 1/2 \mathbf{Z}_{ms} 1/4 \mathbf{A} \sigma_s^2 \mathbf{Z}_{ms}' \\ & + \mathbf{Z}_{ms} 1/4 \mathbf{A} 2\sigma_{s,ms} \mathbf{Z}_s' - \mathbf{Z}_{ms} 1/4 \mathbf{A} \sigma_s^2 \mathbf{Z}_s' + \mathbf{Z}_{ms} 1/4 \mathbf{A} 2\sigma_{s,ms} 1/2 \mathbf{Z}_{ms}' - \mathbf{Z}_{ms} 1/4 \mathbf{A} \sigma_s^2 1/2 \mathbf{Z}_{ms}' \\ & + \mathbf{Z}_{ms} 1/8 \mathbf{A} \sigma_s^2 \mathbf{Z}_{ms}' + \mathbf{Z}_{ms} 1/8 \mathbf{A} 4\sigma_{ms}^2 \mathbf{Z}_{ms}' - 4 \mathbf{Z}_{ms} 1/8 \mathbf{A} \sigma_{s,ms} \mathbf{Z}_{ms}' \\ = & \mathbf{Z}_s 1/2 \mathbf{A} \sigma_s^2 \mathbf{Z}_s' + \mathbf{Z}_{ms} 1/2 \mathbf{A} \sigma_{s,ms} \mathbf{Z}_s' + \mathbf{Z}_s 1/2 \mathbf{A} \sigma_{s,ms} \mathbf{Z}_{ms}' + \mathbf{Z}_{ms} 1/2 \mathbf{A} \sigma_{ms}^2 \mathbf{Z}_{ms}'. \end{aligned} \quad q.e.d$$

The latter is identical to term [18]. Thus, both conditions from Henderson (1985) are satisfied.

SUPPLEMENTARY MATERIAL

MGS mendel model vs MGS imprinting model

In the light of significant parent-of-origin effects the *MGS imprinting model* has to be viewed as more adequate. A comparison of models with respect to the predicted transmitting abilities (PTAs), however, bears the difficulty that the genetic effects from both models have a somewhat different interpretation. From a herdsman's perspective the PTAs of bulls from the *MGS Mendel model* and the PTAs as sire in the *MGS imprinting model* are most relevant, as they summarize the effect of bulls on its immediate progeny. Therefore, consequences of not adopting the *MGS imprinting model* can be examined by comparing the PTAs of bulls from both models. Results are given for the trait net BW gain as an example, where the PTAs were estimated for overall 27,567 (male) ancestors. The rank correlation between the two different kinds of PTAs was 0.98. The amount of re-ranking can be seen from Fig. 1A (in natural units) and Fig. 1B (ranks). In this

context, bulls were decreasingly sorted, thus, the bull with the highest PTA was assigned to rank one, whereas the bull with the lowest PTA was assigned to rank 27,567. The number of bulls that disappear from the top 5%, 10%, 15% and 20% of bulls (ranked according to their PTAs estimated using the *MGS imprinting model*) by re-ranking when the *MGS Mendel model* is employed was:

Portion of top bulls	5%	10%	15%	20%
Amount of bulls disappeared	63	149	220	267.

Further, there are differences in the respective estimated reliabilities, which appear to be overly high by up to 10% for some bulls, when they are taken from the *MGS Mendel model*. The difference in the reliabilities increases with higher reliability levels (Fig. 2).

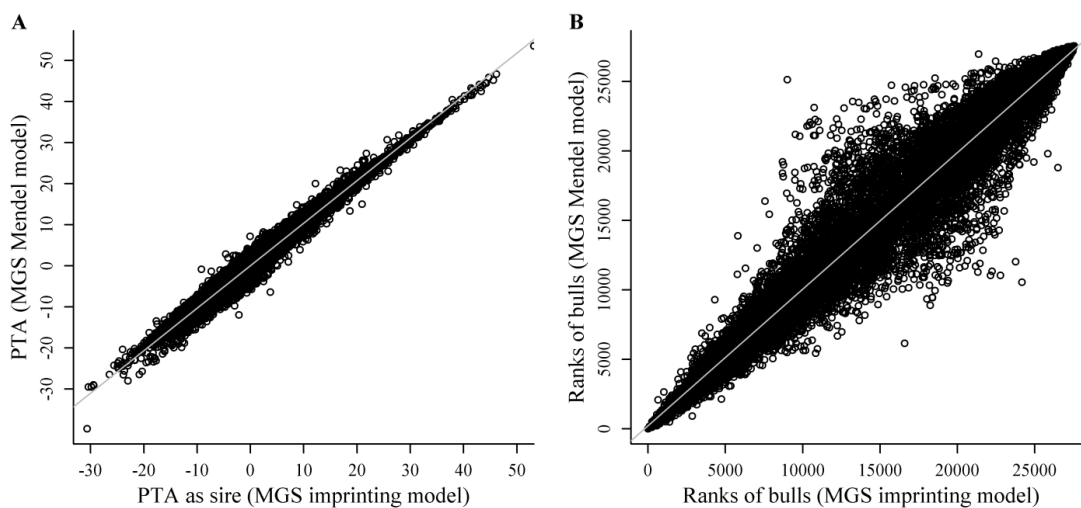


Figure 1. Correlation between the predicted transmitting abilities (PTA) estimated using the *MGS imprinting model* (x-axis) and the *MGS Mendel model* (y-axis) on the left side and between the ranks of the bulls according to the PTA of the *MGS Mendel model* (y-axis) and the ranks of the bulls according to the PTA of the *MGS imprinting model* (x-axis) on the right side.

The average differences in estimated reliabilities were:

Reliabilities (<i>MGS imprinting model</i>)	0.5-0.6	0.6-0.7	0.7-0.8	0.8-0.9	0.9-1.0
Average difference	0.0034	0.0084	0.0133	0.0132	0.0043.

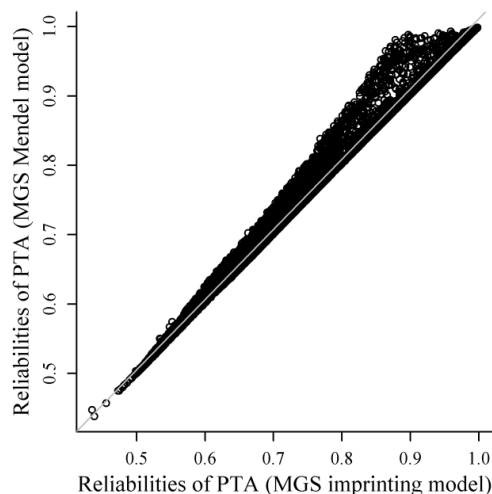


Figure 2. Correlation between the reliabilities of the predicted transmitting abilities (PTA) estimated using the MGS imprinting model (x-axis) and the MGS Mendel model (y-axis).

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CHAPTER 4

Scanning the genomes of parents for imprinted loci acting in their ungenotyped progeny

Scanning the genomes of parents for imprinted loci acting in their ungenotyped progeny

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ABSTRACT

Genomic imprinting is an epigenetic phenomenon, where alleles are fully or partially inactivated depending on their parental origin. Their effects are thus attributed to the family of parent-of-origin effects. Imprinted loci can be mapped by regressing the own phenotype of individuals on their phased marker genotypes, i.e. where the heterozygous genotypes Qq and qQ can be distinguished according to the parental origin of their alleles. Marker genotypes, however, are often not available for individuals with recorded phenotypes, as e.g. for slaughtered offspring in meat animals. Moreover, phase of the marker genotypes may be uncertain. Therefore, we propose estimated parent-of-origin effects (ePOEs) of parents to be analyzed as dependent variables that summarize all information on the impact of imprinted loci in their progeny. In simulated data, we demonstrated that imprinted loci can be mapped, when the ePOEs were used as observations to be regressed on the unphased genotypes of parents, i.e. their simple gene counts. After evaluating this approach theoretically, we adapted ePOEs estimated in Brown Swiss beef performance traits to detect imprinted loci based on the unphased genotypes of fattening bull sires. Within the receptor accessory protein 1 gene on chromosome 11, the analysis suggested a 5% genome-wide significant association with ePOEs on the expression of the net BW gain. With regard to this trait, further associations were found on chromosome 24. A series of loci significantly associated with ePOEs on fatness traits were obtained on chromosome five. These signals were reproduced when, instead of ePOEs, the sire's transmitting abilities were used. To conclude, theory proved ePOEs to be adequate variables to detect imprinted loci based

on unphased genotypes. In practice, this will help cost efficiently scan the genome for imprinted loci as shown in Brown Swiss cattle slaughter house data. Most important, vast repositories of already collected phenotypes become accessible for imprinting analyses by applying our new approach.

Keywords: genomic imprinting, genome-wide association study, parent-of-origin effect, pseudo-phenotype, Brown Swiss

INTRODUCTION

Genomic imprinting is an epigenetic phenomenon, where the expression of genes is partially or entirely limited to one of the two inherited gametes. The effects of imprinted genes can be attributed to the parent-of-origin effects as they appear as phenotypic differences between heterozygotes depending on their parental origin (Lawson *et al.*, 2013). Genomic imprinting has been discovered in a diversity of livestock species. Lopes Pinto *et al.* (2014) found between 500 and 650 imprinted single nucleotide polymorphisms (SNPs) predominantly paternally expressed in liver, hypothalamus and breast muscle in high and low body weight chicken lines. A variance-component analysis by Neugebauer *et al.* (2010a;b) revealed significant contributions of imprinted genes to the total genetic variance in 10 beef performance traits in cattle and 19 slaughter traits in pig. With respect to pig, a pioneering discovery was a purely paternally expressed polymorphism within the porcine insulin-like growth factor 2 gene (*IGF2*), which was found to affect muscle mass and fat deposition traits (Jeon *et al.*, 1999; Nezer *et al.*, 1999). The authors chose a method usually referred to as line-cross design. In this design, inbred lines (assumed to be fixed for the alleles Q and q at the quantitative trait loci (QTLs), respectively) are crossed to create a F_1 generation of individuals being heterozygote at the QTLs (summarized in Sandor and Georges, 2008). Intercrossing these individuals generates a F_2 population with a genotyping ratio of 1:2:1 (qq , Qq and qQ , QQ). When the average phenotypes of Qq and qQ can be statistically distinguished, the existence of an imprinted QTL (iQTL) is indicated. To be able to discriminate Qq and qQ , the knowledge of the phased marker genotypes is needed. In livestock it is assumed that founder lines (which are usually known to be divergently selected for a number of traits) possess alternative homozygous genotypes at the QTL (QQ and qq). Markers, however, are genetically neutral, i.e. they usually have no effect on the phenotype. Therefore,

they can be polymorphic within non-inbred lines, as e.g. different livestock breeds. In these cases phasing of marker genotypes is possible for F_2 individuals, albeit not always with certainty. Inbred strains of laboratory animals or plants behave differently: as they are (ideally) fully inbred, they are also homozygous at all marker loci, not only at the QTLs. In this case, phasing of marker genotypes is impossible for F_2 individuals as they were derived from genetically identical F_1 individuals (Wolf *et al.*, 2008). Phased marker genotypes are not only needed for iQTL mapping purposes but also for genomic selection decisions that consider genomic imprinting. For example, Nishio and Satoh (2014) augmented the commonly used GBLUP method by an imprinting relationship matrix for which phased genotypes were presupposed. In addition to phased marker genotypes, it is a prerequisite for iQTL-mapping purposes that the genotyped individuals deliver the phenotypic information. However, the traits of interest (e.g., milk production traits) are often measured in progeny, whose majority is usually not genotyped (as e.g., in Magee *et al.*, 2010).

In genome scans, the exploitation of estimated breeding values (EBVs) as pseudo-phenotypes has been described earlier (e.g., Becker *et al.*, 2013). In the same way as EBVs, we propose to use estimated parent-of-origin effects (ePOEs) of parents to map iQTLs expressed in their progeny. This exempts iQTL mapping procedures from the necessity of phased genotypes and the fact that phenotyped animals should deliver the genotypes. We examined this theory in simulated and practical data. The latter consisted of genotyped Brown Swiss sires possessing ePOEs for slaughter traits measured in their fattening-progeny.

MATERIALS AND METHODS

Simulated data

Two types of populations were simulated; the first was a two-generation pedigree, while the second contained three generations. Phenotypes were always available for the last generation only. For the two-generation variant 100 unrelated parents were drawn from a base population. These parents were then intermated in a cross-classified manner, resulting in 100×100 full-sib families of size three. In this way each parent was mated as a father to all 100 parents (including itself), acting as mothers and, vice versa, as a mother to all parents acting as father – giving rise

to 100×3 maternal half-sibs per parent. Thus, imprinted alleles were passed from each parent to progeny with both of the two possible parental expression patterns.

For the three-generation pedigree 10 grandparents were randomly chosen from the base population and produced 100 full-sib families, whose variable size was chosen from a Poisson distribution with a mean of 5.0. This led to 514 parents in the second generation, which were again intermated in a cross-classified manner. The number of full-sibs per family in the third generation was also Poisson distributed with a mean of 5.0. Only a fraction of 3% of all possible progeny with phenotypes was retained so that 41,273 records remained for the analysis. The full-sib families contained one to four phenotyped offspring.

Overall, 15 mutually unlinked marker genotypes were simulated, where five were in linkage disequilibrium (LD) with QTLs. The first QTL at marker locus two (minor allele frequency (MAF) = 0.4) was chosen to be biparentally expressed (Mendelian QTL) contributing 30% to a total additive genetic variance of 1.35. A purely paternally and a purely maternally expressed QTL contributed 5% to the total additive genetic variance and 25% to a total imprinting variance of 0.27, respectively. They were linked to the marker loci five (MAF = 0.5) and eight (MAF = 0.5). The marker loci 11 (MAF = 0.5) and 14 (MAF = 0.5) were both linked to contrarily partially imprinted QTLs, which contributed 30% to the total additive genetic variance and 25% to the total imprinting variance, respectively. A residual variance of two generated a heritability of 0.40 for the simulated trait and the imprinting variance accounted for overall 20% of the total additive genetic variance. The trait was created by adding additive and imprinting effects according to the simulated QTL genotypes. All markers were linked to the QTLs with a distance of 10 centiMorgan (cM) in the base population.

For each of the two populations 100 repetitions were simulated by drawing new genotypes and residual effects without altering family sizes and pedigree structures. Four different kinds of analyses labelled 1A, 1B, 2A, and 2B were applied to the two generation data. First, the phenotypes and phased genotypes of the 30,000 offspring were used in two consecutive association analyses (1A, 1B). Analysis 1A considered additive effects only by applying the model:

$$y_i = \mu + b_a x_i^a + g_i^s + g_i^d + e_i,$$

consecutively to each marker. Here y_i is the observed phenotype and x_i^a is the gene content (0, 1 or 2 for the respective genotypes QQ , Qq and qq) of individual i at the particular marker under

consideration, μ is the general mean and e_i represents the residual. The regression coefficient b_a can be interpreted as the additive effect of the locus. It was assumed that the random gametic effects g_i^s (effect of the father's gamete) and g_i^d (effect of the mother's gamete) had different variances σ_s^2 and σ_d^2 from a multivariate normal distribution with the variance:

$$\text{Var} \begin{bmatrix} \mathbf{g}^s \\ \mathbf{g}^d \end{bmatrix} = \mathbf{G} \otimes \begin{bmatrix} \sigma_s^2 & \sigma_{sd} \\ \sigma_{sd} & \sigma_d^2 \end{bmatrix},$$

with \mathbf{G} as the gametic relationship matrix (Gibson *et al.*, 1988; Schaeffer *et al.*, 1989). These random gametic effects were included to account for the stratification of the population into families. The association with a QTL is equivalent to a test of $H_0: b_a = 0$, which was done separately for each marker via a conditional Wald F -test using the ASReml-package (Release 3.0; Gilmour *et al.*, 2009) and ASReml-R (Butler *et al.*, 2009).

To find associations with imprinted loci the same model was augmented with a second regression term $b_p x_i^p$, where x_i^p has the values of 0, 1, -1 and 0 for the phased genotypes QQ , Qq , qQ and qq , respectively and the regression coefficient b_p delivers an estimate for the difference between the two types of heterozygotes Qq and qQ , i.e. for the imprinting effect. A test for $H_0: b_p = 0$ was performed, also for every marker separately in analyses 1B.

Second, in analyses 2A and 2B, we made use only of the unphased genotypes of the 100 parents. The phenotypic information from the 30,000 progeny was summarized by applying a special type of imprinting model (as described in Blunk *et al.*, 2016) that estimates a transmitting ability (TA) as sire and an ePOE for each parent, together with their respective reliabilities. Then the model:

$$y_i = \mu + bx_i^a + u_i + e_i,$$

was applied consecutively for each marker. The dependent variable y_i was either the TA (analysis 2A) or the ePOE of individual i (analysis 2B). The random variable u_i models the unaccounted genetic variability and was assumed to have a variance of $\text{Var}(\mathbf{u}) = \mathbf{A}\sigma^2$ with \mathbf{A} as the numerator relationship matrix in all analyses. A significant test of the hypothesis $H_0: b = 0$ indicates the association of a marker with an additively acting QTL in case y_i is a TA, and the association of a marker with an imprinted QTL in case the dependent variable is an ePOE.

In the three-generation pedigree, sources of family information contributed to the ePOEs of the animals in generation one and two. These sources were the parent-average (PA), records of the

final progeny and records of the progeny's final progeny. The naïve utilization of ePOEs as pseudo-phenotypes would negatively influence the outcome of association studies as shown for EBVs (Ekine *et al.*, 2014). Thus, de-regressed and weighted ePOEs, free from the influence of the PA were needed. The de-regression, PA-correction and weighting were achieved by adapting the approximate method published in Garrick *et al.* (2009) for EBVs, which is described in detail in the Supplementary Material for ePOEs. Finally, to illustrate the effects of the PA-correction, de-regression and weighting the ePOEs from the three-generation data were used for association analyses. In this data reliabilities were somewhat more variable due to the differences in family size and number of progeny per parent. In analysis 3A, ePOEs were left completely untreated, while in 3B they were de-regressed and PA-corrected and in case of 3C the ePOEs were additionally weighted. To define a parameter c , which was needed to calculate the weighting, a grid search was conducted, where c was progressively increased with a step size of 0.05 according to a proposal in Gorjanc *et al.* (2014). The c generating the greatest log-likelihood was chosen for each considered marker.

Brown Swiss data

The ePOEs and parental TAs as well as their reliabilities were derived from an imprinting analysis of Brown Swiss cattle slaughterhouse data published in detail in Blunk *et al.* (2016). Briefly, a reduced imprinting model was used to estimate the effects for up to 428,710 sires and dams based on the routinely recorded performance of their progeny, which were up to 173,051 fattening bulls (exact numbers vary from trait to trait). The imprinting variance contributed a significant proportion to the total genetic variance in the net BW gain (carcass weight divided by age (g/d)), carcass muscularity and carcass fatness. While muscularity was described using five monetary grades reflecting price differences, fatness was categorized by scores ranging from one (lean) to five (very fat). Assuming these traits to be normally distributed, ePOEs were first generated applying a linear imprinting model (these traits are subsequently indicated with the subscript L). Then, ePOEs were generated applying a threshold imprinting model assuming the traits to be binomially distributed (subscript T).

