Institutionen för husdjursgenetik

Genotype by environment interaction for udder health in Swedish Holstein cows

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Handledare:
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Agrovoc: Genotype environment interaction, bovine mastitis, somatic cell count, dairy cows, quantitative genetics, genetic parameters

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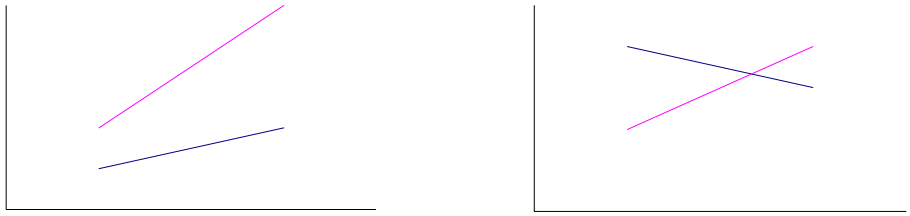
Contents

Abstract

Genotype by environment interaction for somatic cell count and mastitis in the first lactation of the Swedish Holstein breed was studied, using a reaction norm model and multiple trait analysis. Data from the Swedish milk-recording scheme containing more than 200 000 cows having their first calving from 1995-01-01 and onwards was used. Somatic cell count was defined as the average value of the monthly milk sampling results until 150 days after calving expressed in 10 000 cells per millilitre and transformed to a logarithmic scale to the base 10 (LSCC). Mastitis was defined as an all-or-none trait observed from 10 days before calving to 150 days after calving. Environments were defined as: herd-year averages of 305-day protein yield, somatic cell count, and mastitis, all measured in first lactation; and herd size, expressed as the number of cows with first calving per year. Furthermore, overall herd size expressed as the average of 1995-2000 herd-year sizes was used. The multiple trait analysis was done with two models using the highest and lowest quartiles of the environments herd-year protein yield, herd-year somatic cell count, herd-year mastitis, herd size as well as overall herd size. The genetic correlation for LSCC and mastitis between low and high environments indicated GxE for LSCC in somatic cell count environment. Variances of the slope and the level of the reaction norm were analyzed by regressing phenotypic values of somatic cell count and mastitis on herd-year environments of protein yield, somatic cell count, mastitis and on the environment overall herd size. Significant genetic variation in slope was also detected for LSCC in somatic cell count environments and the correlation between predicted offspring performance in low and high somatic cell count environments showed GxE and indicated re-ranking of sires. The heritability of somatic cell count and mastitis estimated as functions of the environments tended to be lowest in average environments and increased with the distance from the average. Neither the multiple trait analysis nor the reaction norm model provided us with complete results when using the environmental scale mastitis. This is probably due to that the mastitis frequency is close to zero in low mastitis environments, resulting in a lack of phenotypic variation.

Introduction

Living organisms respond to changes in their environment, and the ability to alter the phenotype in response to changes in the environment is called plasticity or environmental sensitivity. Differences in environmental sensitivity between individuals result in genotype by environment interaction (GxE), *i.e.* the difference between the phenotypic values of two genotypes is not the same in two environments. If the difference changes sign between environments, the effect of GxE is re-ranking of individuals. If the difference changes in magnitude, but not in sign, there is a scaling effect (Falconer & Mackay, 1996; Kolmodin, 2003). This is illustrated in Figure 1.



a) b)

Figure 1. An illustration of scaling (a) and re-ranking of individuals (b) due to GxE.

The increasing co-operation and exchange of semen between countries has raised the question of GxE in dairy cattle breeding evaluation. If GxE is large, this would mean that the same bulls should not be selected in all countries. Futhermore, if there is GxE within country, this would indicate the need for selection of different bulls for different environments (Kolmodin *et al*., 2002).

Previous studies have examined the existence of GxE for production and fertility traits (Kolmodin, 2003) and longevity (Peterson, 2002) in dairy cattle for Swedish and Nordic countries. To make the picture more complete knowledge about GxE for mastitis and somatic cell count (SCC) is desirable. The objective of this study was therefore to quantify GxE for both mastitis and SCC between different environments in the first lactation of Swedish Holstein cows. The methods used were multiple trait analysis and an analysis with a reaction norm model.

Literature review

Methods to study genotype by environment interaction

There are basically three different methods to describe the extent of GxE. For all methods, observations on the same or related individuals in two or more different environments are needed to study GxE. The common use of artificial insemination in dairy cattle makes it possible to compare the performance of daughters of the same sires in different environments (de Jong, 1995).