Upon agreement of all involved organizations, genotypes could be made available from the central genome database, which is maintained for breeding purposes at LKV Bayern in Munich. Data retrieval was assisted by the Institute for Animal Breeding at the Bavarian State Research Center for Agriculture. Using PLINK version v1.07 (Purcell *et al.*, 2007), genotypes were

excluded based on low frequencies ($MAF < 0.05$) and a Hardy-Weinberg equilibrium test ($p \leq 1e-005$). Neither animals nor genotypes were dismissed due to low genotyping as missing genotypes were imputed beforehand (BEAGLE version 3.3.2; Browning and Browning, 2009). Note that 30 genotypes were found to be located at the same position (base pairs) and that they were kept for the analyses. After the quality control, 37,443 genotypes remained for 1,857 sires for the net BW gain and 37,433 genotypes remained for 1,831 sires for the muscularity and fatness traits. The genome was scanned via a single marker regression, where the pseudo-phenotypes of sires were regressed on their marker gene counts individually. The pseudo-phenotypes were ePOEs defined as deviations of the TAs as dam from the TAs as sire. With respect to the TAs, both kinds (TAs as sire and TAs as dam) were used as pseudo-phenotypes. The pseudo-phenotypes were PA-corrected, de-regressed and weighted (Supplementary Material). When their de-regressed reliabilities were smaller than 3%, they were discarded. Thus, 1,793 ePOEs remained for the net BW gain with an average reliability of 25.86%; 1,033 ePOEs remained for muscularity_(L) with an average reliability of 9.73%; 1,720 ePOEs remained for muscularity_(T) with an average reliability of 14.01%; 1,277 ePOEs remained for fatness_(L) with an average reliability of 10.63%; and 1,649 ePOEs remained for fatness_(T) with an average reliability of 12.51%. As discussed in detail later, the sensitivity to c , which was chosen to calculate the weighting of ePOEs, was not particularly high in the simulation study. Therefore, c was limited to 0.1, 0.5 and 0.8. These values were only applied during the genome scan for ePOEs on the net BW gain to investigate the effect of changing c within a practical data framework. A c of 0.1 was chosen to analyze the ePOEs on all other traits. The marker effect's deviation from zero was tested conducting a conditional Wald F -test using ASReml (Release 3.0; Gilmour *et al.*, 2009). The unexplained genetic variance due to the relationships between sires was captured by including the inverse of the additive genomic relationship matrix (VanRaden, 2008). As it was not of full rank, its blending with 5% of the numerator relationship matrix A was required. Matrix A only contained the relationships between the animals with genotypes and was generated using the kinship2 R-package version 1.6.4 (Sinnwell *et al.*, 2014) in R (R Core Team, 2015). Except otherwise specified, all SNP positions and further genetic information were subsequently obtained from the UCSC Genome Browser (<http://genome.ucsc.edu/>) based on the Bos Taurus UMD3.1.1/bosTau8 (assembly date: Dec. 2009). Pairwise LD between SNPs was specified as r^2 estimated using Haploview (version 4.2;

Barrett *et al.*, 2005). Information about the known imprinting status of genes was derived from the geneimprint database (<http://www.geneimprint.org>; R.L. Jirtle).

RESULTS AND DISCUSSION

Simulated data

To investigate whether ePOEs are suitable to detect iQTLs, they were used as dependent variables to be regressed on the gene counts of the 100 unrelated parents in the two-generation pedigree (scenario 2B). As a result, the mean estimated effect at marker locus two (linked to a Mendelian QTL) did not significantly deviate from zero. In contrast, at the fully imprinted marker loci, the average p -values ranged from $p = 0.012$ to $p = 0.025$ indicating the presence of imprinted loci (Fig. 1A; Table 1). The signals at the marker positions 11 and 14 (linked to partial iQTLs) were visible with average p -values of 0.010, respectively. Mendelian and imprinted loci could thus be distinguished. This distinction was also possible when the phenotypes and phased genotypes of offspring were used to detect iQTLs in scenario 1B (Fig. 1B; Table 1). In comparison to scenario 2B, higher average signals (in terms of $-\log_{10} p$ -values) indicated the positions of iQTLs. However, phased marker genotypes of 30,000 individuals with records were necessary to observe this result, whereas unphased genotypes of only 100 parents without records were used in scenario 2B. Therefore, using ePOEs as pseudo-phenotypes constitutes a suitable alternative to detect iQTLs when phased genotypes are not available for animals with phenotypes or phenotypes are not observed for the animals with genotypes (e.g., selection candidates). The ePOEs and reliabilities can be directly estimated for parents by applying a special type of imprinting model as mentioned above (Blunk *et al.*, 2016). This model is also available in a sire-maternal grandsire version, which helps especially in case of large data sets. This was demonstrated in Blunk *et al.* (2017), where more than 1.3Mio records of Simmental fattening bulls were analyzed. When, in contrast to ePOEs, TAs were used as dependent variables (scenario 2A), mean p -values ranging from 0.002 to 0.011 indicated QTLs at all Mendelian and partially imprinted loci (Fig. 1C; Table 1). The mean effects estimated for the two fully imprinted markers did not significantly deviate from zero with mean p -values of 0.328 and 0.369. Thus, TAs are suitable to detect QTLs contributing to the Mendelian genetic variation but not to detect fully imprinted loci.

The model in scenario 1A did not consider imprinting as only an additive effect was included. At all marker loci the mean test statistics indicated the existence of QTLs without possibility to distinguish Mendelian and imprinted loci (Fig. 1D; Table 1). With regard to the mean estimated

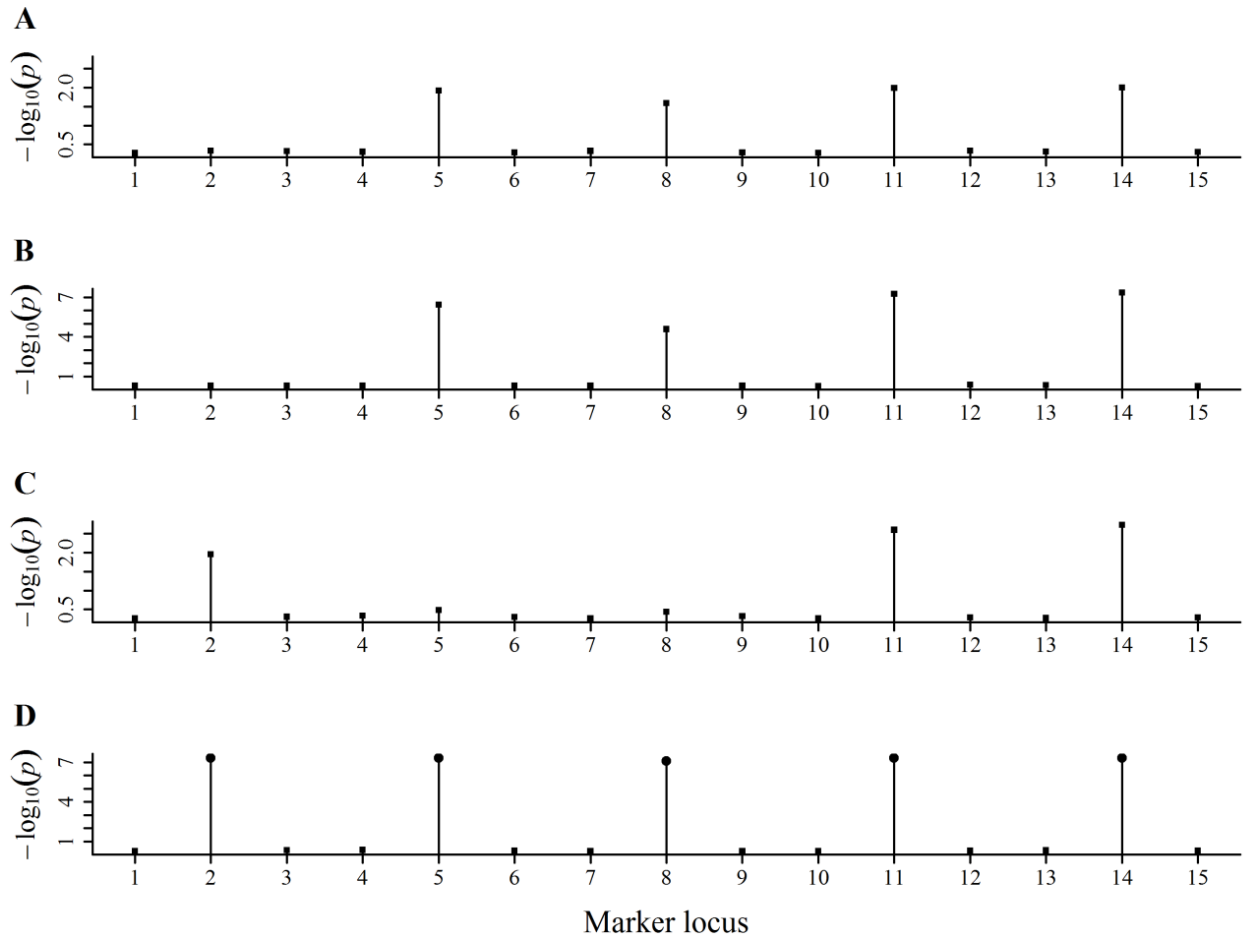


Figure 1. A) Average $-\log_{10} p$ -values generated by regressing the ancestor's parent-of-origin effects on their own genotypes B) Average $-\log_{10} p$ -values generated by regressing the offspring's phenotypes on their own genotypes via a model that considers parent-of-origin effects via an imprinting effect C) Average $-\log_{10} p$ -values generated by regressing the ancestor's transmitting abilities on their own genotypes D) Average $-\log_{10} p$ -values generated by regressing the offspring's phenotypes on their own genotypes via a model that neglects parent-of-origin effects. Round tops indicate $-\log_{10} p$ -values of $< 1.0e-45$.

effects in scenario 1A, inflations were observed at all marker positions. The same was observed for the additive effect when the model in scenario 1A was augmented by an imprinting effect in scenario 1B (Table 2). Regarding the imprinting effect, inflated values were observed at all markers linked to iQTLs. The mean estimated effect at marker position two (Mendelian QTL) equaled zero, which was expected. Although the signs were reversed, a similar pattern of inflated estimates was observed when ePOEs were used as dependent variables in scenario 2B (Table 2). The sign of ePOEs depends on their definition in the imprinting model (Blunk *et al.*, 2016).

However, as the imprinting variance is independent, the definitions of ePOEs need not necessarily be the same for the detection of iQTLs.

In scenario 3A, ‘untreated’ ePOEs of parents were regressed on their own genotypes. Again, the mean p -values indicated that ePOEs are suitable pseudo-phenotypes to detect imprinted loci, although a further loss of power must be noted in comparison to scenario 2B (Table 1). Whereas the mean marker effects were inflated in scenario 2B, the mean effects in scenario 3A were deflated (Table 2). Both, the loss of power as well as the deflation, may be explained by a loss of LD between the QTLs and their markers due to more recombination events happening since the base generation until alleles are transmitted to the last (third) generation. With an initial LD of 0.1 among founders, their distance was large (10cM), which let the LD decrease rapidly from one generation to the next. As the ePOEs of individuals in generation two showed an undesired regression to their PA with an average reliability of 0.80, the ePOEs were de-regressed and PA-corrected in scenario 3B. In comparison to the results in scenario 3A, only minor changes of the mean F -values and p -values were observed (Table 1). However, the de-regression and PA-correction increased the mean estimates of marker effects by almost one-third so that they nearly approximated the simulated values (Table 2). Due to their heterogeneous variance, ePOEs were additionally weighted in scenario 3C. With regard to the test statistics, no particular differences could be observed in comparison to scenario 3B (Table 1). Moreover, neither the mean marker effect estimates nor their variation and standard errors differed (Table 2). In examination of the mean c -parameters across all replications (Table 1) demonstrated that the grid-search did not favor c -values in a certain range because no particular differences could be observed between markers. However, the variances reflected strong fluctuations among replications. The log-likelihoods changed little in relation to the development of c . This indicated a flat log-likelihood function and thus a low sensitivity to c . According to Garrick *et al.* (2009), this sensitivity depends on the heterogeneity of the information content in the data. Thus, due to the straightforward simulation design, a low sensitivity could have been expected from the outset. To summarize, no particular changes of the test statistics could be observed due to the de-regression, PA-correction and weighting of ePOEs. Perhaps, the additive genetic effect within the model captured part of the genetic variance lessening the impact of the PA. With regard to the effect estimates, an impact of the de-regression and PA correction was obtained, whereas a minor role of the weighting was indicated (Table 2).

Table 1. Average F -statistics (F), p -values (p) and c -parameters (c) with their variance (var) and log-likelihoods ($logL$) for all scenarios and each locus with biparental (Men), maternal (Pat), paternal (Mat), partial maternal (Pat/Mend) and partial paternal (Mat/Mend) expression patterns. Subscript i corresponds to the imprinting parameter in the model used in scenario 1B.

	Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Scenario	effect	-	Men	-	-	Pat	-	-	Mat	-	-	Pat/Men	-	-	Mat/Men	-
1A	F	1.035	634.262	1.080	1.168	66.349	1.005	0.888	59.002	0.900	0.950	757.273	1.082	1.106	794.083	1.249
	p	0.518	0.000	0.457	0.432	0.000	0.479	0.519	0.000	0.520	0.542	0.000	0.481	0.470	0.000	0.472
1B	F	1.036	634.309	1.081	1.166	66.215	1.005	0.886	58.98	0.900	0.951	755.583	1.083	1.108	792.784	1.247
	p	0.519	0.000	0.456	0.434	0.000	0.479	0.520	0.000	0.521	0.543	0.000	0.482	0.470	0.000	0.472
1B	F_i	1.115	1.068	1.190	1.023	48.277	0.930	1.156	40.688	0.954	0.985	51.290	1.481	1.116	51.969	1.137
	p_i	0.484	0.473	0.497	0.503	0.000	0.503	0.504	0.000	0.480	0.511	0.000	0.407	0.452	0.000	0.514
2A	F	0.743	15.928	1.099	1.358	2.153	0.925	0.902	1.913	1.094	0.755	20.235	0.967	0.791	21.465	1.066
	p	0.541	0.011	0.496	0.465	0.328	0.498	0.542	0.369	0.478	0.546	0.002	0.511	0.534	0.002	0.518
2B	F	0.798	1.137	1.224	0.984	14.210	0.912	1.145	13.478	1.002	0.985	15.168	1.154	1.049	14.411	0.958
	p	0.535	0.459	0.476	0.496	0.012	0.515	0.469	0.025	0.511	0.530	0.010	0.460	0.490	0.010	0.500
3A	F	0.826	1.647	1.023	0.864	25.836	1.026	0.989	22.913	1.153	1.353	27.271	0.806	0.952	33.387	0.945
	p	0.544	0.415	0.479	0.527	0.030	0.488	0.499	0.049	0.507	0.450	0.018	0.564	0.524	0.017	0.484
3B	F	0.813	1.664	1.028	0.862	26.101	0.998	1.014	23.19	1.201	1.341	27.428	0.836	0.938	33.679	0.945
	p	0.549	0.402	0.479	0.525	0.026	0.489	0.486	0.046	0.502	0.451	0.017	0.559	0.528	0.017	0.488
3C	F	0.852	1.632	1.034	0.864	26.082	1.037	1.016	23.287	1.187	1.379	27.48	0.803	0.946	33.674	0.951
	p	0.531	0.411	0.488	0.522	0.027	0.485	0.494	0.046	0.500	0.449	0.016	0.559	0.522	0.017	0.479
	c	0.319	0.328	0.321	0.324	0.340	0.316	0.322	0.31	0.327	0.32	0.328	0.325	0.319	0.324	0.322
	var_c	0.108	0.110	0.108	0.109	0.121	0.108	0.107	0.11	0.109	0.11	0.118	0.111	0.108	0.111	0.109
	$logL$	340.967	341.34	341.091	340.995	353.061	341.092	341.048	351.759	341.152	341.339	353.671	340.997	341.018	356.445	340.997

Table 2. Mean, variation (var) and standard errors (se) of regression coefficients generated for each locus in relation to the simulated genetic effects contributing to the Mendelian (Men) and imprinting (Imp) variance. Subscript i corresponds to the imprinting parameter in the model used in scenario 1B.

Scenario	Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Men	0	0.461	0	0	0	0	0	0	0	0	0.300	0	0	0.300	0
	Imp	0	0	0	0	0.130	0	0	-0.133	0	0	0.130	0	0	-0.130	0
1A	mean	-0.002	0.469	0.000	0.002	0.158	0.002	0.000	0.149	-0.002	-0.004	0.534	-0.001	0.000	0.543	0.002
	var	0.000	0.003	0.000	0.001	0.001	0.000	0.000	0.001	0.000	0.000	0.002	0.000	0.000	0.002	0.000
	±se	0.002	0.006	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.004	0.002	0.002	0.004	0.002
1B	mean	-0.002	0.469	0.000	0.002	0.158	0.002	0.000	0.149	-0.002	-0.004	0.534	-0.001	0.000	0.542	0.002
	var	0.000	0.003	0.0000	0.001	0.001	0.000	0.000	0.001	0.000	0.000	0.002	0.000	0.000	0.002	0.000
	±se	0.002	0.006	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.004	0.002	0.002	0.004	0.002
1B	mean _{i}	0.003	0.000	0.001	0.001	0.167	-0.001	-0.003	-0.152	0.003	0.004	0.169	-0.001	0.000	-0.170	-0.003
	var _{i}	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	±se _{i}	0.003	0.002	0.003	0.003	0.002	0.002	0.003	0.003	0.002	0.002	0.002	0.003	0.003	0.003	0.003
2A	mean	-0.004	0.272	-0.007	0.000	0.086	0.000	0.002	0.082	0.017	0.006	0.320	0.011	-0.008	0.333	-0.013
	var	0.004	0.005	0.007	0.010	0.007	0.006	0.005	0.006	0.006	0.005	0.006	0.006	0.005	0.005	0.006
	±se	0.007	0.007	0.008	0.010	0.008	0.008	0.007	0.008	0.008	0.007	0.008	0.008	0.007	0.007	0.008
2B	mean	-0.001	0.004	0.002	-0.001	-0.176	-0.002	0.005	0.169	0.004	0.007	-0.181	-0.001	0.003	0.178	0.003
	var	0.002	0.003	0.003	0.003	0.003	0.002	0.003	0.003	0.002	0.003	0.002	0.003	0.003	0.003	0.002
	±se	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
3A	mean	0.001	0.003	-0.001	-0.001	-0.082	-0.003	0.000	0.077	0.003	-0.002	-0.082	0.001	-0.003	0.087	0.001
	var	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.001	0.001	0.001	0.001	0.000	0.000	0.001	0.000
	±se	0.002	0.002	0.002	0.002	0.003	0.002	0.002	0.004	0.002	0.002	0.004	0.002	0.002	0.003	0.002
3B	mean	0.001	0.005	-0.002	-0.001	-0.136	-0.005	0.000	0.126	0.004	-0.003	-0.135	0.002	-0.005	0.143	0.001
	var	0.001	0.001	0.001	0.001	0.003	0.001	0.001	0.004	0.001	0.001	0.004	0.001	0.001	0.003	0.001
	±se	0.003	0.004	0.003	0.003	0.006	0.003	0.003	0.006	0.004	0.004	0.006	0.003	0.003	0.006	0.003
3C	mean	0.001	0.005	-0.002	-0.002	-0.135	-0.005	0.000	0.126	0.004	-0.003	-0.135	0.002	-0.005	0.142	0.001
	var	0.001	0.001	0.001	0.001	0.003	0.001	0.001	0.004	0.001	0.001	0.004	0.001	0.001	0.003	0.001
	±se	0.003	0.004	0.003	0.003	0.006	0.003	0.003	0.006	0.004	0.004	0.006	0.003	0.003	0.006	0.003

Brown Swiss data

Parent-of-origin effects: Of 37,443 SNPs, one SNP (*ARS-BFGL-NGS-101636*) on BTA11 was associated with ePOEs estimated in the net BW gain at the 5% genome-wide significance level (Table 3; Fig. 2). To control the type I error rate, the significance threshold was adjusted according to the concept of the false discovery rate (Benjamini and Hochberg, 1995). As shown in Fig. SF1 (Supplementary Material), SNP *ARS-BFGL-NGS-101636* can be assigned to an intron of the receptor accessory protein 1 gene (*REEP1*). The imprinting status of *REEP1* is unknown. This applies also to the human and mouse orthologs. Imumorin *et al.* (2011) identified bovine iQTLs containing orthologs of imprinted genes in mice and human. One ortholog (hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), beta subunit) was located on BTA11 and was found in a region harboring iQTLs with an effect on weaning weight. However, this gene is located 24.7Mb away from marker locus *ARS-BFGL-NGS-101636*. Furthermore, *ARS-BFGL-NGS-101636* displayed low LD to its surrounding markers ($r^2 < 0.2$). The highest LD ($r^2 = 0.34$) was calculated for SNP *BTA-97072-no-rs* located in a non-coding area at 47.52Mb (Fig. SF1). Thus, a causal variant may rather be expected within *REEP1*. Mutations in the human ortholog of the bovine *REEP1* are related to neurodegenerative disorders such as hereditary spastic paraplegia, a syndrome characterized by progressive lower-limb spastic paralysis (Züchner *et al.*, 2006). Furthermore, the expression of *Reep1* was suggested to regulate the adipogenesis in white adipose tissue in mice. In *Reep1*-null mice the expression of pro-adipogenesis markers was observed to be reduced, whereas the expression of anti-adipogenesis markers was upregulated. Thereby, *Reep1*-null mice were observed to be thinner, albeit not lighter than their wild-type counterparts. Males showed a significant decrease in the proportion of total adipose tissue (Renvoisé *et al.*, 2016). To investigate the effect of c within a practical data framework, we scanned the genome for ePOEs on net BW gain varying this parameter. With a change from $c = 0.1$ to $c = 0.8$, the estimated effects increased, whereas the p -values decreased (Table 3). Thus, as expected, real data with an increasing amount of heterogeneous information, leads to a higher sensitivity to c . As a result, in addition to *ARS-BFGL-NGS-101636*, a 5% chromosome-wide association was found for *BTA-97072-no-rs* on BTA11. When c equaled 0.8, further 5% chromosome-wide significant markers were detected on BTA24 (Fig. 2). Except for two SNPs, they are closely located in an area containing the genes myomesin 1 gene and the tyrosine-protein kinase Yes gene (*YES1*). The

first encodes the Myomesin-1 protein, which was found, among others, to provide scaffold of myosin filaments (Grove *et al.*, 1985). The latter is involved in cytokinesis and cell cycle mechanisms (Jung *et al.*, 2011). The imprinting status of these genes is neither known in cattle nor in mice. However, with regard to the *YES1* orthologue in mice, an adjacent gene (interleukin 6 gene) on chromosome 5 at 30.01Mb is imprinted (<http://www.geneimprint.org/>). With regard to muscularity_(L), two 5% chromosome-wide significant SNPs (Table 3; Fig. 2) were found in non-coding areas on BTA7 (74.53Mb) and BTA18 (35.58Mb). While the existence of imprinted genes on BTA7 is unknown, four maternally imprinted genes on BTA18 were reported (<http://www.geneimprint.org/>). However, these genes are clustered in a region from 64.26Mb to 64.54Mb. Single nucleotide polymorphisms within this cluster and the SNP found on BTA18 were independent with $r^2 < 0.02$. Furthermore, the SNP found on BTA18 could not be reproduced when the ePOEs estimated in muscularity_(T) were analyzed. Instead, a 5% chromosome-wide significant SNP was found in a non-coding area on BTA7. As this marker is distantly located at 38.41Mb, no relationship can be assumed to the SNP found on BTA7 when ePOEs on muscularity_(L) were analyzed.

With regard to fatness_(L), no significant SNPs were found. However, for fatness_(L) the smallest number of records was available because 798 ePOEs were discarded from the analysis due to their low reliabilities. This might have considerably reduced the power to detect imprinted loci. With regard to fatness_(T), 45 5% chromosome-wide significant SNPs were found on BTA5. As described in detail below, a series of these SNPs were also observed to be significantly associated with the parental TAs in fatness_(L) and fatness_(T). The strongest signals were displayed by SNPs surrounding the trophinin associated protein gene (*TROAP*) and the protein lifeguard 2 gene (*FAIM2*). These genes are located in regions from 30.65Mb to 30.66Mb and 30.15Mb to 30.18Mb. Some SNPs, being significantly associated with ePOEs, are located within or proximal to these genes (Table 3; Fig. SF2). So far, no imprinted loci on BTA5 are known in cattle. However, the Sodium-coupled neutral amino acid transporter 4 gene (*SLC38A4*) is known to be imprinted in mice (<http://www.geneimprint.org/>). The bovine orthologue is located on BTA5 close to *TROAP* and *FAIM2* in a region from 33.61Mb to 33.65Mb (Fig. SF2). This may suggest the existence of an imprinting cluster on BTA5 that possibly incorporates *TROAP*, *FAIM2* and *SLC38A4*. The fact that associations were detected for ePOEs as well as for TAs may indicate the existence of partial imprinting (which may be concluded from the patterns observed in the

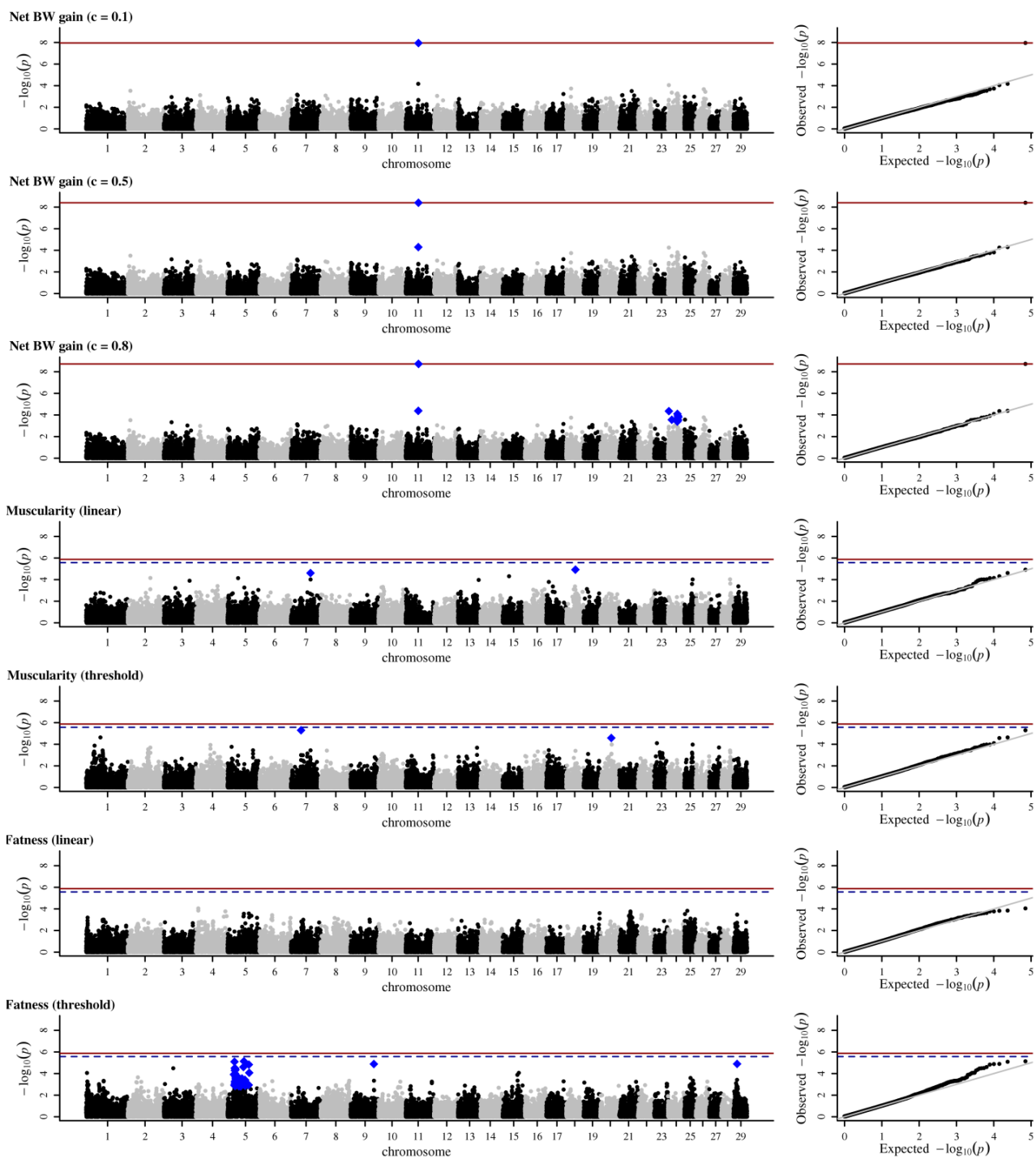


Figure 2. Marker loci in relation to their $-\log_{10} p$ -values generated by regressing parent-of-origin effects on genotypes. The net BW gain was analyzed with varying c -parameters of 0.1, 0.5 and 0.8. Red line = 5% genome-wide significance; dashed line = 10% genome-wide significance; blue diamonds = 5% chromosome-wide significant markers.

Table 3. Significant single nucleotide polymorphisms associated with parent-of-origin effects estimated in the net BW gain, muscularity and fatness analyzed using a linear (L) and threshold model (T). The net BW gain was analyzed with varying c -parameters of 0.1, 0.5 and 0.8.

Trait	SNP	Chr	Position (bp)	p -value	effect	se	gene
Net BW gain ₀₁	ARS-BFGL-NGS-101636	11	48473153	1.109e-08***	-7.4336	1.3011	<i>REEPI</i>
Net BW gain ₀₅	ARS-BFGL-NGS-101636	11	48473153	3.946e-09***	-7.9069	1.3433	
	BTA-97072-no-rs	11	47522289	5.048e-05*	3.6728	0.9061	
Net BW gain ₀₈	ARS-BFGL-NGS-101636	11	48473153	1.894e-09***	-8.2353	1.3701	
	BTA-97072-no-rs	11	47522289	4.110e-05*	3.7979	0.9260	
	ARS-BFGL-NGS-111462	24	3601352	4.378e-05*	3.0791	0.7535	
	ARS-BFGL-NGS-37285	24	36201079	7.951e-05*	-3.7057	0.9391	
	ARS-BFGL-NGS-96321	24	37709361	1.1890-04*	3.5995	0.9355	<i>MYOM1</i>
	ARS-BFGL-NGS-104327	24	40056388	1.510e-04*	-3.5138	0.9273	
	BTA-05662-no-rs	24	36077466	2.463e-04*	3.1706	0.8647	
	Hapmap59495-rs29020511	24	33038121	2.543e-04*	-4.1534	1.1353	
	ARS-BFGL-NGS-31453	24	14704121	2.740e-04*	4.4021	1.2100	
	Hapmap48158-BTA-102889	24	38899975	3.001e-04*	-3.3790	0.9348	
	BTA-57995-no-rs	24	36003718	3.736e-04*	3.3137	0.9313	<i>YES1</i>
	BTA-57998-no-rs	24	36024062	4.091e-04*	3.2913	0.9311	<i>YES1</i>
Muscularity _(L)	BTB-01217732	7	74537346	2.441e-05*	-1.1920	0.2825	
	Hapmap34397-BES7_Contig335_1167	18	35583594	1.208e-05*	-1.0794	0.2467	
Muscularity _(T)	Hapmap41111-BTA-94756	7	38413207	5.072e-06*	-0.2527	0.0554	
	ARS-BFGL-BAC-33671	20	41576197	2.655e-05*	-0.1407	0.0335	
Fatness _(T)	BTA-73718-no-rs	5	61790994	7.311e-06*	0.1610	0.0359	
	Hapmap41950-BTA-72999	5	26082666	8.118e-06*	-0.1663	0.0373	
	Hapmap39431-BTA-74343	5	80695234	1.482e-05*	0.2050	0.0473	<i>FAR2</i>
	ARS-BFGL-NGS-55084	5	60513092	2.493e-05*	-0.1464	0.0347	
	Hapmap39353-BTA-73120	5	27777281	3.093e-05*	-0.1477	0.0354	
	ARS-BFGL-NGS-91751	5	28573792	3.400e-05*	-0.1624	0.0392	
	ARS-BFGL-NGS-112542	5	30159843	4.332e-05*	0.1729	0.0423	<i>FAIM2</i>
	ARS-BFGL-NGS-113311	5	27633265	5.210e-05*	-0.1490	0.0368	<i>KRT75</i>
	Hapmap41784-BTA-17439	5	82036800	8.383e-05*	0.1663	0.0423	
	ARS-BFGL-NGS-7567	5	28331294	1.025e-04*	-0.1685	0.0433	<i>SCN8A</i>
	ARS-BFGL-NGS-77906	5	24533402	1.208e-04*	-0.1367	0.0356	<i>TMCC3</i>
	ARS-BFGL-NGS-10291	5	24512405	1.281e-04*	0.1525	0.0398	<i>TMCC3</i>
	ARS-BFGL-NGS-43909	5	30879776	2.168e-04*	-0.1748	0.0473	
	BTB-00226634	5	44695924	2.815e-04*	0.1250	0.0344	
	Hapmap42984-BTA-58358	5	54449817	2.830e-04*	-0.2632	0.0725	
	BTA-73464-no-rs	5	42918584	2.860e-04*	0.1294	0.0357	<i>PTPRR</i>
	ARS-BFGL-NGS-7799	5	44936099	2.906e-04*	0.1250	0.0345	
	ARS-BFGL-NGS-53461	5	30659497	3.049e-04*	0.1242	0.0344	<i>TROAP</i>
	ARS-USMARC-614	5	45722126	3.411e-04*	0.2543	0.0710	<i>IL22</i>
	ARS-BFGL-NGS-15520	5	65986618	3.878e-04*	0.1263	0.0356	<i>GNPTAB</i>
	ARS-BFGL-NGS-15787	5	66157408	3.899e-04*	-0.1319	0.0372	
	Hapmap47760-BTA-85576	5	65998778	4.180e-04*	-0.1253	0.0355	
	ARS-USMARC-657	5	45834943	4.653e-04*	0.1523	0.0435	<i>IFNG</i>
	Hapmap42150-BTA-25138	5	32819286	4.703e-04*	-0.1239	0.0354	
	ARS-BFGL-NGS-20969	5	62920850	5.207e-04*	0.1508	0.0435	
	ARS-BFGL-NGS-38038	5	27992179	5.235e-04*	-0.1817	0.0524	<i>NR4A1</i>
	ARS-BFGL-NGS-8796	5	29095603	5.987e-04*	0.1202	0.0350	
	ARS-BFGL-NGS-118817	5	31739928	6.420e-04*	-0.1885	0.0552	

Trait	SNP	Chr	Position (bp)	<i>p</i> -value	effect	se	gene
	ARS-USMARC-652	5	45807839	6.490e-04*	0.1630	0.0478	
	ARS-USMARC-675	5	31164990	6.631e-04*	0.2044	0.0600	<i>ADCY6</i>
	ARS-BFGL-NGS-717	5	32861996	6.812e-04*	0.1202	0.0354	
	ARS-BFGL-NGS-30677	5	43565856	7.627e-04*	0.1163	0.0346	
	BTA-73685-no-rs	5	60929878	7.751e-04*	0.1234	0.0367	
	ARS-BFGL-NGS-7725	5	27839649	7.835e-04*	-0.1154	0.0344	<i>KRT7</i>
	ARS-BFGL-NGS-38020	5	29264678	9.111e-04*	0.1147	0.0346	
	ARS-BFGL-NGS-92420	5	33078266	9.721e-04*	0.1136	0.0344	
	ARS-BFGL-NGS-45045	5	63899453	9.880e-04*	-0.1177	0.0357	
	Hapmap24085-BTA-143102	5	46364432	1.043e-04*	-0.2532	0.0772	
	BTA-74351-no-rs	5	80256799	1.168e-03*	-0.1511	0.0466	<i>TMTC1</i>
	BTA-72978-no-rs	5	25699752	1.175e-03*	-0.1293	0.0398	<i>NCKAP1L</i>
	BTA-73296-no-rs	5	26526934	1.200e-03*	-0.1248	0.0386	
	ARS-BFGL-NGS-23025	5	66976106	1.338e-03*	0.1127	0.0351	<i>PAH</i>
	ARS-BFGL-NGS-107085	5	28660813	1.420e-03*	-0.1545	0.0484	<i>BIN2</i>
	BTB-01477536	5	43417275	1.500e-03*	-0.1139	0.0359	<i>CNOT2</i>
	ARS-BFGL-NGS-34352	5	49842685	1.507e-03*	0.1115	0.0351	<i>SRGAP1</i>
	ARS-BFGL-NGS-36861	9	88814138	1.321e-05*	-0.2174	0.0499	
	Hapmap33865-BES2_Contig389_1251	29	13959142	1.280e-05*	-0.1857	0.0426	

***5% genome-wide; *5% chromosome-wide; se = standard error

simulation study as shown in Fig. 1). However, whether an imprinting cluster actually exists and whether causal variants are fully or partially imprinted is not clear. Another fact giving rise to some uncertainty is that the SNPs found for $\text{fatness}_{(T)}$ on BTA5 were not observed, when ePOEs on $\text{fatness}_{(L)}$ were analyzed. However, as mentioned above, the lowest number of records and the smallest average of reliabilities was observed for ePOEs estimated in $\text{fatness}_{(L)}$. Furthermore, the heritability estimated for $\text{fatness}_{(L)}$ ($h^2 = 0.23$) in Blunk *et al.* (2016) was far below the heritability estimated for $\text{fatness}_{(T)}$ ($h^2 = 0.46$). These facts might have considerably reduced the power to detect imprinted loci, when ePOEs on $\text{fatness}_{(L)}$ were analyzed. With regard to $\text{fatness}_{(T)}$, another 5% chromosome-wide significant SNP (*ARS-BFGL-NGS-36861*) was found in a non-coding region on BTA9 (Table 3). This chromosome hosts the imprinted *PLAG1* like zinc finger 1 gene (*PLAGL1*) and the imprinted insulin like growth factor 2 receptor gene (*IGF2R*). They are located in areas from 82.41Mb to 82.47Mb and from 97.63Mb to 97.74Mb. *ARS-BFGL-NGS-36861* lays in between at 88.81Mb. The LD to SNPs proximal to *PLAGL1* ($r^2 \leq 0.03$) and to SNPs within *IGF2R* ($r^2 \leq 0.02$), however, suggests full independence. Another 5% chromosome-wide significant SNP was found in a non-coding region at 13.95Mb on BTA29. An imprinting cluster is located on BTA29 incorporating the *IGF2* and the H19 gene. However, this cluster is distantly located in a region from 49.32Mb to 50.15Mb.

To conclude, the results mainly suggest imprinted genes on BTA5 and BTA11 affecting the carcass fatness and net BW gain. Their imprinting status cannot be established with certainty. Moreover, as ePOEs were used as pseudo-phenotypes, it remains unclear whether their effects actually arose from genomic imprinting or another parent-specific genetic phenomenon (e.g., maternal genetic effects). To answer this question, follow-up studies are necessary to gain deeper insights.

Transmitting abilities: Due to strong similarities between the results for the TAs as sire and the TAs as dam, findings for the latter are subsequently omitted from Tables. The same holds true for the results found in traits analyzed via the threshold model. Moreover, only markers found to be significant at the 5% and 10% genome-wide significance level are listed. Detailed illustrations can be found in the Supplementary Material (Table ST1 and ST2; Fig. SF4). With regard to net BW gain, only one SNP, located in a non-coding region on BTA28, was found to be significantly associated with the parental TAs at the 5% chromosome-wide significance level (Table 4; Fig. 3).

With regard to muscularity_(L), six SNPs on BTA10 were associated with the parental TAs at the 5% genome-wide significance level (Table 4; Fig. 3). Except for one SNP, these findings were reproduced for both parental TAs estimated in muscularity_(T). Three SNPs can be assigned to the Unc-13 Homolog C gene (*UNC13C*) and one SNP to the aquaporin-9 gene (*AQP9*). Their effects are of similar magnitude (Table 4), where slightly higher effects were observed, when the TAs as dam were analyzed. This holds true for muscularity_(L) as well as for muscularity_(T). Both genes are protein coding; however, their functions are still unclear in cattle. They are located in regions from 52.15Mb to 52.19Mb (*AQP9*) and from 55.71Mb to 56.41Mb (*UNC13C*). Moderate lambdas (up to 1.042 for the TAs as sire) indicate almost no genome-wide inflation of *p*-values (Yang *et al.*, 2011) as shown for the muscularity traits in the Q-Q plot in Fig. 3. However, SNPs located in *UNC13C* and *AQP9* are not fully independent with $r^2 > 0.24$ (for LD patterns and gene assignments on BTA10 see Fig. SF3). Therefore, whether causal variants are located in either *UNC13C*, *AQP9* or in both cannot clearly be determined. According to the CattleQTLdb (Release 32; Hu *et al.*, 2016), a QTL expressed in lean meat yield was found in Holstein-Friesian on BTA10 at 56.10Mb, a region which is located within *UNC13C* (Doran *et al.*, 2014). Apart from the findings on BTA10, further markers significantly associated with the TAs estimated in the muscularity traits were found on BTA20, BTA23 and BTA28. Associations on BTA20 were

Table 4. Significant single nucleotide polymorphisms associated with transmitting abilities as sire estimated in the net BW gain, muscularity and fatness analyzed using a linear (L) and threshold model (T).