1) Interaction term model. In the first method the phenotypic value of an individual is simply described as the sum of the genotypic value, the environmental value and the residual.

$$P = G + E + e \qquad [1]$$

When interaction between genotype and environment exists an interaction component, GxE, is added to the equation:

$$P = G + E + GxE + e \qquad [2]$$

The phenotypic variance (σ_P^2) of the observed phenotypes (P) can be derived from [2] as:

$$\sigma_P^2 = \sigma_G^2 + \sigma_E^2 + \sigma_{GE}^2 + \sigma_e^2 \qquad [3]$$

assuming all covariance being zero.

2) Multiple trait model. The second method used to describe GxE is based on phenotypic values in different environments and genetic correlations (r_g) between these. The phenotypic expression in the two environments is seen as two separate traits and r_g can be studied to see whether GxE exists. When r_g between the phenotypic values of the same genotype expressed in different environments is high, the phenotypic expression is considered as the same trait in the different environments (Falconer & Mackay, 1996). In other words, if r_g between the phenotypic expressions of the trait in two different environments is equal or close to 1 there is no GxE (Robertson, 1959). When r_g is low, the phenotypic expressions in the different environments are not the same trait and this is an indication of GxE. The genetic correlation (r_g) can

be estimated using a multiple trait analysis based on grouping herds with similar production environments to clusters and treating the observation from the different clusters as separate traits. GxE is indicated by low r_g between clusters (Falconer & Mackay, 1996). If the environment cannot be described as a continuous variable, it is common to describe the change in the phenotype that depends on the environment as a series of character states (Kolmodin, 2003).

In the international sire evaluation for bulls, performed by the International Bull Evaluation Service (INTERBULL), GxE is important. The evaluation is based on a multiple trait across-country evaluation (MACE) and a sire model. Accordingly to Robertson (1959), if the genetic correlation between countries is less than 0.8, there is a biologically important GxE. The current estimates, among the member countries, for SCC and mastitis are in the range from 0.85 to 0.96 and 0.73 to 0.86, respectively (Interbull, 2004).

3) Reaction norm model. When the production environment can be described as a continuous variable, a third method called the reaction norm model, is possible to use (de Jong, 1995). The phenotypic expression of a genotype as a function of the environment is described by the reaction norm (Kolmodin, 2003). A given difference of an environment can have a greater effect on one genotype than on another (Falconer & Mackay, 1996). The reaction norm model has an advantage in describing the environment on a continuous scale that the multiple trait model does not possess (Fikse *et al.*, 2003).

Mastitis

Mastitis is an inflammation of the mammary gland caused by the introduction and multiplication of pathogenic microorganisms, mainly bacteria. The causative bacteria can be divided into major and minor pathogens. Major pathogens that are contagious are *Staphylococcus aureus* and *Streptococcus agalactiae* while coliforms, streptococci and enterococci come from the cow's environment like bedding, manure and soil. Minor pathogens are coagulase-negative staphylococci and *Corynebacterium bovis*. Clinical mastitis arises mostly from major pathogens, and shows symptoms like abnormal milk, swelling or pain in the udder and, in some cases, increased rectal temperature, lethargy, anorexia and even death. Subclinical mastitis does not lead to visible changes. However, both clinical and subclinical mastitis causes deterioration in milk quality and a reduced quantity of milk synthesized (Harmon, 1994).

The disease has a complex nature, since it is affected by both genetic and environmental factors. Various factors like age, season and management have an impact on the infection status (Harmon, 1994). The risk of infection varies also with the stage of lactation and is greater in the beginning of the lactation when cows are metabolically stressed (Detilleux *et al.,* 1997). Some conformation traits like fore udder attachment, teat placement and teat shape also have an impact on the cow's risk of being infected (Schukken *et al.*, 1997). This was shown by Carlén *et al.* (2004) as a peak of veterinary treated cases of mastitis a few days after calving.

The heritability for mastitis is low, around 0.001-0.06 (Heringstad *et al*., 2000). Carlén *et al*. (2004) estimated the heritability for mastitis in Swedish Holstein cows to 0.03 in first parity and 0.01 in later parities. Mastitis is and generally unfavorably correlated to milk production traits (Emanuelsson *et al.,* 1988; Pösö & Mäntysaari, 1996; Nielsen *et al*, 1997; Carlén *et al*, 2004). Since breeding for increased production increases the clinical mastitis occurrence, breeding with regard for mastitis is desirable (Strandberg & Shook, 1989). Denmark, Finland,

Norway and Sweden are the only countries with a well-established national recording system for health data in dairy cattle. In these countries a breeding value for mastitis is included in the total merit bull index. Breeding for improved mastitis resistance can be performed either by direct or indirect selection, or by a combination. Direct selection is usually performed by using veterinary treated clinical mastitis cases, and clinical mastitis is treated as an all-or-none trait. Indirect selection can be performed by using somatic cell count measurements or other correlated traits such as certain conformation traits (Heringstad *et al.*, 2000). When breeding for an improvement in mastitis resistance using a combination of direct and indirect selection can be expected to be most efficient.