Trait	SNP	Chr	Position (bp)	p-value	effect	se	gene
Net BW gain	Hapmap49247-BTA-63647	28	3637555	4.2470e-06*	-8.3648	1.8187	
Muscularity _(L)	BTB-00429961	10	56093653	4.3065e-09***	-1.2325	0.2099	<i>UNC13C</i>
	ARS-BFGL-NGS-21100	10	56116909	6.2669e-09***	-1.2186	0.2098	<i>UNC13C</i>
	BTB-01911175	10	56140822	8.7539e-09***	1.2083	0.2100	<i>UNC13C</i>
	BTB-00430730	10	55535781	4.2323e-08***	1.1607	0.2118	
	Hapmap51030-BTA-69263	10	52190618	1.0970e-06***	1.2283	0.2521	<i>AQP9</i>
	Hapmap50767-BTA-72346	10	60145660	8.0751e-06***	1.2731	0.2852	<i>HDC</i>
	ARS-BFGL-NGS-24556	10	51980270	1.9888e-05**	-0.8659	0.2029	
	Hapmap36252-SCAFFOLD195517_10504	10	58707718	2.0960e-05**	-1.0007	0.2352	<i>TMOD2</i>
	ARS-BFGL-NGS-27708	10	55510249	2.1071e-05**	-0.8953	0.2105	
	Hapmap58597-rs29013533	10	58097440	2.5192e-05**	-0.8634	0.2049	
	BTB-01125630	10	57912228	3.4723e-05**	1.5872	0.3834	
	Hapmap59786-rs29012019	10	55611885	3.7975e-05**	-0.8747	0.2123	
	ARS-USMARC-Parent-DQ984827-rs29012019	10	55611885	3.9402e-05**	-0.8721	0.2121	
	ARS-BFGL-NGS-114479	16	33402551	2.3777e-05**	1.1821	0.2798	
ARS-BFGL-NGS-40515	23	10593430	6.3823e-06***	-0.9244	0.2048		
Fatness _(L)	ARS-BFGL-NGS-67309	2	114710664	2.5727e-05***	0.0240	0.0057	
	ARS-BFGL-NGS-38020	5	29264678	2.9461e-10***	0.0328	0.0052	
	Hapmap39353-BTA-73120	5	27777281	7.9597e-10***	-0.0335	0.0055	
	ARS-BFGL-NGS-53461	5	30659497	1.7002e-08***	0.0292	0.0052	<i>TROAP</i>
	ARS-BFGL-NGS-91751	5	28573792	1.9337e-08***	-0.0332	0.0059	
	ARS-BFGL-NGS-92420	5	33078266	2.5670e-08***	0.0293	0.0053	
	ARS-BFGL-NGS-30168	5	111313740	8.8196e-07***	0.0266	0.0054	<i>SYNGR1</i>
	ARS-BFGL-NGS-98156	5	27869236	1.5540e-06***	0.0255	0.0053	
	Hapmap42150-BTA-25138	5	32819286	2.2840e-06***	-0.0257	0.0054	
	ARS-USMARC-675	5	31164990	2.5085e-06***	0.0410	0.0087	<i>ADCY6</i>
	Hapmap51043-BTA-73218	5	29622395	2.8574e-06***	0.0230	0.0049	<i>LARP4</i>
	ARS-BFGL-NGS-107085	5	28660813	2.9176e-06***	-0.0330	0.0071	<i>BIN2</i>
	ARS-BFGL-NGS-112542	5	30159843	4.0947e-06***	0.0288	0.0063	<i>FAIM2</i>
	BTA-72978-no-rs	5	25699752	4.2470e-06***	-0.0274	0.0060	<i>NCKAP1L</i>
	Hapmap23022-BTA-161235	5	31613026	4.2916e-06***	-0.0245	0.0053	
	ARS-USMARC-629	5	25738874	5.6301e-06***	0.0310	0.0068	<i>GTSF1</i>
	ARS-USMARC-657	5	45834943	5.8398e-06***	0.0281	0.0062	<i>IFNG</i>
	BTA-73209-no-rs	5	29496625	6.6548e-06***	0.0230	0.0051	<i>DIP2B</i>
	ARS-BFGL-NGS-68582	5	111380409	7.7038e-06***	-0.0251	0.0056	
	ARS-BFGL-NGS-717	5	32861996	1.0656e-05***	0.0240	0.0055	
	ARS-BFGL-NGS-7725	5	27839649	1.3210e-05***	-0.0220	0.0051	<i>KRT7</i>
	BTA-73392-no-rs	5	32104484	1.4981e-05***	0.0243	0.0056	
	ARS-BFGL-NGS-8796	5	29095603	2.9035e-05***	0.0227	0.0054	
	ARS-USMARC-652	5	45807839	4.0453e-05**	0.0282	0.0069	
	BTA-28787-no-rs	5	37925443	4.4479e-05**	0.0221	0.0054	
	ARS-BFGL-NGS-11851	5	32426908	4.5188e-05**	0.0226	0.0055	
	UA-IFASA-2781	5	29989860	5.3496e-05**	0.0218	0.0054	<i>ASIC1</i>
	ARS-BFGL-NGS-43909	5	30879776	5.4350e-05**	-0.0282	0.0070	
	Hapmap55179-rs29024483	13	38830375	2.9496e-05***	0.0231	0.0055	<i>DZANK1</i>
	Hapmap55095-rs29010810	13	27589301	7.0403e-05**	0.0260	0.0065	
ARS-BFGL-NGS-4866	16	50095343	5.3215e-05**	-0.0317	0.0079		
ARS-BFGL-NGS-18515	19	29219211	2.2798e-05***	0.0231	0.0055	<i>NTN1</i>	

***5% genome-wide; **10% genome-wide; *5% chromosome-wide; se = standard error

only found for the TAs as dam at the 5% chromosome-wide level. They cannot be assigned to genes. However, Doran *et al.* (2014) detected SNPs on BTA20, which were strongly associated with carcass conformation and less than 1Mb away from the growth hormone receptor gene (*GHR*). In this study, one of the SNPs on BTA20 is located only 41.75Kb away from *GHR*. A SNP on BTA23 was found to be significantly associated with both parental TAs estimated in muscularity_(L) and muscularity_(T) at the 5% genome-wide significance level. However, it is located within a non-coding region and markers found to be significantly associated with carcass conformation in Doran *et al.* (2014), are distantly located. Another marker significantly associated (5% chromosome-wide) with both TAs estimated in the muscularity_(T) was found BTA28 within the calcium-activated potassium channel subunit alpha-1 gene (32.82Mb to 33.58Mb). Its function with regard to the carcass conformation in cattle is unknown. However, Doran *et al.* (2014) identified a QTL with an effect on lean meat yield close to this gene at 33.60Mb (CattleQTLdb; Release 32; Hu *et al.*, 2016). With respect to fatness_(L), 22 SNPs were found to be associated with the TAs as sire at the 5% genome-wide significance level on BTA5. The smallest *p*-values ($p < 2.6e-08$) were displayed by markers surrounding *TROAP* located in a region from 30.65Mb to 30.66Mb. Overall, 41 further SNPs on BTA5 were 10% genome-wide or 5% chromosome-wide significant. They were replicated for the TAs as dam as well as for the parental TAs estimated in fatness_(T) (Fig. 3). Some SNPs are located within genes other than *TROAP* (Table 4). However, due to they moderate to high LD to SNPs located within *TROAP*, most of which may not be assumed causal but indicators for causal variants within or proximal to *TROAP* (Fig. SF2). According to the CattleQTLdb (Release 32; Hu *et al.*, 2016), haplotypes with an effect on backfat breeding values are closely located to *TROAP* in a region from 32.6 to 34.2Mb (Li *et al.*, 2004). Further significant SNPs (5% and 10% genome-wide) were found on BTA2, BTA13, BTA16 and BTA19 when the TAs as sire estimated in fatness_(L) were used as pseudo-phenotypes (Table 4; Fig. 3). The SNP on BTA13 is located in the double zinc ribbon and ankyrin repeat-containing protein 1 gene, which is positioned in an area from 38.78Mb to 38.83Mb. McClure *et al.* (2010) found a QTL with an effect on fat thickness within this area (CattleQTLdb; Release 32; Hu *et al.*, 2016). With regard to SNPs solely found when TAs as dam were analyzed, a SNP significant at the 5% chromosome-wide level was revealed for fatness_(T) on BTA29 (Fig. SF4). The SNP (13.59Mb) is located in a great distance from *IGF2* (50.04Mb to 50.06Mb). Therefore, a connection may be doubted.

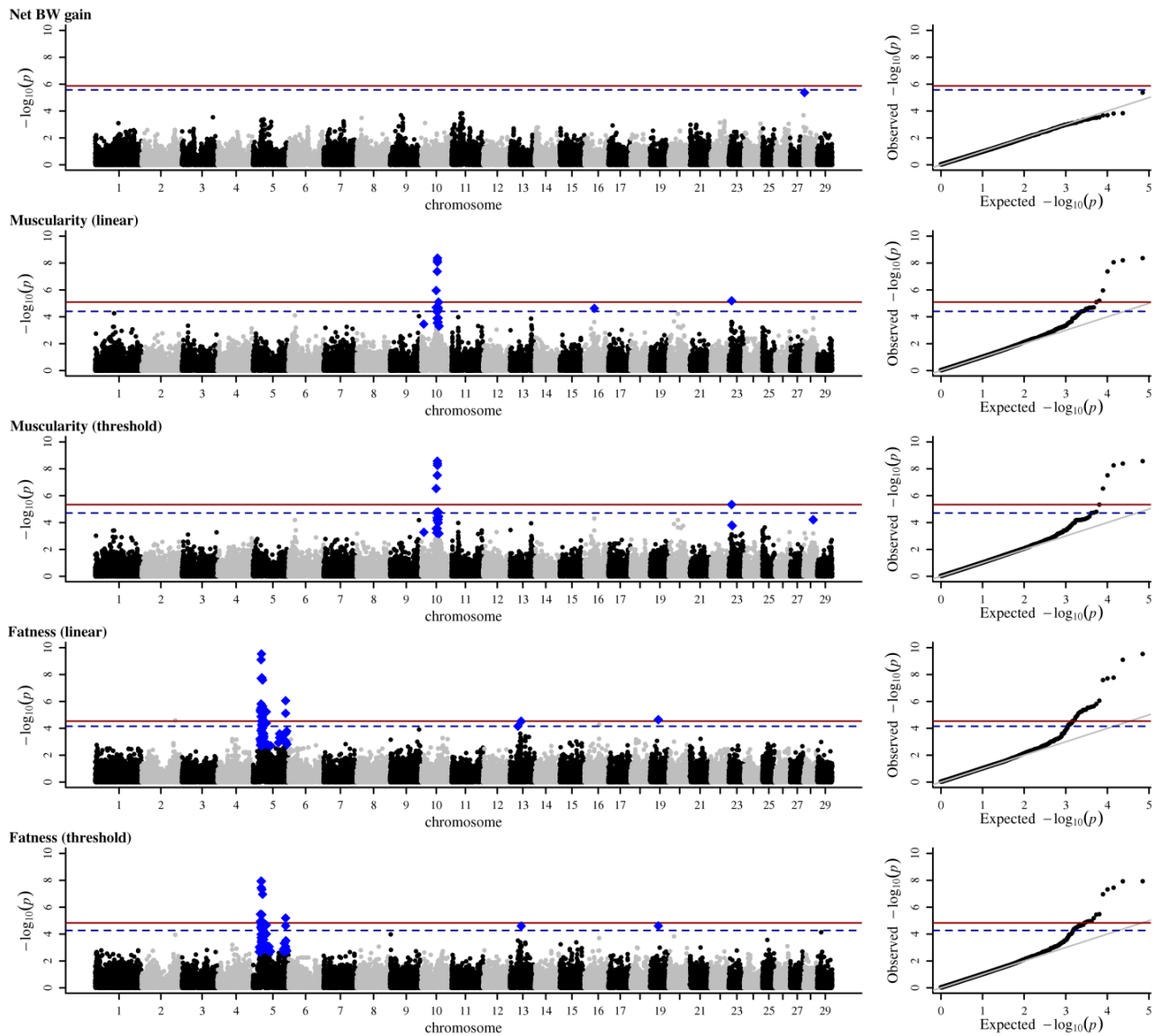


Figure 3. Marker loci in relation to their $-\log_{10} p$ -values generated by regressing transmitting abilities as sire on genotypes. Red line = 5% genome-wide significance; dashed line = 10% genome-wide significance; blue diamonds = 5% chromosome-wide significant markers.

To conclude, the strongest indications for markers associated with TAs observed in muscularity and fatness traits were found on BTA10 and BTA5. The signals mainly pointed to variants located within or proximal to the genes *UNC13C* (BTA10) and *TROAP* (BTA5). Scanning the genome for effects on TAs, which were estimated in muscularity and fatness traits via linear and threshold models led to results of high consistency. This suggests – at least for the categorical traits under consideration – that the linear and threshold models perform equally well capturing the underlying genetic variation.

General discussion

The simulation analysis suggests ePOEs to constitute convenient pseudo-phenotypes to detect imprinted loci in unphased genomes. As shown for EBVs in Ekine *et al.* (2014), preprocessing the ePOEs is, however, necessary to achieve reliable marker effect estimates and to avoid large false-positive rates. Apart from the PA-correction, de-regression and weighting, this includes also the consideration of the information contributed from the genotyped offspring to the ePOEs of their genotyped parents. This information was not accounted for in this study. This may lead to the double counting of the descendant's information, which is, however, of negligible impact (Garrick *et al.*, 2009). With regard to the weighting, c was limited to 0.1 to analyze the muscularity and fatness traits. When the ePOEs on the net BW gain were analyzed, c was varied, which led to changes in p -values and effects. As this indicates a certain sensitivity to c , varying this parameter with regard to the muscularity and fatness traits, might have resulted in further associations. However, as no effect of the weighting was observed in the simulation study, it remains questionable whether changing c actually results in true associations or in an inflation of effects. Thus, whether the associations found on BTA24 for ePOEs on the net BW gain constitute true associations or false-positives remains unclear when c was set to 0.8. Analyses with greater amounts of data would help investigating the true imprinting status of loci, especially regarding the SNPs found on BTA24. Due to the utilization of ePOEs as dependent variables it is possible that not only imprinted loci but also QTLs with a different nature of ePOEs (e.g., maternal genetic effects) were found. Follow-up studies could help to establish whether findings were solely based on imprinting effects or on effects mimicking genomic imprinting. Such nuisance factors could be also accounted for by including them in the model used to estimate the ePOEs.

The regression of de-regressed ePOEs on the simple gene counts of parents (e.g., AI-bulls) provides an unprecedented opportunity to cost-efficiently detect imprinted loci, which are expressed in their progeny without genotypes (e.g., fattening progeny). Most important, vast repositories of already collected phenotypes become accessible for imprinting analyses by applying our new approach.

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SUPPLEMENTARY MATERIAL

De-regression, parent-average correction and weighting

The reliability of an estimated parent-of-origin effect (ePOE) of an individual j was determined with $r_j^2 = 1 - PEV / (1/2 \sigma_i^2 (1 + F_j))$, where the PEV is the prediction error variance and F_j the inbreeding coefficient (generated using the pedigree R-package version 1.4 (Coster, 2013) in R (R Core Team, 2015)). To compute the parent average (PA) corrected ePOEs, the PA reliabilities (r_{PA}^2) and the PA ePOEs (\hat{i}_{PA}) were necessary. Moreover, a lambda was needed, which in our case corresponds to $\lambda = \sigma_R^2 / 1/2 \sigma_i^2$, where σ_R^2 is the residual variance obtained from the reduced imprinting model and σ_i^2 is the estimated imprinting variance. The additive genetic covariance

matrix for the PA and descendants was $\mathbf{A}^{-1} = \begin{bmatrix} 4 & -2 \\ -2 & 2 \end{bmatrix} \sigma_i^{-2}$. Then the following equations were set

up:

$$\begin{bmatrix} Z'_{PA} Z_{PA} + 4\lambda & -2\lambda \\ -2\lambda & Z'_j Z_j + 2\lambda \end{bmatrix} \begin{bmatrix} \hat{i}_{PA} \\ \hat{i}_j \end{bmatrix} = \begin{bmatrix} y_{PA}^* \\ y_j^* \end{bmatrix}, \quad [1]$$

where \hat{i}_j corresponds to the ePOE of individual j . According to Garrick *et al.* (2009), the direct solution for $Z'_j Z_j$ is $Z'_j Z_j = \delta Z'_{PA} Z_{PA} + 2\lambda(2\delta - 1)$ with $\delta = (0.5 - r_{PA}^2) / (1 - r_j^2)$. The direct solution for $Z'_{PA} Z_{PA}$ is $Z'_{PA} Z_{PA} = \lambda(0.5\alpha - 4) + 0.5\lambda \sqrt{(\alpha^2 + 16/\delta)}$ with $\alpha = 1 / (0.5 - r_{PA}^2)$. The left-hand-side

of Eq. [1] was then reconstructed and y_{PA}^* and y_j^* were calculated. The equation $[Z_j'Z_j + \lambda][\hat{i}_{j-PA}] = [y_j^*]$ was solved for the PA corrected ePOE \hat{i}_{j-PA} and the corresponding PA-corrected reliability r_j^{2*} was achieved as $r_j^{2*} = 1 - \lambda / (Z_j'Z_j + \lambda)$. Following the PA correction, the \hat{i}_{j-PA} were de-regressed, which involved their division by r_j^{2*} . In Garrick *et al.* (2009) this is simplified by $y_j^* / Z_j'Z_j$. Then, each de-regressed pseudo-phenotype needed to be weighted. The polygenic part of the ePOE not explained by markers is $var(\varepsilon_j)$. Adding the de-regressed prediction error variance, the residual variance of a de-regressed ePOE by applying the principles that were outlined in Garrick *et al.* (2009) for de-regressed EBVs is:

$$var(\varepsilon_j + k_j \hat{i}_{j-PA} - i_j) = var(\varepsilon_j) + \frac{(1 - r_j^{2*})}{r_j^{2*}} var(i_j) = \sigma_\varepsilon^2 + \frac{(1 - r_j^{2*})}{r_j^{2*}} \sigma_i^2 = \left[c + \frac{(1 - r_j^{2*})}{r_j^{2*}} \right] \sigma_i^2.$$

The parameter c defines the proportion of σ_i^2 not captured by markers so that $var(\varepsilon_j) = c\sigma_i^2$. To define c , a grid search was conducted, where c was progressively increased with a step size of 0.05 according to a proposal in Gorjanc *et al.* (2014). The c generating the greatest log-likelihood was chosen for each considered marker. Eventually, the inverse weights can be written as:

$$w_j^{-1} = \frac{\sigma_R^2}{\left[c + (1 - r_j^{2*}) / r_j^{2*} \right] \sigma_i^{2*}}.$$

Figures

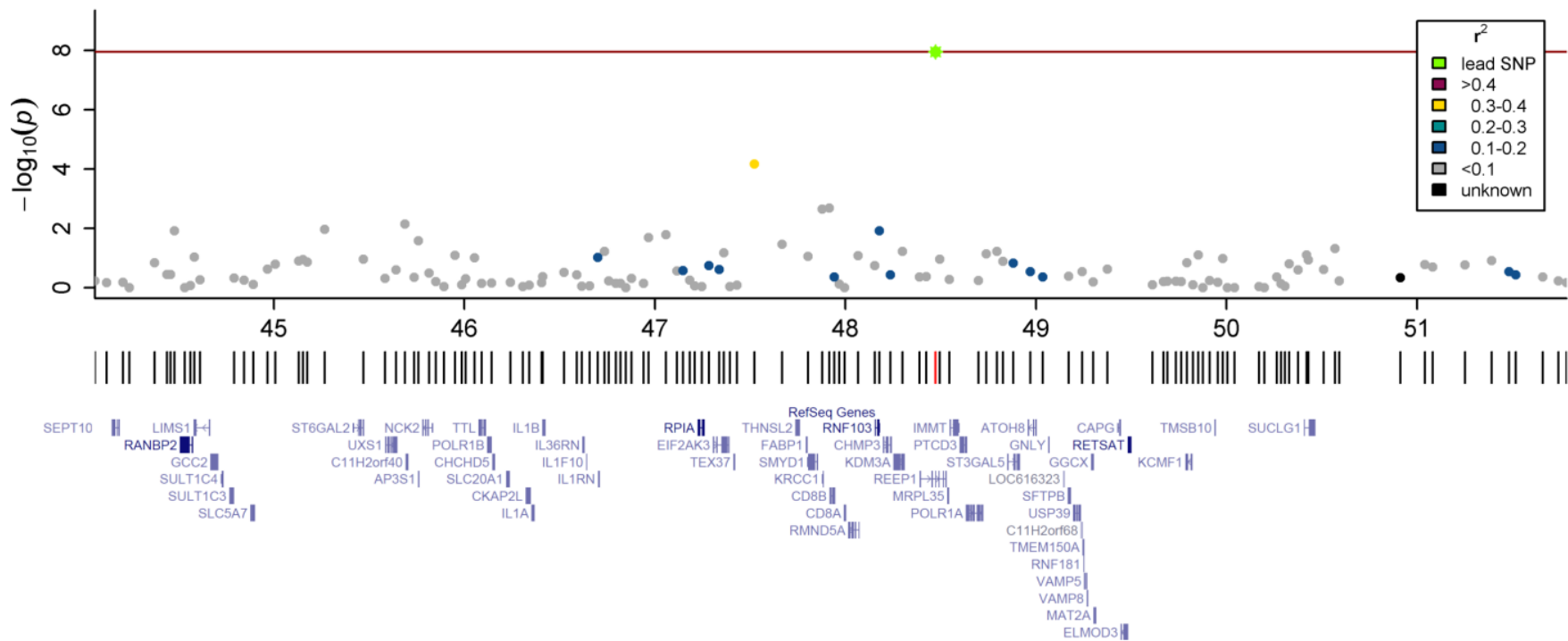


Figure SF1. Pairwise linkage disequilibrium (r^2) between the lead SNP and its adjacent markers at their mega base pair positions on BTA11. They are shown in relation to their $-\log_{10} p$ -values, which were calculated by regressing parent-of-origin effects of parents estimated in net BW gain on their genotypes when the c -parameter equaled 0.1. The red line is the 5% genome-wide significance level. Genes are displayed depending upon their physical positions (Bos Taurus UMD3.1.1/bosTau8; UCSC Genome Browser; Assembly date: Dec. 2009; <http://genome.ucsc.edu/> [last accessed in July 2017]). The red bars indicate significant SNPs.

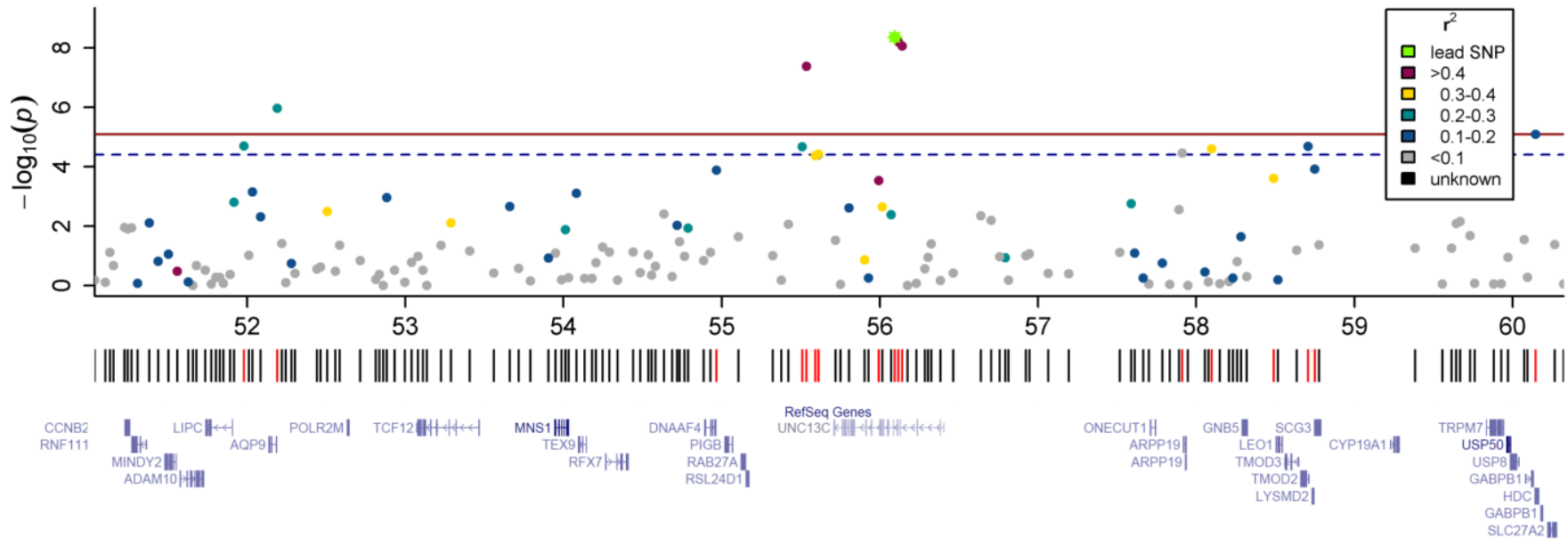


Figure SF3. Pairwise linkage disequilibrium (r^2) between the lead SNP and its adjacent markers at their mega base pair positions on BTA10. They are shown in relation to their $-\log_{10} p$ -values, which were calculated by regressing transmitting abilities as sire estimated in muscularity (analyzed via a linear model) on genotypes. The red line is the 5% genome-wide significance level. The blue dashed line is the 10% genome-wide significance level. Genes are displayed depending upon their physical positions (Bos Taurus UMD3.1.1/bosTau8; UCSC Genome Browser; Assembly date: Dec. 2009; <http://genome.ucsc.edu/> [last accessed in July 2017]). The red bars indicate significant SNPs.

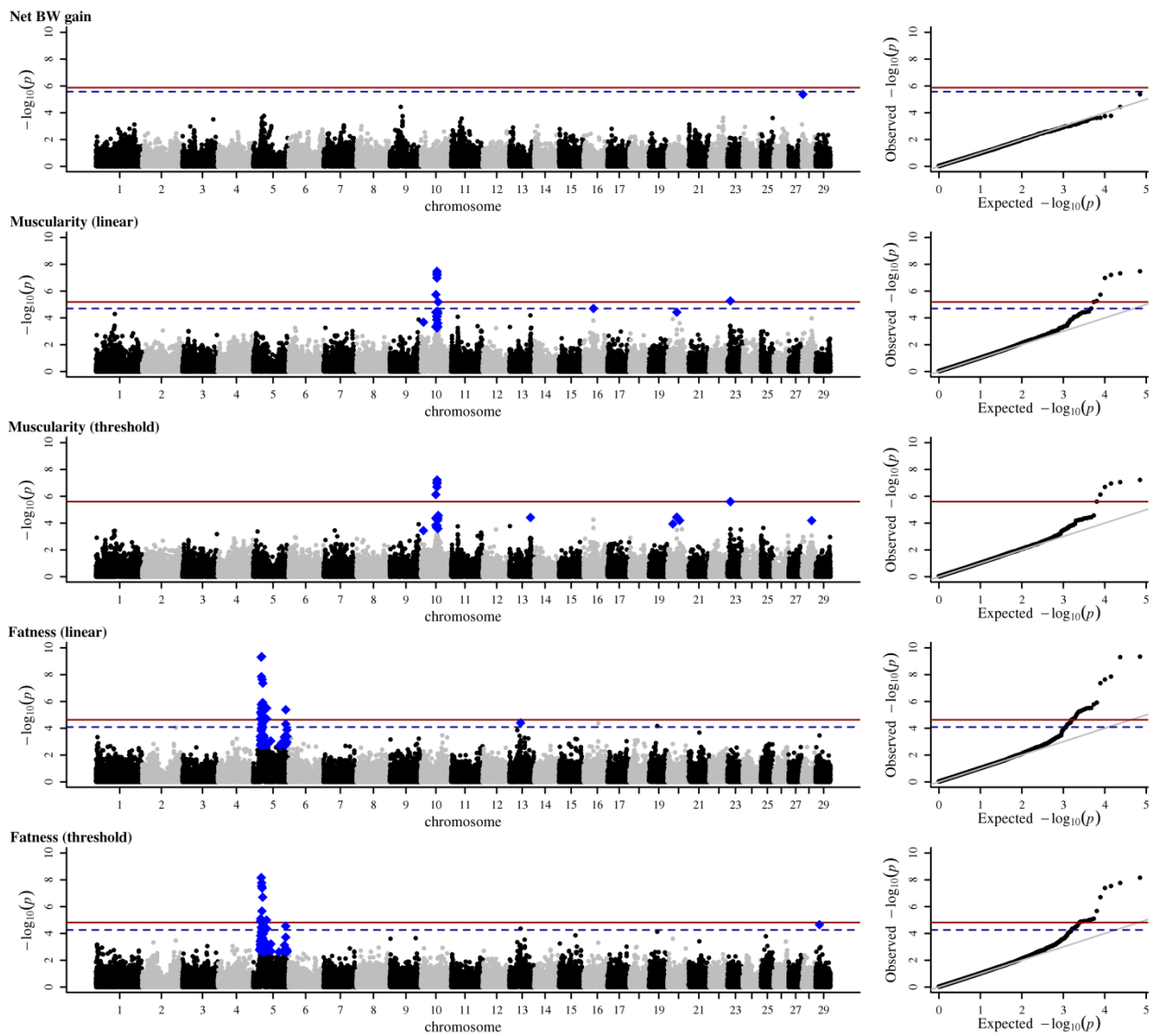


Figure SF4. Marker loci in relation to their $-\log_{10} p$ -values generated by regressing estimated transmitting abilities as dam on genotypes. Red line = 5% genome-wide significance; dashed line = 10% genome-wide significance; blue diamonds = 5% chromosome-wide significant markers.

Tables

Table ST1. Significant single nucleotide polymorphisms (SNPs) associated with transmitting abilities as sire estimated in net BW gain, muscularity and fatness analyzed via linear (L) and threshold model (T).

Trait	SNP	Chr	Position (bp)	<i>p</i> -value	effect	se	gene
Net BW gain	Hapmap49247-BTA-63647	28	3637555	4.2470e-06*	-8.3648	1.8187	
Muscularity _(L)	BTB-00429961	10	56093653	4.3065e-09***	-1.2325	0.2099	<i>UNC13C</i>
	ARS-BFGL-NGS-21100	10	56116909	6.2669e-09***	-1.2186	0.2098	<i>UNC13C</i>
	BTB-01911175	10	56140822	8.7539e-09***	1.2083	0.2100	<i>UNC13C</i>
	BTB-00430730	10	55535781	4.2323e-08***	1.1607	0.2118	
	Hapmap51030-BTA-69263	10	52190618	1.0970e-06***	1.2283	0.2521	<i>AQP9</i>
	Hapmap50767-BTA-72346	10	60145660	8.0751e-06***	1.2731	0.2852	<i>HDC</i>
	ARS-BFGL-NGS-24556	10	51980270	1.9888e-05**	-0.8659	0.2029	
	Hapmap36252-SCAFFOLD195517_10504	10	58707718	2.0960e-05**	-1.0007	0.2352	<i>TMOD2</i>
	ARS-BFGL-NGS-27708	10	55510249	2.1071e-05**	-0.8953	0.2105	
	Hapmap58597-rs29013533	10	58097440	2.5192e-05**	-0.8634	0.2049	
	BTB-01125630	10	57912228	3.4723e-05**	1.5872	0.3834	
	Hapmap59786-rs29012019	10	55611885	3.7975e-05**	-0.8747	0.2123	
	ARS-USMARC-Parent-DQ984827-rs29012019	10	55611885	3.9402e-05**	-0.8721	0.2121	
	BTB-00428180	10	55591993	4.2418e-05*	0.8700	0.2126	
	BTA-114684-no-rs	10	58749431	1.2210e-04*	0.8611	0.2241	<i>SCG3</i>
	BTB-00429005	10	54968099	1.3082e-04*	1.1514	0.3010	<i>DNAAF4</i>
	ARS-BFGL-NGS-55539	10	58488593	2.5029e-04*	0.8171	0.2231	
	BTB-00430147	10	55993255	2.9061e-04*	-0.7769	0.2144	<i>UNC13C</i>
	ARS-BFGL-NGS-55845	10	9821501	3.4662e-04*	0.7200	0.2013	<i>ARSB</i>
	ARS-BFGL-NGS-31807	10	61684704	4.9617e-04*	-0.7336	0.2106	
ARS-BFGL-NGS-114479	16	33402551	2.3777e-05**	1.1821	0.2798		
ARS-BFGL-NGS-40515	23	10593430	6.3823e-06***	-0.9244	0.2048		
Muscularity _(T)	BTB-00429961	10	56093653	2.6987e-09***	0.1888	0.0317	<i>UNC13C</i>
	ARS-BFGL-NGS-21100	10	56116909	4.0909e-09***	0.1865	0.0317	<i>UNC13C</i>
	BTB-01911175	10	56140822	5.5682e-09***	-0.1851	0.0318	<i>UNC13C</i>
	BTB-00430730	10	55535781	3.0586e-08***	-0.1770	0.0320	
	Hapmap51030-BTA-69263	10	52190618	2.9533e-07***	-0.1963	0.0383	<i>AQP9</i>
	Hapmap36252-SCAFFOLD195517_10504	10	58707718	1.5870e-05**	0.1532	0.0355	<i>TMOD2</i>
	ARS-BFGL-NGS-24556	10	51980270	1.8382e-05**	0.1315	0.0307	
	ARS-BFGL-NGS-27708	10	55510249	1.9373e-05**	0.1359	0.0318	
	Hapmap50767-BTA-72346	10	60145660	3.4541e-05*	-0.1794	0.0433	<i>HDC</i>
	BTB-00429005	10	54968099	4.4479e-05*	-0.1866	0.0457	<i>DNAAF4</i>
	BTB-01125630	10	57912228	5.6099e-05*	-0.2336	0.0580	
	Hapmap58597-rs29013533	10	58097440	5.7296e-05*	0.1246	0.0310	
	BTB-00428180	10	55591993	6.5038e-05*	-0.1284	0.0322	
	Hapmap59786-rs29012019	10	55611885	6.5038e-05*	0.1283	0.0321	
	ARS-USMARC-Parent-DQ984827-rs29012019	10	55611885	7.0776e-05*	0.1275	0.0321	
	BTA-114684-no-rs	10	58749431	1.0305e-04*	-0.1314	0.0338	<i>SCG3</i>
	BTB-00430147	10	55993255	2.7698e-04*	0.1178	0.0324	<i>UNC13C</i>
	ARS-BFGL-NGS-5130	10	52033595	2.9061e-04*	0.1788	0.0494	
	ARS-BFGL-NGS-55845	10	9821501	5.2633e-04*	-0.1055	0.0304	<i>ARSB</i>
	BTA-95978-no-rs	10	54082665	5.7661e-04*	0.1696	0.0493	
ARS-BFGL-NGS-55539	10	58488593	6.2165e-04*	-0.1154	0.0337		
ARS-BFGL-NGS-31807	10	61684704	6.6307e-04*	0.1083	0.0318		
ARS-BFGL-NGS-40515	23	10593430	4.6169e-06***	0.1409	0.0308		

Trait	SNP	Chr	Position (bp)	p-value	effect	se	gene
Fatness _(L)	ARS-BFGL-NGS-11408	23	11007995	1.5670e-04*	0.1514	0.0400	
	Hapmap49110-BTA-29232	23	12519690	1.7151e-04*	0.1478	0.0394	
	ARS-BFGL-NGS-88771	28	33359220	6.1044e-05*	-0.1243	0.0310	<i>KCNMA1</i>
	ARS-BFGL-NGS-67309	2	114710664	2.5727e-05***	0.0240	0.0057	
	ARS-BFGL-NGS-38020	5	29264678	2.9461e-10***	0.0328	0.0052	
	Hapmap39353-BTA-73120	5	27777281	7.9597e-10***	-0.0335	0.0055	
	ARS-BFGL-NGS-53461	5	30659497	1.7002e-08***	0.0292	0.0052	<i>TROAP</i>
	ARS-BFGL-NGS-91751	5	28573792	1.9337e-08***	-0.0332	0.0059	
	ARS-BFGL-NGS-92420	5	33078266	2.5670e-08***	0.0293	0.0053	
	ARS-BFGL-NGS-30168	5	111313740	8.8196e-07***	0.0266	0.0054	<i>SYNGR1</i>
	ARS-BFGL-NGS-98156	5	27869236	1.5540e-06***	0.0255	0.0053	
	Hapmap42150-BTA-25138	5	32819286	2.2840e-06***	-0.0257	0.0054	
	ARS-USMARC-675	5	31164990	2.5085e-06***	0.0410	0.0087	<i>ADCY6</i>
	Hapmap51043-BTA-73218	5	29622395	2.8574e-06***	0.0230	0.0049	<i>LARP4</i>
	ARS-BFGL-NGS-107085	5	28660813	2.9176e-06***	-0.0330	0.0071	<i>BIN2</i>
	ARS-BFGL-NGS-112542	5	30159843	4.0947e-06***	0.0288	0.0063	<i>FAIM2</i>
	BTA-72978-no-rs	5	25699752	4.2470e-06***	-0.0274	0.0060	<i>NCKAP1L</i>
	Hapmap23022-BTA-161235	5	31613026	4.2916e-06***	-0.0245	0.0053	
	ARS-USMARC-629	5	25738874	5.6301e-06***	0.0310	0.0068	<i>GTSF1</i>
	ARS-USMARC-657	5	45834943	5.8398e-06***	0.0281	0.0062	<i>IFNG</i>
	BTA-73209-no-rs	5	29496625	6.6548e-06***	0.0230	0.0051	<i>DIP2B</i>
	ARS-BFGL-NGS-68582	5	111380409	7.7038e-06***	-0.0251	0.0056	
	ARS-BFGL-NGS-717	5	32861996	1.0656e-05***	0.0240	0.0055	
	ARS-BFGL-NGS-7725	5	27839649	1.3210e-05***	-0.0220	0.0051	<i>KRT7</i>
	BTA-73392-no-rs	5	32104484	1.4981e-05***	0.0243	0.0056	
	ARS-BFGL-NGS-8796	5	29095603	2.9035e-05***	0.0227	0.0054	
	ARS-USMARC-652	5	45807839	4.0453e-05**	0.0282	0.0069	
	BTA-28787-no-rs	5	37925443	4.4479e-05**	0.0221	0.0054	
	ARS-BFGL-NGS-11851	5	32426908	4.5188e-05**	0.0226	0.0055	
	UA-IFASA-2781	5	29989860	5.3496e-05**	0.0218	0.0054	<i>ASIC1</i>
	ARS-BFGL-NGS-43909	5	30879776	5.4350e-05**	-0.0282	0.0070	
	Hapmap46916-BTA-105154	5	33246646	9.7217e-05*	-0.0220	0.0056	<i>PCED1B</i>
	Hapmap39286-BTA-73191	5	30275164	1.0866e-04*	-0.0210	0.0054	
	ARS-BFGL-NGS-100971	5	28153566	1.3942e-04*	0.0287	0.0075	
	ARS-BFGL-NGS-7567	5	28331294	1.5504e-04*	-0.0242	0.0064	<i>SCN8A</i>
	ARS-BFGL-NGS-93481	5	115223183	1.6525e-04*	0.0241	0.0064	
	Hapmap56154-rs29018650	5	35234997	2.2861e-04*	0.0379	0.0103	
	ARS-BFGL-NGS-104808	5	111635921	2.3478e-04*	-0.0223	0.0061	
	ARS-BFGL-NGS-29931	5	36528436	2.5162e-04*	-0.0189	0.0052	<i>TMEM117</i>
	BTA-111859-no-rs	5	91044792	2.5297e-04*	0.0267	0.0073	
ARS-BFGL-NGS-112163	5	31074760	2.5568e-04*	-0.0191	0.0052		
BTB-01248388	5	37440296	4.0262e-04*	0.0198	0.0056		
BTB-01495858	5	33981125	4.5784e-04*	-0.0204	0.0058		
BTA-05125-rs29019289	5	90866134	4.8564e-04*	-0.0191	0.0055		
ARS-BFGL-NGS-113311	5	27633265	5.3201e-04*	-0.0195	0.0056	<i>KRT75</i>	
ARS-BFGL-NGS-40375	5	36041533	5.5833e-04*	-0.0187	0.0054	<i>NELL2</i>	
Hapmap58633-rs29009713	5	97828652	6.0843e-04*	0.0202	0.0059	<i>BORCS5</i>	
ARS-BFGL-NGS-10291	5	24512405	6.1832e-04*	0.0203	0.0059	<i>TMCC3</i>	
ARS-BFGL-NGS-111020	5	106558376	6.9596e-04*	-0.0172	0.0051		
ARS-BFGL-NGS-33993	5	107031270	7.6677e-04*	0.0176	0.0052	<i>TSPAN11</i>	

Trait	SNP	Chr	Position (bp)	p-value	effect	se	gene
Fatness _(F)	BTB-01205531	5	33762720	8.6795e-04*	-0.0204	0.0061	
	ARS-BFGL-NGS-115973	5	28757093	9.2599e-04*	-0.0323	0.0098	
	ARS-BFGL-NGS-118817	5	31739928	9.4110e-04*	-0.0263	0.0080	
	ARS-BFGL-NGS-34382	5	26661043	9.5132e-04*	0.0340	0.0104	<i>ATF7</i>
	Hapmap33270-BTA-75139	5	112559317	1.0205e-03*	0.0300	0.0091	
	ARS-BFGL-NGS-100028	5	86753605	1.1682e-03*	-0.0183	0.0057	<i>SOX5</i>
	BTA-21377-no-rs	5	35657214	1.4120e-03*	-0.0237	0.0074	
	Hapmap41340-BTA-71012	5	115693793	1.4197e-03*	-0.0213	0.0067	
	ARS-BFGL-NGS-5790	5	26986116	1.5318e-03*	-0.0174	0.0055	
	ARS-BFGL-NGS-89539	5	50175236	1.5739e-03*	0.0178	0.0056	
	ARS-BFGL-NGS-72724	5	116005043	1.5911e-03*	-0.0152	0.0048	
	Hapmap41951-BTA-73168	5	28442563	1.6891e-03*	0.0176	0.0056	
	BTB-02015191	5	36553084	1.7169e-03*	0.0166	0.0053	<i>TMEM117</i>
	ARS-BFGL-NGS-21191	5	26263552	1.8730e-03*	-0.0297	0.0096	
	ARS-BFGL-NGS-20135	5	29839619	1.8832e-03*	0.0170	0.0055	<i>LIMA1</i>
	ARS-BFGL-NGS-119788	5	30185840	1.9458e-03*	-0.0221	0.0071	
	ARS-BFGL-NGS-55084	5	60513092	1.9778e-03*	-0.0171	0.0055	
	Hapmap55179-rs29024483	13	38830375	2.9496e-05***	0.0231	0.0055	<i>DZANK1</i>
	Hapmap55095-rs29010810	13	27589301	7.0403e-05**	0.0260	0.0065	
	ARS-BFGL-NGS-4866	16	50095343	5.3215e-05**	-0.0317	0.0079	
	ARS-BFGL-NGS-18515	19	29219211	2.2798e-05***	0.0231	0.0055	<i>NTN1</i>
	Hapmap39353-BTA-73120	5	27777281	1.1676e-08***	-0.1948	0.0342	
	ARS-BFGL-NGS-38020	5	29264678	1.1858e-08***	0.1858	0.0326	
	ARS-BFGL-NGS-91751	5	28573792	3.5517e-08***	-0.2030	0.0368	
	ARS-BFGL-NGS-53461	5	30659497	4.7653e-08***	0.1769	0.0324	<i>TROAP</i>
	ARS-BFGL-NGS-92420	5	33078266	1.0884e-07***	0.1746	0.0329	
	ARS-USMARC-629	5	25738874	3.3064e-06***	0.1985	0.0427	<i>GTSF1</i>
	ARS-BFGL-NGS-112542	5	30159843	3.5568e-06***	0.1814	0.0391	<i>FAIM2</i>
	ARS-BFGL-NGS-30168	5	111313740	6.5171e-06***	0.1530	0.0339	<i>SYNGR1</i>
	ARS-BFGL-NGS-107085	5	28660813	1.0769e-05***	-0.1945	0.0442	<i>BIN2</i>
	ARS-USMARC-675	5	31164990	1.0997e-05***	0.2390	0.0544	<i>ADCY6</i>
	BTA-72978-no-rs	5	25699752	1.1527e-05***	-0.1631	0.0372	<i>NCKAP1L</i>
	ARS-BFGL-NGS-43909	5	30879776	1.3004e-05***	-0.1900	0.0436	
	Hapmap42150-BTA-25138	5	32819286	1.4670e-05***	-0.1466	0.0338	
	ARS-BFGL-NGS-8796	5	29095603	2.0420e-05**	0.1442	0.0338	
	ARS-USMARC-657	5	45834943	2.0850e-05**	0.1661	0.0390	<i>IFNG</i>
	ARS-BFGL-NGS-68582	5	111380409	2.3777e-05**	-0.1481	0.0351	
	ARS-BFGL-NGS-7725	5	27839649	2.9808e-05**	-0.1324	0.0317	<i>KRT7</i>
	ARS-BFGL-NGS-98156	5	27869236	3.2770e-05**	0.1379	0.0332	
	Hapmap51043-BTA-73218	5	29622395	3.4000e-05**	0.1282	0.0309	<i>LARP4</i>
	BTA-73209-no-rs	5	29496625	4.1973e-05**	0.1310	0.0320	<i>DIP2B</i>
	ARS-BFGL-NGS-717	5	32861996	4.1973e-05**	0.1386	0.0338	
	Hapmap56154-rs29018650	5	35234997	5.3779e-05**	0.2576	0.0638	
	Hapmap23022-BTA-161235	5	31613026	9.1717e-05*	-0.1308	0.0334	
	ARS-USMARC-652	5	45807839	9.9826e-05*	0.1676	0.0431	
	ARS-BFGL-NGS-100971	5	28153566	1.0582e-04*	0.1828	0.0472	
	ARS-BFGL-NGS-7567	5	28331294	1.8477e-04*	-0.1496	0.0400	<i>SCN8A</i>
ARS-BFGL-NGS-29931	5	36528436	2.5568e-04*	-0.1184	0.0324	<i>TMEM117</i>	
UA-IFASA-2781	5	29989860	2.7550e-04*	0.1226	0.0337	<i>ASIC1</i>	
Hapmap33270-BTA-75139	5	112559317	3.0983e-04*	0.2046	0.0567		

Trait	SNP	Chr	Position (bp)	<i>p</i> -value	effect	se	gene
	ARS-BFGL-NGS-11851	5	32426908	3.1652e-04*	0.1252	0.0348	
	ARS-BFGL-NGS-115973	5	28757093	3.8369e-04*	-0.2171	0.0611	
	BTB-01205531	5	33762720	3.9199e-04*	-0.1353	0.0382	
	ARS-BFGL-NGS-112163	5	31074760	4.6030e-04*	-0.1148	0.0328	
	BTA-73392-no-rs	5	32104484	4.6277e-04*	0.1228	0.0351	
	ARS-BFGL-NGS-33993	5	107031270	4.8825e-04*	0.1143	0.0328	<i>TSPAN11</i>
	BTA-28787-no-rs	5	37925443	5.7045e-04*	0.1165	0.0338	
	ARS-BFGL-NGS-40375	5	36041533	6.8115e-04*	-0.1146	0.0337	<i>NELL2</i>
	BTB-01495858	5	33981125	7.2658e-04*	-0.1227	0.0363	
	BTB-01205481	5	33808700	7.4640e-04*	-0.1301	0.0386	
	Hapmap46916-BTA-105154	5	33246646	7.5855e-04*	-0.1187	0.0353	<i>PCED1B</i>
	ARS-BFGL-NGS-10291	5	24512405	8.1797e-04*	0.1244	0.0372	<i>TMCC3</i>
	ARS-BFGL-NGS-89539	5	50175236	8.3131e-04*	0.1176	0.0352	
	ARS-BFGL-NGS-119788	5	30185840	8.4486e-04*	-0.1490	0.0446	
	BTA-54940-no-rs	5	55263796	8.4486e-04*	-0.1084	0.0325	
	ARS-BFGL-NGS-104808	5	111635921	8.5864e-04*	-0.1265	0.0380	
	Hapmap41346-BTA-72920	5	22196364	9.4620e-04*	0.1175	0.0356	
	ARS-USMARC-Parent-DQ500958-no-rs	5	27825118	9.7208e-04*	-0.1225	0.0371	
	ARS-BFGL-NGS-113311	5	27633265	1.0041e-03*	-0.1150	0.0350	<i>KRT75</i>
	ARS-BFGL-NGS-5790	5	26986116	1.0316e-03*	-0.1123	0.0342	
	Hapmap41950-BTA-72999	5	26082666	1.0541e-03*	-0.1158	0.0354	
	Hapmap39286-BTA-73191	5	30275164	1.0541e-03*	-0.1111	0.0339	
	ARS-BFGL-NGS-66566	5	36122701	1.1248e-03*	-0.1279	0.0393	
	BTA-75143-no-rs	5	112647134	1.3595e-03*	-0.1143	0.0357	<i>XPNPEP3</i>
	ARS-BFGL-NGS-52457	5	104116518	1.3818e-03*	-0.1167	0.0365	<i>PIANP</i>
	ARS-BFGL-NGS-34382	5	26661043	1.4509e-03*	0.2074	0.0651	<i>ATF7</i>
	ARS-BFGL-NGS-34352	5	49842685	1.5318e-03*	0.1064	0.0336	<i>SRGAP1</i>
	ARS-BFGL-NGS-93481	5	115223183	1.8227e-03*	0.1251	0.0401	
	Hapmap43887-BTA-58386	5	54045462	1.8527e-03*	-0.1004	0.0323	<i>SLC16A7</i>
	ARS-BFGL-NGS-21191	5	26263552	1.8935e-03*	-0.1858	0.060	
	ARS-BFGL-NGS-77906	5	24533402	1.9352e-03*	-0.1070	0.0345	<i>TMCC3</i>
	ARS-BFGL-NGS-55084	5	60513092	1.9352e-03*	-0.1069	0.0345	
	Hapmap47087-BTA-73116	5	27898166	1.9564e-03*	-0.1809	0.0584	<i>KRT80</i>
	ARS-BFGL-NGS-111020	5	106558376	2.0436e-03*	-0.0980	0.0318	
	BTA-72912-no-rs	5	22020313	2.0886e-03*	-0.1519	0.0494	
	ARS-BFGL-NGS-110506	5	105152085	2.0886e-03*	-0.1053	0.0342	<i>NTF3</i>
	Hapmap55179-rs29024483	13	38830375	2.4928e-05**	0.1452	0.0344	<i>DZANK1</i>
	ARS-BFGL-NGS-18515	19	29219211	2.4410e-05**	0.1445	0.0342	<i>NTN1</i>

***5% genome-wide; **10% genome-wide; *5% chromosome-wide; se = standard error

Table ST2. Significant single nucleotide polymorphisms associated with transmitting abilities as dam estimated in net BW gain, muscularity and fatness analyzed using a linear (L) and threshold model (T).

Trait	SNP	Chr	Position (bp)	p-value	effect	se	gene
Net BW gain	Hapmap49247-BTA-63647	28	3637555	4.2249e-06*	-9.7952	2.1293	
Muscularity _(L)	BTB-00429961	10	56093653	3.3044e-08***	-1.3037	0.2360	<i>UNC13C</i>
	ARS-BFGL-NGS-21100	10	56116909	4.7164e-08***	-1.2881	0.2358	<i>UNC13C</i>
	BTB-01911175	10	56140822	6.2317e-08***	1.2778	0.2361	<i>UNC13C</i>
	BTB-00430730	10	55535781	1.0443e-07***	1.2633	0.2375	
	Hapmap51030-BTA-69263	10	52190618	1.8450e-06***	1.3499	0.2830	<i>AQP9</i>
	Hapmap50767-BTA-72346	10	60145660	6.4493e-06***	1.4423	0.3197	<i>HDC</i>
	Hapmap58597-rs29013533	10	58097440	3.3467e-05*	-0.9521	0.2295	
	ARS-BFGL-NGS-27708	10	55510249	3.4723e-05*	-0.9781	0.2363	
	ARS-BFGL-NGS-24556	10	51980270	3.7380e-05*	-0.9369	0.2272	
	BTB-01125630	10	57912228	4.0241e-05*	1.7619	0.4291	
	Hapmap36252-SCAFFOLD195517_10504	10	58707718	4.7886e-05*	-1.0755	0.2645	<i>TMOD2</i>
	Hapmap59786-rs29012019	10	55611885	7.2671e-05*	-0.9452	0.2383	
	ARS-USMARC-Parent-DQ984827-rs29012019	10	55611885	7.7023e-05*	-0.9413	0.2381	
	BTB-00428180	10	55591993	8.3383e-05*	0.9387	0.2390	
	BTB-00429005	10	54968099	1.3649e-04*	1.2890	0.3379	<i>DNAAF4</i>
	ARS-BFGL-NGS-55845	10	9821501	2.0994e-04*	0.8355	0.2254	<i>ARSB</i>
	BTA-114684-no-rs	10	58749431	2.5162e-04*	0.9208	0.2515	<i>SCG3</i>
	BTA-95978-no-rs	10	54082665	3.9833e-04*	-1.2853	0.3600	
	ARS-BFGL-NGS-55539	10	58488593	4.5296e-04*	0.8760	0.2498	
	ARS-BFGL-NGS-5130	10	52033595	4.6526e-04*	-1.2770	0.3649	
	BTB-00430147	10	55993255	5.6436e-04*	-0.8299	0.2407	<i>UNC13C</i>
	ARS-BFGL-NGS-114479	16	33402551	1.9784e-05**	1.3420	0.3145	
	ARS-BFGL-NGS-10108	20	31848979	3.7184e-05*	0.9655	0.2341	
ARS-BFGL-NGS-40515	23	10593430	5.4280e-06***	-1.0428	0.2293		
Muscularity _(T)	BTB-00429961	10	56093653	6.0106e-08***	0.2219	0.0410	<i>UNC13C</i>
	ARS-BFGL-NGS-21100	10	56116909	8.8069e-08***	0.2189	0.0409	<i>UNC13C</i>
	BTB-01911175	10	56140822	1.1111e-07***	-0.2174	0.0410	<i>UNC13C</i>
	BTB-00430730	10	55535781	2.0136e-07***	-0.2141	0.0412	
	Hapmap51030-BTA-69263	10	52190618	7.4310e-07***	-0.2447	0.0494	<i>AQP9</i>
	Hapmap50767-BTA-72346	10	60145660	2.6974e-05*	-0.2340	0.0557	<i>HDC</i>
	Hapmap36252-SCAFFOLD195517_10504	10	58707718	4.2868e-05*	0.1881	0.0460	<i>TMOD2</i>
	ARS-BFGL-NGS-24556	10	51980270	4.3094e-05*	0.1613	0.0394	
	BTB-00429005	10	54968099	4.6640e-05*	-0.2396	0.0588	<i>DNAAF4</i>
	ARS-BFGL-NGS-27708	10	55510249	5.4350e-05*	0.1656	0.0410	
	BTB-01125630	10	57912228	5.7296e-05*	-0.2993	0.0744	
	Hapmap58597-rs29013533	10	58097440	6.1367e-05*	0.1594	0.0398	
	Hapmap59786-rs29012019	10	55611885	1.4547e-04*	0.1571	0.0414	
	BTB-00428180	10	55591993	1.4624e-04*	-0.1573	0.0414	
	ARS-BFGL-NGS-5130	10	52033595	1.4859e-04*	0.2405	0.0634	
	ARS-USMARC-Parent-DQ984827-rs29012019	10	55611885	1.6263e-04*	0.1559	0.0413	
	BTA-95978-no-rs	10	54082665	2.1675e-04*	0.2333	0.0631	
	BTA-114684-no-rs	10	58749431	2.6259e-04*	-0.1591	0.0440	<i>SCG3</i>
	ARS-BFGL-NGS-55845	10	9821501	3.6177e-04*	-0.1399	0.0392	<i>ARSB</i>
	Hapmap54667-rs29016013	13	72431970	3.7975e-05*	0.2172	0.0527	
	ARS-BFGL-NGS-10108	20	31848979	3.6408e-05*	-0.1674	0.0405	
	ARS-BFGL-BAC-33671	20	41576197	6.4015e-05*	-0.1595	0.0399	
	ARS-BFGL-NGS-25782	20	18537789	1.1518e-04*	0.2377	0.0617	

Trait	SNP	Chr	Position (bp)	<i>p</i> -value	effect	se	gene
Fatness _(L)	ARS-BFGL-NGS-40515	23	10593430	2.4954e-06***	0.1866	0.0396	
	ARS-BFGL-NGS-88771	28	33359220	6.5729e-05*	-0.1597	0.0400	<i>KCNMA1</i>
	ARS-BFGL-NGS-38020	5	29264678	4.5299e-10***	0.0362	0.0058	
	Hapmap39353-BTA-73120	5	27777281	4.9168e-10***	-0.0378	0.0061	
	ARS-BFGL-NGS-91751	5	28573792	1.3981e-08***	-0.0373	0.0066	
	ARS-BFGL-NGS-53461	5	30659497	2.2920e-08***	0.0323	0.0058	<i>TROAP</i>
	ARS-BFGL-NGS-92420	5	33078266	4.2982e-08***	0.0321	0.0059	
	Hapmap42150-BTA-25138	5	32819286	1.2171e-06***	-0.0294	0.0061	
	ARS-BFGL-NGS-98156	5	27869236	1.6542e-06***	0.0283	0.0059	
	ARS-BFGL-NGS-107085	5	28660813	3.0737e-06***	-0.0367	0.0079	<i>BIN2</i>
	ARS-USMARC-657	5	45834943	3.1059e-06***	0.0323	0.0069	<i>IFNG</i>
	Hapmap23022-BTA-161235	5	31613026	3.1879e-06***	-0.0278	0.0060	
	ARS-BFGL-NGS-112542	5	30159843	3.6508e-06***	0.0323	0.0070	<i>FAIM2</i>
	ARS-BFGL-NGS-30168	5	111313740	4.1161e-06***	0.0279	0.0061	<i>SYNGRI</i>
	ARS-USMARC-675	5	31164990	4.5928e-06***	0.0445	0.0097	<i>ADCY6</i>
	Hapmap51043-BTA-73218	5	29622395	5.3997e-06***	0.0250	0.0055	<i>LARP4</i>
	ARS-BFGL-NGS-717	5	32861996	5.4850e-06***	0.0276	0.0061	
	BTA-72978-no-rs	5	25699752	6.3823e-06***	-0.0300	0.0066	<i>NCKAP1L</i>
	ARS-BFGL-NGS-7725	5	27839649	8.6887e-06***	-0.0251	0.0056	<i>KRT7</i>
	BTA-73392-no-rs	5	32104484	1.1527e-05***	0.0275	0.0063	
	BTA-73209-no-rs	5	29496625	1.6122e-05***	0.0246	0.0057	<i>DIP2B</i>
	ARS-USMARC-652	5	45807839	1.9577e-05***	0.0328	0.0077	
	ARS-USMARC-629	5	25738874	1.9784e-05***	0.0325	0.0076	<i>GTSE1</i>
	ARS-BFGL-NGS-8796	5	29095603	2.1745e-05***	0.0256	0.0060	
	BTA-28787-no-rs	5	37925443	2.3282e-05***	0.0254	0.0060	
	ARS-BFGL-NGS-11851	5	32426908	3.6601e-05**	0.0256	0.0060	
	ARS-BFGL-NGS-43909	5	30879776	4.5667e-05**	-0.0316	0.0078	
	ARS-BFGL-NGS-68582	5	111380409	4.8394e-05**	-0.0255	0.0063	
	UA-IFASA-2781	5	29989860	5.4637e-05**	0.0243	0.0060	<i>ASIC1</i>
	Hapmap46916-BTA-105154	5	33246646	8.1206e-05**	-0.0248	0.0063	<i>PCED1B</i>
	ARS-BFGL-NGS-100971	5	28153566	9.5685e-05*	0.0327	0.0084	
	ARS-BFGL-NGS-7567	5	28331294	1.0251e-04*	-0.0276	0.0071	<i>SCN8A</i>
	Hapmap39286-BTA-73191	5	30275164	1.0808e-04*	-0.0234	0.0061	
	ARS-BFGL-NGS-93481	5	115223183	1.3151e-04*	0.0274	0.0072	
	ARS-BFGL-NGS-113311	5	27633265	1.4938e-04*	-0.0237	0.0063	<i>KRT75</i>
	ARS-BFGL-NGS-104808	5	111635921	3.4293e-04*	-0.0243	0.0068	
	ARS-BFGL-NGS-29931	5	36528436	3.5411e-04*	-0.0206	0.0058	<i>TMEM117</i>
	ARS-BFGL-NGS-72724	5	116005043	4.0262e-04*	-0.0191	0.0054	
	ARS-BFGL-NGS-10291	5	24512405	4.1799e-04*	0.02333	0.0066	<i>TMCC3</i>
	ARS-BFGL-NGS-112163	5	31074760	4.2933e-04*	-0.0206	0.0059	
ARS-BFGL-NGS-118817	5	31739928	4.3629e-04*	-0.0312	0.0089		
ARS-BFGL-NGS-111020	5	106558376	4.5539e-04*	-0.0199	0.0057		
BTB-01248388	5	37440296	4.7790e-04*	0.0217	0.0062		
BTB-01495858	5	33981125	5.2633e-04*	-0.0225	0.0065		
ARS-BFGL-NGS-34382	5	26661043	6.9972e-04*	0.0394	0.0116	<i>ATF7</i>	
Hapmap56154-rs29018650	5	35234997	7.0349e-04*	0.0388	0.0114		
ARS-BFGL-NGS-5790	5	26986116	7.1880e-04*	-0.0207	0.0061		
Hapmap41950-BTA-72999	5	26082666	8.0486e-04*	-0.0211	0.0063		
ARS-BFGL-NGS-55084	5	60513092	8.7736e-04*	-0.0205	0.0062		
BTB-02015191	5	36553084	9.0134e-04*	0.0197	0.0059	<i>TMEM117</i>	

Trait	SNP	Chr	Position (bp)	<i>p</i> -value	effect	se	gene
Fatness _T	BTB-01205531	5	33762720	9.4110e-04*	-0.0225	0.0068	
	Hapmap41340-BTA-71012	5	115693793	1.0947e-03*	-0.0244	0.0075	
	BTB-00225371	5	36578127	1.1873e-03*	-0.0192	0.0059	<i>TMEM117</i>
	Hapmap58633-rs29009713	5	97828652	1.2602e-03*	0.0213	0.0066	<i>BORCS5</i>
	ARS-BFGL-NGS-115973	5	28757093	1.3303e-03*	-0.0349	0.0109	
	Hapmap41951-BTA-73168	5	28442563	1.3521e-03*	0.0200	0.0063	
	Hapmap44526-BTA-17619	5	50731517	1.4827e-03*	-0.0197	0.0062	
	ARS-BFGL-NGS-21191	5	26263552	1.5485e-03*	-0.0337	0.0107	
	ARS-BFGL-NGS-15778	5	111227019	1.6349e-03*	-0.0338	0.0107	
	ARS-BFGL-NGS-4809	5	111682678	1.6349e-03*	0.0309	0.0098	<i>ENTHD1</i>
	Hapmap24085-BTA-143102	5	46364432	1.7357e-03*	-0.0404	0.0129	
	ARS-BFGL-NGS-33993	5	107031270	1.7357e-03*	0.0184	0.0059	<i>TSPAN11</i>
	ARS-BFGL-NGS-40375	5	36041533	1.8129e-03*	-0.0189	0.0061	<i>NELL2</i>
	ARS-BFGL-NGS-77906	5	24533402	1.8935e-03*	-0.0192	0.0062	<i>TMCC3</i>
	Hapmap47087-BTA-73116	5	27898166	1.9247e-03*	-0.0324	0.0105	<i>KRT80</i>
	BTA-111859-no-rs	5	91044792	1.9352e-03*	0.0252	0.0081	
	Hapmap55179-rs29024483	13	38830375	3.9195e-05**	0.0253	0.0062	<i>DZANK1</i>
	ARS-BFGL-NGS-4866	16	50095343	4.2868e-05**	-0.0359	0.0088	
	ARS-BFGL-NGS-18515	19	29219211	6.7846e-05**	0.0243	0.0061	<i>NTN1</i>
	Hapmap39353-BTA-73120	5	27777281	6.9099e-09***	-0.2447	0.0423	
	ARS-BFGL-NGS-38020	5	29264678	1.7002e-08***	0.2280	0.0404	
	ARS-BFGL-NGS-91751	5	28573792	2.9049e-08***	-0.2528	0.0456	
	ARS-BFGL-NGS-53461	5	30659497	4.0822e-08***	0.2209	0.0403	<i>TROAP</i>
	ARS-BFGL-NGS-92420	5	33078266	1.9826e-07***	0.2122	0.0408	
	ARS-BFGL-NGS-112542	5	30159843	2.1124e-06***	0.2300	0.0485	<i>FAIM2</i>
	ARS-USMARC-629	5	25738874	7.8667e-06***	0.2372	0.0531	<i>GTSF1</i>
	ARS-BFGL-NGS-107085	5	28660813	9.1077e-06***	-0.2434	0.0548	<i>BIN2</i>
	ARS-USMARC-657	5	45834943	1.0007e-05***	0.2146	0.0486	<i>IFNG</i>
	Hapmap42150-BTA-25138	5	32819286	1.1895e-05***	-0.1837	0.0420	
	BTA-72978-no-rs	5	25699752	1.1958e-05***	-0.2020	0.0461	<i>NCKAP1L</i>
	ARS-BFGL-NGS-43909	5	30879776	1.2275e-05***	-0.2359	0.0540	
	ARS-BFGL-NGS-7725	5	27839649	1.2868e-05***	-0.1716	0.0393	<i>KRT7</i>
	ARS-USMARC-675	5	31164990	1.3141e-05***	0.2939	0.0675	<i>ADCY6</i>
	ARS-BFGL-NGS-8796	5	29095603	1.5218e-05***	0.1810	0.0419	
	ARS-BFGL-NGS-30168	5	111313740	2.8281e-05**	0.1769	0.0423	<i>SYNGR1</i>
	ARS-BFGL-NGS-717	5	32861996	3.0283e-05**	0.1750	0.0419	
	ARS-BFGL-NGS-98156	5	27869236	3.5276e-05**	0.1705	0.0412	
	Hapmap51043-BTA-73218	5	29622395	4.0667e-05**	0.1580	0.0385	<i>LARP4</i>
	ARS-USMARC-652	5	45807839	4.3781e-05**	0.2198	0.0538	
	Hapmap23022-BTA-161235	5	31613026	5.4927e-05**	-0.1681	0.0417	
BTA-73209-no-rs	5	29496625	7.2288e-05*	0.1581	0.0398	<i>DIP2B</i>	
ARS-BFGL-NGS-100971	5	28153566	8.2505e-05*	0.2301	0.0584		
ARS-BFGL-NGS-7567	5	28331294	8.6988e-05*	-0.1945	0.0496	<i>SCN8A</i>	
Hapmap56154-rs29018650	5	35234997	1.0251e-04*	0.3068	0.0790		
Hapmap41950-BTA-72999	5	26082666	1.4702e-04*	-0.1661	0.0438		
ARS-BFGL-NGS-68582	5	111380409	1.8874e-04*	-0.1633	0.0437		
ARS-BFGL-NGS-11851	5	32426908	2.1791e-04*	0.1600	0.0433		
ARS-BFGL-NGS-113311	5	27633265	2.5842e-04*	-0.1585	0.0434	<i>KRT75</i>	
BTA-73392-no-rs	5	32104484	2.7258e-04*	0.1591	0.0437		
UA-IFASA-2781	5	29989860	2.8906e-04*	0.1520	0.0419	<i>ASIC1</i>	

Trait	SNP	Chr	Position (bp)	<i>p</i> -value	effect	se	gene
	ARS-BFGL-NGS-29931	5	36528436	3.4662e-04*	-0.1441	0.0403	<i>TMEM117</i>
	ARS-BFGL-NGS-10291	5	24512405	3.8575e-04*	0.1635	0.0461	<i>TMCC3</i>
	BTA-28787-no-rs	5	37925443	4.4336e-04*	0.1471	0.0419	
	BTB-01205531	5	33762720	5.2633e-04*	-0.1641	0.0473	
	ARS-BFGL-NGS-115973	5	28757093	5.3487e-04*	-0.2631	0.0760	
	ARS-BFGL-NGS-5790	5	26986116	5.6436e-04*	-0.1463	0.0424	
	ARS-BFGL-NGS-55084	5	60513092	6.0193e-04*	-0.1462	0.0426	
	Hapmap46916-BTA-105154	5	33246646	6.7386e-04*	-0.1493	0.0439	<i>PCED1B</i>
	ARS-BFGL-NGS-77906	5	24533402	6.8482e-04*	-0.1452	0.0428	<i>TMCC3</i>
	BTB-01495858	5	33981125	7.3444e-04*	-0.1524	0.0451	
	ARS-USMARC-Parent-DQ500958-no-rs	5	27825118	7.4239e-04*	-0.1556	0.0461	
	ARS-BFGL-NGS-33993	5	107031270	7.4239e-04*	0.1379	0.0409	<i>TSPAN11</i>
	ARS-BFGL-NGS-34352	5	49842685	7.7091e-04*	0.1401	0.0417	<i>SRGAP1</i>
	BTA-54940-no-rs	5	55263796	8.1358e-04*	-0.1352	0.0404	
	BTB-01205481	5	33808700	8.7264e-04*	-0.1593	0.0479	
	ARS-BFGL-NGS-34382	5	26661043	9.1605e-04*	0.2680	0.0808	<i>ATF7</i>
	ARS-BFGL-NGS-21191	5	26263552	1.0598e-03*	-0.2432	0.0743	
	ARS-BFGL-NGS-119788	5	30185840	1.0656e-03*	-0.1809	0.0552	
	Hapmap47087-BTA-73116	5	27898166	1.1127e-03*	-0.2367	0.0726	<i>KRT80</i>
	Hapmap39286-BTA-73191	5	30275164	1.1248e-03*	-0.1373	0.0422	
	ARS-BFGL-NGS-112163	5	31074760	1.1809e-03*	-0.1323	0.0408	
	ARS-BFGL-NGS-104808	5	111635921	1.3231e-03*	-0.1517	0.0473	
	ARS-BFGL-NGS-72504	5	24605672	1.3595e-03*	-0.1684	0.0526	
	Hapmap33270-BTA-75139	5	112559317	1.5654e-03*	0.2224	0.0703	
	ARS-BFGL-NGS-66566	5	36122701	1.6800e-03*	-0.1534	0.0488	
	ARS-BFGL-NGS-89539	5	50175236	1.6800e-03*	0.1371	0.0436	
	BTA-72912-no-rs	5	22020313	1.6891e-03*	-0.1927	0.0614	
	Hapmap41346-BTA-72920	5	22196364	1.7642e-03*	0.1378	0.0441	
	BTA-73296-no-rs	5	26526934	1.7738e-03*	-0.1405	0.0450	
	ARS-BFGL-NGS-93481	5	115223183	1.7738e-03*	0.1565	0.0501	
	Hapmap51042-BTA-73045	5	24579644	1.7835e-03*	0.1642	0.0526	
	ARS-BFGL-NGS-101539	5	24628072	1.7835e-03*	0.1642	0.0526	
	Hapmap24085-BTA-143102	5	46364432	1.7835e-03*	-0.2800	0.0900	
	ARS-BFGL-NGS-111020	5	106558376	1.8730e-03*	-0.1231	0.0396	
	ARS-BFGL-NGS-38038	5	27992179	1.9671e-03*	-0.1840	0.0594	<i>NR4A1</i>
	BTA-75143-no-rs	5	112647134	2.0104e-03*	-0.1369	0.0444	<i>XPNPEP3</i>
	ARS-BFGL-NGS-52457	5	104116518	2.0660e-03*	-0.1403	0.0455	<i>PIANP</i>
	BTB-02015191	5	36553084	2.0772e-03*	0.1272	0.0413	<i>TMEM117</i>
	BTA-73685-no-rs	5	60929878	2.0886e-03*	0.1277	0.0415	
	Hapmap41340-BTA-71012	5	115693793	2.1937e-03*	-0.1602	0.0523	
	ARS-BFGL-NGS-72724	5	116005043	2.3167e-03*	-0.1150	0.0378	
	ARS-BFGL-NGS-40375	5	36041533	2.3294e-03*	-0.1277	0.0419	<i>NELL2</i>
	ARS-BFGL-NGS-118817	5	31739928	2.3421e-03*	-0.1889	0.0621	
	BTB-00234784	5	88311948	2.4334e-03*	-0.1331	0.0439	<i>ST8SIA1</i>
	BTB-00225371	5	36578127	2.5008e-03*	-0.1252	0.0414	<i>TMEM117</i>
	Hapmap55179-rs29024483	13	38830375	4.3781e-05**	0.1749	0.0428	<i>DZANK1</i>
	Hapmap33865-BES2_Contig389_1251	29	13959142	2.1860e-05**	-0.2103	0.0496	

***5% genome-wide; **10% genome-wide; *5% chromosome-wide; se = standard error

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GENERAL DISCUSSION

In 2010 Neugebauer *et al.* (2010a;b) published a model (Neugebauer model) that facilitates the estimation of the imprinting variance considering all kinds of imprinting simultaneously. The model and its properties are introduced and demonstrated in simulated data in chapter one. In chapter two the *Neugebauer model* was applied to slaughterhouse data to investigate the relevance of imprinting for the expression of carcass quality and beef performance traits in Brown Swiss cattle. In chapter three it is described how the *Neugebauer model* facilitated the estimation of imprinting variances in large data sets solely based on male ancestors. The analysis of Simmental cattle slaughterhouse data observed in 1,366,160 fattening bulls with 2,637,761 ancestors led to significant imprinting variances in all carcass quality and beef performance traits.

When imprinting effects are analyzed applying the *Neugebauer model*, the derivation of their prediction error variances (PEVs) – which are used to calculate their reliabilities – is difficult and computational detours are needed (Neugebauer, 2010). Therefore, apart from the estimation of the imprinting variances using the *Neugebauer model*, a new model (equivalent model) that incorporates a random imprinting effect and thus delivers its prediction error variance directly was developed and introduced in chapter two. A linear and generalized linear version of this model provided reliable parent-of-origin effects (ePOEs; a broader term for imprinting effects) on the slaughter traits in the Brown Swiss cattle. In chapter three the *equivalent model* provided reliable ePOEs solely based on male ancestors in the Simmental cattle slaughterhouse data.

Chapter four addresses challenges encountered with regard to the mapping of imprinted loci. To overcome these challenges, a new approach was introduced that exempts the mapping of imprinted loci from the necessity of phased genotypes (where heterozygotes can be distinguished according to the parental origin of their alleles). For this approach, genotypes do not need to be available for animals with records because this phenotypic information is summarized in the parental ePOEs (e.g., estimated via the *equivalent model*). The ePOEs of parents can then be used as de-regressed pseudo-phenotypes to locate the imprinted loci solely based on their simple gene counts. As the *equivalent model* delivered the ePOEs on slaughter traits earlier (chapter two), they were used as de-regressed pseudo-phenotypes to scan the genomes of Brown Swiss ancestors for the underlying imprinted loci (chapter four). One genome-wide and multiple

chromosome-wide associations were found for ePOEs on all analyzed slaughter traits. The following discussion approaches the contents of this thesis and addresses the obstacles and difficulties still impeding genomic imprinting analyses.

Results of the imprinting variance analyses

To investigate the relevance of imprinting for the expression of slaughter traits in Brown Swiss cattle, the *Neugebauer model* (Neugebauer *et al.*, 2010a;b) was applied to slaughterhouse data to estimate the imprinting variances (chapter two). The analysis resulted in significant imprinting variances in the net BW gain, fat score and carcass muscularity. In chapter three, the *Neugebauer model* was applied in a parsimonious version to slaughter data in Austrian and German Simmental cattle after replacing the genetic effect as dam with the corresponding genetic effect as maternal grand-sire. The analysis led to significant estimates of the genomic imprinting variance in all analyzed traits (net BW gain, fat score, carcass muscularity and killing out percentage). When comparing these findings with the literature, it should be stated that comparisons across different breeds ought to be drawn with caution. Moreover, divergent definitions of traits and the application of different statistical models complicate the comparison across studies. Nonetheless, consistent results were obtained for carcass quality traits (fat score and carcass muscularity) leading to the conclusion that imprinted genes affect their expression, while breed differences are inconsiderable (Engellandt and Tier, 2002; Neugebauer *et al.*, 2010b; Tier and Meyer, 2012; Blunk *et al.*, 2016; Blunk *et al.*, 2017). The meaning of imprinted genes for the expression of carcass quality traits was underpinned by the findings that the Insulin-like growth-factor 2 gene (IGF2) is maternally imprinted in cattle (Dindot *et al.*, 2004) and that gene variants are known to affect the expression of carcass quality traits, such as rib eye area and percent fat (Godall and Schmutz, 2007). In contrast to the carcass quality traits, discrepancies exist for the beef performance traits net BW gain and killing out percentage. For the latter, a moderate proportion of the genetic variance attributed to imprinted genes arose for Simmental cattle with 24.56% in Neugebauer *et al.* (2010b). Our study confirmed this finding in Simmental cattle although a lower proportion of the genetic variance was observed (chapter three). Imprinting variance analyses in Gelbvieh and Brown Swiss cattle did not result in significant outcomes for the killing out percentage (Engellandt and Tier, 2002; Blunk *et al.*, 2016), which may be, e.g., due to breed differences. However, it should be considered that another model was used in the Gelbvieh study that only provided an estimate for the proportion of the phenotypic

variance attributed to paternally expressed genes. With regard to our study in Brown Swiss cattle (chapter two), the data set might have been too small to capture the imprinting variance properly. For the net BW gain, we found significant imprinting variance contributions to the total genetic variance in Brown Swiss and Simmental cattle (chapter two and three). This result could neither be confirmed by Neugebauer *et al.* (2010b) in Simmental cattle, although they used the same model, nor by Engellandt and Tier (2002) in Gelbvieh. Therefore, inconsistent findings exist regarding the relevance of genomic imprinting for beef performance traits in literature that are probably attributable to breed differences or different statistical models used in the particular studies. To conclude, the results for carcass quality traits (fat score and carcass muscularity) seem consistent across breeds (Gelbvieh, Austrian and German Simmental, Brown Swiss, Australian Angus and Hereford), whereas inconsistencies were found for the beef performance traits net BW gain and killing out percentage.

A new model for imprinting variance analyses

The *Neugebauer model* (Neugebauer *et al.*, 2010a;b) contains two correlated additive genetic effects; one as sire and one as dam. Whereas the imprinting effect can easily be derived as the difference between the two parental genetic effects, the determination of its PEV requires an inconvenient detour as mentioned in Neugebauer (2010). The reason is that the imprinting effect constitutes a contrast. To achieve its PEV, which is given by $Var[(\hat{a}_d - a_d) - (\hat{a}_s - a_s)]$, the two diagonal elements $Var(\hat{a}_d - a_d)$ and $Var(\hat{a}_s - a_s)$ as well as the off-diagonal element $Cov((\hat{a}_d - a_d), (\hat{a}_s - a_s))$ are required from the inverted coefficient matrix. This is not straightforward using standard software packages such as ASReml because in ASReml the variance matrix for estimates is only available for equations in the dense portion (Gilmour *et al.*, 2009). The *equivalent model*, developed in this thesis, overcomes this inconvenience. It considers the maternal gamete to be inherited under a paternal expression pattern, which, however, leaves the deviation from the genetic effect as sire unconsidered. Therefore, the dam's imprinting effect is added, which offers the opportunity to derive its PEV from the diagonal elements of the inverted coefficient matrix. The *equivalent model* can be written as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_s\mathbf{a}_s + \mathbf{Z}_t\mathbf{i}_d + \mathbf{e},$$

where \mathbf{i}_d is the vector of half of the imprinting effects of dams; \mathbf{a}_s is a vector of transmitting abilities (TAs) as sire; and \mathbf{y} , $\boldsymbol{\beta}$ and \mathbf{e} are vectors of records, fixed effects and residuals. The incidence matrices \mathbf{X} and \mathbf{Z}_i link the fixed effects and the imprinting effects to the respective records, where \mathbf{Z}_i is equal to \mathbf{Z}_d , which is an incidence matrix in the *Neugebauer model* linking the records to the genetic effects as dam. Matrix \mathbf{Z}_s is an incidence matrix containing two ones: the first links records to the TAs of sires as sire, the second connects records to the TAs of dams as sire. Moreover, \mathbf{Z}_s can be considered as a composition of the two incidence matrices contained in the *Neugebauer model*, where the first links records to the genetic effects as sire and the second corresponds to \mathbf{Z}_d .

From a biological point of view one should acknowledge that the *Neugebauer model* may be more intuitive than the *equivalent model* and the imprinting effect can be easily derived as the difference between the parental genetic effects. Furthermore, applying both models to the same data set using the same starting values, the *Neugebauer model* required less iterations until convergence was achieved. Thus, for approximately the same iteration time, the *Neugebauer model* took less time until the variance parameters stabilized. However, although it took longer to converge, the *equivalent model* provided the PEVs of ePOEs without a detour so that the desired results could be achieved quicker and more conveniently. This is advantageous, e.g., when the ePOEs and their reliabilities are to be used in subsequent analyses. For example, in chapter four the de-regressed ePOEs determined using the *equivalent model* were used as pseudo-phenotypes to locate imprinted genome regions. From a technical point of view, the *equivalent model* was as easy to apply as the *Neugebauer model* using the standard software package ASReml (Gilmour *et al.*, 2009). The only difference was the additional entry in the incidence matrix \mathbf{Z}_s . At this point it should be mentioned that, to the best of the author's knowledge, ASReml constituted the most suitable tool to apply the *equivalent model* (for job files see the appendix). The reason is that this program allows a so called "overlay" of incidence matrices (Gilmour *et al.*, 2009), which was needed to construct \mathbf{Z}_s and to generate an appropriate correlation between the model terms \mathbf{a}_s and \mathbf{a}_d where the latter corresponds to the TA as dam in the *Neugebauer model*. Moreover, an overlay was needed to construct a null model, which was necessary to test for the significance of the imprinting variance using a REML log-likelihood ratio test (chapter two and three).

To conclude, the *equivalent model* does not only consider all kinds of imprinting simultaneously, but also provides the desired ePOEs accompanied by their PEVs directly. It is easy to apply using the software package ASReml (Gilmour *et al.*, 2009) and thus constitutes a convenient tool to provide components, e.g. needed for genomic imprinting analyses.

Estimated parent-of-origin effects as pseudo-phenotypes

Genomic scans like association studies and linkage analyses that aim to identify imprinted loci “traditionally” require the knowledge – at least with some probability – of the phased genotype of each individual with a phenotypic record. In particular, the two types of heterozygous genotypes Qq and qQ , where the first allele is of paternal origin, have to be distinguished. The imprinting effect at a locus can then be estimated as the regression on the difference between the allele counts (1 or 0) on the paternal and maternal chromosomes. At a bi-allelic marker these differences are 0, 1, -1 and 0 for the phased genotypes QQ , Qq , qQ and qq , respectively (see e.g., the review of Wolf *et al.*, 2008). A significance test for a non-zero regression coefficient then indicates the presence or absence of an imprinting effect, equivalent to the existence or non-existence of a systematic phenotypic difference between the Qq and qQ heterozygotes. However, the phase of genotypes is often unknown or at least uncertain and genotypes are frequently unavailable for individuals with phenotypic measures (e.g., slaughter animals). Assuming that the regression of an animal’s ePOE on the unphased genotype (thus, the simple gene count with 0, 1 or 2) is the imprinting effect, it was proposed in chapter four to use de-regressed and weighted ePOEs of parents as pseudo-phenotypes to be regressed on their unphased genotypes. The existence of an imprinted locus is then indicated, when the regression coefficient significantly deviates from zero. In an imprinting variance analysis in ungenotyped Brown Swiss fattening bulls (chapter two), the *equivalent model* provided ePOEs for their parents on the net BW gain, fat score and carcass muscularity. To locate the underlying imprinted loci, the de-regressed ePOEs were then regressed on the simple gene counts of sires, where each marker effect was individually tested for its deviation from zero (chapter four). With regard to the de-regression, weighting and parent-average correction of the ePOEs, an approximation method published in Garrick *et al.* (2009) for breeding values was applied. This method was a convenient choice as no access to the equation system was needed and all necessary information was previously provided by the *equivalent model*. Using this approach did not allow the de-regression of two effects simultaneously, which would, however, be appropriate using models

that consider imprinting by including two genetic effects. Thus, a corresponding method still needs to be developed. The genome scan for the underlying imprinted loci in Brown Swiss cattle led multiple chromosome-wide and one genome-wide significant association with the ePOEs on slaughterhouse data. Whether these findings actually point to imprinted loci or another kind of parent-of-origin effect remains to be discussed, especially because maternal genetic effects were not considered in the model when the ePOEs were estimated for Brown Swiss cattle in chapter two. As the inheritance of maternal genetic effects is sex-limited, relationships caused by these effects are different for males and females. Therefore, in case maternal genetic effects are neglected, they contribute to the imprinting variance (Hager *et al.*, 2008). According to literature, the relevance of maternal genetic effects seems, however, to be limited to traits such as birth and weaning weight (Meyer, 1992). Thus, an inflation of the imprinting variance attributed to maternal genetic effects on slaughter traits (measured at an average slaughter age of fattening bulls of approximately 18 months) may be of minor magnitude. This applies also to non-genetic maternal effects, as Brown Swiss and Simmental calves are separated from their dams shortly after birth and raised with a formula diet. Inflations of the imprinting variance may also be caused by X-chromosomal additive genetic effects as they were not considered in the model when the ePOEs were estimated in chapter two and three. X-chromosomal contributions to the imprinting variance have, to the author's knowledge, not yet been discussed in the literature in contrast to other potential nuisance factors such as maternal effects (Hager *et al.*, 2008), Y-chromosomal effects (Reinsch *et al.*, 1999) and mitochondrial effects (Neugebauer *et al.*, 2010a;b). With regard to imprinted loci on the X-chromosome, their existence could not be analyzed as only phenotypes of fattening bulls were available for the analysis (chapter four). They received their X-chromosome from their dams so that there was only a maternal methylation pattern affecting the measured phenotype. In this case only X-chromosomal breeding values as dam could be estimated and no difference, i.e. imprinting effect, was available. However, when genotypes were also available for dams and their X-chromosomal genotypes were analyzed, signals on the X-chromosome would indicate nuisances of ePOEs generated by X-chromosomal contributions. The reason is that no signals on the X-chromosome are expected because no paternal methylation patterns and thus no imprinting effects are expected as explained above. Knowledge of X-chromosomal effects in cattle is limited to findings of significant contributions to the genetic variance in milk production and fertility (VanRaden, 1987; Fernando and Grossman, 1990). The only reference suggesting the existence

of significant X-chromosomal variance contributions in slaughter traits are X-chromosomal effects on carcass composition traits in pigs (Harlizius *et al.*, 2000).

In the genome scan for imprinted loci in Brown Swiss cattle, chromosome-wide and genome-wide significant associations were found for ePOEs on the fat class, carcass muscularity and net BW gain (chapter four). Despite these findings, many imprinted loci may still be undetected. In genome scans such as association studies, the ability to detect markers in the close vicinity of causal variants depends, among others, on the variance explained by the considered marker. The variance itself depends on the effect size of a locus and its allelic variation in the population (Zondervan and Cardon, 2004; Korte and Farlow, 2013). These conditions also apply for the mapping of imprinted loci. Until now, there is little information about the actual number and frequency of imprinted variants in cattle and it is questionable to what extent they affect the phenotypes of interest. That imprinted loci can have considerable effects on the expression of agriculturally important traits was demonstrated in a study that revealed a purely paternally expressed variant within the *IGF2* gene in pigs. This variant explained up to 30% of the variance for lean meat content, lean meat mass and back fat thickness (Jeon *et al.*, 1999; Nezer *et al.*, 1999). When estimated values are used as dependent variables in genome scans, their own reliability is another factor influencing the ability of finding true associations. In chapter two, the average reliabilities of ePOEs ranged between 9.73% and 25.86% so that they cannot be assumed to fully reflect true imprinting effects.

It should be noted that apart from the reduced versions (Quaas and Pollak, 1980) of models that consider imprinting, gametic versions are available. A gametic version of the *equivalent model* would allow the prediction of ePOEs for individuals with phenotypes. Thus, if genotypes and ePOEs were available for individuals carrying, e.g., a particular disorder, it would be possible to scan their genomes for imprinted loci affecting the expression of this phenotype, even if the genotypes were unphased.

With regard to livestock, the new approach of using ePOEs as pseudo-phenotypes to detect imprinted loci allows the exploitation of a large amount of already collected phenotypes, which otherwise would remain idle for imprinting analyses if only the “traditional approach” was followed. For example, with regard to dual-purpose and dairy breeds in Germany, Austria and Czechia, genotypes are usually only available for bulls (e.g., AI-bulls and selection candidates) and the traits of interest (e.g., milk production traits, somatic cell count or beef performance) can only be observed in their progeny (a problem, which was e.g., encountered in an imprinting

analysis in Magee *et al.*, 2010). Cows are genotyped to a lesser extent (bull dams and selection candidates), although more cows may be expected to be genotyped in the future. In some populations large numbers of females have already been genotyped so that the “traditional approach” is an opportunity to detect imprinted loci (e.g., Jiang *et al.*, 2017). Although it is of course a matter of cost, slaughter animals (e.g., fattening bulls) are not genotyped and may not be expected to be genotyped in the future. The situation is similar with regard to pigs, although for some breeds (e.g., Danish breeds) genotypes are available for several thousands of fattening pigs (e.g., Guo *et al.*, 2016). Nonetheless, with the “traditional approach” a lot of phenotypic data would remain unused, whereas our new approach opens the door to their exploitation for imprinting analyses.

Conclusion

The *Neugebauer model* is suitable to take every variant of genomic imprinting into account as shown in chapter one. The *equivalent model* introduced in chapter two grants the same advantage and facilitates also the direct prediction of ePOEs and PEVs for a large number of animals using the existing software. With the ePOEs and PEVs the *equivalent model* provides the necessary features for an approach introduced in chapter four that enables the detection of imprinted loci when phased genotypes are not available and genotyped animals do not have records. The approaches developed in this thesis open up new possibilities for investigating genomic imprinting and allow the exploitation of information, which otherwise would remain unused for imprinting studies.

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SUMMARY

Genomic imprinting is an epigenetic phenomenon that arises when the expression of genes depends on the parental origin of their alleles. Thus, genetic imprinting effects are attributed to wider class of the parent-of-origin effects (POEs). So far, the derivation of their standard errors – which are needed to calculate their reliabilities – was of high computational effort. Therefore, a model (equivalent model) was proposed that directly provides the estimates of POEs (ePOEs) and thus easily delivers their standard errors. The application of this model to slaughter data resulted in new findings on the impact of imprinting on beef traits in Brown Swiss cattle. On average, the imprinting variances accounted for 9.6% of the total genetic variance in the net body weight gain, fat score and carcass conformation. The equivalent model was then modified to enable the analyses of great sets of Simmental cattle slaughterhouse data provided by up to 1.36 million fattening bulls solely based on male ancestors. A mean proportion of 13.2% of the total genetic variance was found to be attributable to POEs on the killing out percentage, net body weight gain, fat score and carcass conformation.

After investigating the role of imprinting in variance component analyses, the underlying imprinted loci were analyzed in a genome-wide association study in Brown Swiss cattle. In earlier approaches of such studies, it was a general prerequisite that phased genotypes are available for the animals with phenotypes, i.e. the parental origin of the alleles of heterozygote genotypes is known. However, only unphased genotypes and no own records were available for the sires of the Brown Swiss fattening bulls. In simulated data, it was, however, shown that imprinted loci can be detected and distinguished from biparentally expressed loci, when ePOEs of parents are regressed on their own unphased genotypes, i.e. their simple gene counts. The variation of all ePOEs can be adjusted using their reliabilities derived via the equivalent model. This approach has made it possible to detect potentially imprinted loci within the unphased genomes of the Brown Swiss sires. The *equivalent model* efficiently provides the necessary features for this approach that facilitates the exploitation of large amounts of data, which already exist and otherwise would remain idle for imprinting analyses.

ZUSAMMENFASSUNG

Beim genomischen Imprinting (dt. genomische Prägung) hängt die Expression der Gene von der elterlichen Herkunft ihrer Allele ab. Daher werden genetische Imprintingeffekte den sogenannten ‚Parent-of-origin effects‘ zugeordnet (POEs). Bisher war die direkte Schätzung der Imprintingeffekte nicht möglich, so dass die Berechnung ihrer Standardfehler – die auch für die Berechnung ihrer Sicherheiten benötigt werden – mit einem hohen rechnerischen Aufwand verbunden war. Aus diesem Grund wurde ein Modell (äquivalentes Modell) entwickelt, das die Schätzwerte für POEs (ePOEs) gemeinsam mit ihren Standardfehlern direkt und mit deutlich verringertem rechnerischem Aufwand liefert. Die Anwendung dieses Modells auf Schlachtdaten führte zu neuen Erkenntnissen über den Einfluss des Imprintings auf Schlachtmerkmale beim Braunvieh. Im Durchschnitt erklärte die Imprintingvarianz 9,6 % der genetischen Varianz in Nettozunahme, Fett- und Handelsklasse. In einem darauffolgenden Schritt wurde das Modell abgewandelt, um – ausschließlich basierend auf männlichen Verwandtschaftsstrukturen – die Imprintingvarianzen in großen Datensätzen zu schätzen. Dies erlaubte die Analyse von Schlachtdaten von bis zu 1,36 Millionen Fleckviehbullen. Geprägte Loci trugen im Durchschnitt 13,2 % zur genetischen Varianz der Ausschachtung, Nettozunahme, Fett- und Handelsklasse bei. Nachdem die Bedeutung des Imprintings in Varianzkomponentenanalysen untersucht wurde, sollten die zugrunde liegenden geprägten Loci im Rahmen einer genomweiten Assoziationsstudie beim Braunvieh analysiert werden. Bekannte Ansätze für solche Analysen setzen voraus, dass von den Mastnachkommen selbst geordnete Genotypen verfügbar sind, d.h. die elterliche Herkunft der Allele heterozygoter Markergenotypen bekannt ist. Zur Verfügung standen jedoch ausschließlich ungeordnete Genotypen von Vätern der Mastnachkommen, also von Tieren ohne eigenen Phänotyp. In simulierten Daten konnte aber gezeigt werden, dass geprägte Genorte als solche erkannt und kartiert werden können, wenn ePOEs von Elterntieren als abhängige Variable auf ihre eigenen ungeordneten Genotypen regressiert werden. Die Streuung aller ePOEs wurde vorher mit Hilfe der mit dem äquivalenten Modell geschätzten Sicherheiten auf ein gleiches Niveau gebracht. So wurde es möglich potentiell geprägte Loci in den Genomen von Braunvieh-Vätern zu detektieren, die in ihren Nachkommen exprimiert wurden. Das äquivalente Modell liefert somit auf rechnerisch effiziente Art alle Komponenten, die im Rahmen des neuen Kartierungsansatzes für geprägte Genorte benötigt werden. Dieser Ansatz ermöglicht die Nutzung großer bereits vorhandener Datenmengen, die bisher für Imprintinganalysen nicht zugänglich waren.

APPENDIX

ASReml (Gilmour *et al.*, 2009) – Job control files (.as file) and files to calculate functions of variance components (.pin file)

ASReml job control file for the linear imprinting model (Neugebauer *et al.*, 2010a;b) with weights exemplarily for the fat class:

```
!Workspace 32000 !DEBUG !LOG
Analyse Fettklasse Braunvieh
tier !A 133671      # animal ID
trait              # records
weight !M0         # weighting
VATER 420626 !P    # Father in pedigree file
MUTTER 420626 !P   # Mother in pedigree file
sd 11214 !I        # fixed effects (farm x date of slaughter)
pn 3 !I           # parity number
bt 2 !I           # birth type
alter1            # linear regression on slaughter age
alter2            # quadratic regression on slaughter age
alter3            # cubic regression on slaughter age

# ==== pedigree ====
PED.txt !SKIP 1 !ALPHA !MAKE

# ==== data ====
SDAT.txt !SKIP 1 !MAXIT 100 !AISING !CONTINUE

# ==== linear model ====
trait !WT weight ~ sd pn bt alter1 alter2 alter3 !r VATER MUTTER

# ==== variance-covariance-structure ===
0 0 1             # R-structure header
VATER 2           # G-structure header
2 0 US !GP        # First structure definition
V11 C21 V22       # Initial cov values (V11= $\sigma_s^2$ ; C21= $\sigma_{s,d}$ ; V22= $\sigma_d^2$ )
VATER 0 AINV      # Second structure definition
```


ASReml job control file for the linear imprinting model containing an imprinting effect (Blunk *et al.*, 2016) with weights exemplarily for the fat class:

```

!Workspace 32000 !DEBUG !LOG
Analyse Fettklasse Braunvieh
tier !A 133671
trait
weight !M0
VATER 420626 !P
MUTTER 420626 !P
IMP 420626 !P
sd 11214 !I
pn 3 !I
bt 2 !I
alter1
alter2
alter3

# ==== pedigree ====
PED.txt !SKIP 1 !ALPHA

# ==== data ====
SDAT.txt !SKIP 1 !BLUP 3 !AISING !CONTINUE

# ==== linear model ====
trait !WT weight ~ sd pn bt leg(alter1,-3) !r VATER and(MUTTER,1)
IMP

# ==== variance-covariance-structure ===
1 1 1
0 0 ID !S2==r #!S2==r sets the initial error variance to r
VATER 2
2 0 US !GP
V11 C21 V22      # (V11= $\sigma_s^2$ ; C21=( $\sigma_{s,d} - \sigma_s^2$ ); V22= $\sigma_i^2$ )
VATER 0 AINV

```

ASReml job control file for the generalized linear imprinting model containing an imprinting effect (Blunk *et al.*, 2016) with weights exemplarily for the fat class:

```

!Workspace 32000 !DEBUG !LOG
Analyse Fettklasse2 Braunvieh
tier !A 133671
trait
weight !M0
VATER 420626 !P
MUTTER 420626 !P
IMP 420626 !P
sd 11214 !I
pn 3 !I
bt 2 !I
alter1
alter2
alter3

# ==== pedigree ====
PED.txt !SKIP 1 !ALPHA

# ==== data ====
SDAT.txt !SKIP 1 !MAXIT 2 !AISING !CONTINUE

# ==== linear model ====
trait !BINOMIAL !LOGIT !WT weight ~ sd pn bt leg(alter1,-3) !r
VATER and(MUTTER,1) IMP

# ==== variance-covariance-structure ===
1 1 1
0 0 ID !S2==3.289868

VATER 2
2 0 US !GP
V11 C21 V22          # (V11= $\sigma_s^2$ ; C21=( $\sigma_{s,d} - \sigma_s^2$ ); V22= $\sigma_i^2$ )
VATER 0 AINV

```

ASReml job control file for the linear sire-maternal-grandsire model containing an imprinting effect (Blunk *et al.*, 2016) with weights exemplarily for the fat class:

```

!Workspace 32000 !DEBUG !LOG
Analyse PRECISION FETTKLASSE_5
tier !A 1366160
trait
weight !M0
VATER 27567 !P
MVATER 27567 !P          #maternal grandsire in pedigree file
IMP 27567 !P
sd 87654 !I
pn 3 !I
bt 3 !I
alter1
alter2
alter3

# ==== pedigree ====
PED.txt !SKIP 1 !MGS !ALPHA

# ==== data ====
DAT.txt !SKIP 1 !BLUP 3 !AISING !CONTINUE

!PART 1 # New model
# ==== linear model ====
trait !WT weight ~ pn bt leg(alter1,-3) !r IMP VATER
and(MVATER,0.5) !F sd

# ==== variance-covariance-structure ===
1 1 1
0 0 ID !S2==r

IMP 2
2 0 US !GP
V11 C21 V22          # (V11=0.25* $\sigma_i^2$ ; C21=0.5*( $\sigma_{ms,s} - \sigma_s^2$ ); V22= $\sigma_s^2$ )
VATER 0 AINV

```

ASReml .pin file to calculate functions of variance components estimated using the linear imprinting model (Neugebauer *et al.*, 2010a;b)

```

F sirevar 2*2           # 5 add. gen. var as sire ( $\sigma_s^2$ )
F damvar 4*2           # 6 add. gen. var as dam ( $\sigma_d^2$ )
F covar 3*2           # 7 covariance ( $\sigma_{sd}$ )
F genvar 5 + 6         # 8 total add. gen. var ( $\sigma_a^2$ )
F impvar 5 + 6 - 7*2   # 9 imprinting variance ( $\sigma_i^2$ )
F restvar 1 - 5*0.5 - 6*0.5 # 10 residual variance ( $\sigma_e^2$ )
F phenvar 10 + 8       # 11 phenotypic var ( $\sigma_p^2$ )
F damvar-covar 6 - 7   # 12 ( $\sigma_d^2 - \sigma_{sd}$ )
F sirvar-covar 5 - 7   # 13 ( $\sigma_s^2 - \sigma_{sd}$ )
H matBeitragivar 12 9  # 14 ( $\sigma_d^2 - \sigma_{sd} / \sigma_i^2$ )
H patBeitragivar 13 9  # 15 ( $\sigma_s^2 - \sigma_{sd} / \sigma_i^2$ )
H ivarangvar 9 8       # 16 ( $\sigma_i^2 / \sigma_a^2$ )
H heritab 8 11         # 17 heritability ( $h^2$ )
R corre 2:4           # 18 correlation betw. s and d

```

ASReml .pin file to calculate functions of variance components estimated using the generalized linear imprinting model (Neugebauer *et al.*, 2010a;b)

```

F restvar 4*3.29       # 4 imaginary component, so ASReml
                        # assumes this component being 1
F sirevar 1*2          # 5 ( $\sigma_s^2$ )
F damvar 3*2           # 6 ( $\sigma_d^2$ )
F covar 2*2           # 7 ( $\sigma_{sd}$ )
F genvar 5 + 6         # 8 ( $\sigma_a^2$ )
F impvar 5 + 6 - 7*2   # 9 ( $\sigma_i^2$ )
F phenvar 8 + 4        # 10 ( $\sigma_p^2$ )
F damvar-covar 6 - 7   # 11 ( $\sigma_d^2 - \sigma_{sd}$ )
F sirvar-covar 5 - 7   # 12 ( $\sigma_s^2 - \sigma_{sd}$ )
H matBeitragivar 11 9  # 13 ( $\sigma_d^2 - \sigma_{sd} / \sigma_i^2$ )
H patBeitragivar 12 9  # 14 ( $\sigma_s^2 - \sigma_{sd} / \sigma_i^2$ )
H ivarangvar 9 8       # 15 ( $\sigma_i^2 / \sigma_a^2$ )
H heritab 8 10         # 16 heritability ( $h^2$ )
R corre 1:3           # 17 correlation between s and d

```

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