Somatic cell count

Somatic cell count in milk (mainly the number of white blood cells) is the most common way of measuring milk quality as well as udder health (Harmon, 1994). When bacteria invade the udder the first line of defense is the teat canal and the second line of defense is phagocytes and leukocytes in the teat cistern (Detilleux *et al.*, 1997). In a healthy udder macrophages and lymphocytes are dominating, but in a diseased udder more than 95 % of the somatic cells are neutrophils (Kehrli & Shuster, 1994).

Different genetic and environmental factors affect the amount of SCC (Kehrli & Shuster, 1994). SCC varies with the stage of lactation in the inverted way of milk production. There is also a seasonal variation in SCC, which can be related to the distribution of calvings throughout the year. The risk of being infected increases with age in infected cows because previous infection allows easier access to the mammary gland and because the immune system of older cows is not as effective as that of younger cows. The herd level influences the SCC since different management groups have different level of infection. Further, the pathogen species to which the cow is exposed to is affecting SCC, contagious pathogens causing the most sustainable increase (Detilleux *et al.*, 1997).

The heritability for SCC is higher than the heritability for mastitis. In a review by Heringstad *et al.* (2000) estimates range from 0.08 to 0.19. Due to the fact that SCC has a higher heritability than mastitis, SCC is favourable to use as an indirect trait to improve mastitis resistance. A high genetic correlation between the index trait and the breeding goal trait is necessary to have a successful indirect selection. In previous studies the genetic correlation between these two traits is in the range of 0.5 to 0.7 (Emanuelsson *et al.,* 1988; Nielsen *et al.*, 1997; Carlén *et al.,* 2004). Since the genetic correlation between SCC and mastitis is moderate to high, selection against SCC should result in decreased occurrence of mastitis (Pösö & Mäntysaari, 1996).

Genotype by environment interaction for mastitis and somatic cell count

Few studies have been reported on GxE for SCC (Weigel *et al.,* 2001). The effect of GxE for SCC between automatic milking systems and conventional milking systems has been studied and estimated to be small (Mulder *et al.*, 2003). Neither Boettcher *et al*. (2003) nor Kearney *et al*. (2004) could give any evidence for the existence of GxE for SCC between grazing and confinement systems. Castillo-Juarez *et al*. (2000) could not show any GxE for SCC when studying low and high environments based on different levels of herd mature equivalent milk**.** When it comes to GxE for mastitis, no previous studies have been found in the literature.

Materials and methods

Data editing

The data set was collected from a previous study (Carlén *et al.,* 2004) and contained more than 200 000 cows of the Swedish Holstein breed. The data, originally received from the Swedish milk-recording scheme, contained information about identification number and year of birth of the cows and their sires, as well as the cow's proportion North American Holstein and proportion heterosis. There were also records of herd, year, month and age of first calving and information about protein production, SCC and clinical mastitis from first lactation. Mastitis was defined as an all-or-none trait observed from 10 days before calving to 150 days after calving. Since mastitis is a binary trait the record of a cow can be either 1 (the cow has at least one treatment of mastitis) or 0 (when the cow has not been treated for mastitis). The somatic cell count was defined as the average value of the monthly milk sampling results until 150 days after calving expressed in 10,000 cells per millilitre and transformed to a logarithmic scale to the base 10 (LSCC). The production of protein was defined as kg of the completed 305-days first lactation. Average LSCC in the data set was 0.787 (SD 0.426), which corresponds to about 60 000 cells per ml, and average mastitis was 0.099 (SD 0.299).

Herd-year and herd classes were excluded if there were less than two cows with observations on either LSCC, protein production or mastitis in that herd-year or herd. After editing the dataset contained 221 104 cows belonging to 27 410 herd-year and 7 054 herd classes.

The phenotypic values of LSCC, protein production and mastitis were pre-adjusted before estimating herd-year and herd mean values for the random regression model. The following fixed model using the GLM procedure in the SAS package (SAS Institute Inc., 2000) was used for the pre-adjustment:

$$y_{ijk} = \mu + a_i + m_j + am_{ij} + e_{ijk} \qquad [4]$$

where,

y_{ijk} = either the value of LSCC, protein production or mastitis in first lactation of cow k
μ = overall mean of LSCC, protein production or mastitis in first lactation
a_i = fixed effect of i^{th} age in months at first calving
m_j = fixed effect of j^{th} month at first calving
am_{ij} = interaction effect between age and month at first calving
e_{ijk} = random residual effect

The residuals were used to calculate mean values of LSCC, protein production and mastitis for each herd-year and herd class. For herd size, a more representative herd-year size was received by calculating a mean from the 1995-2000 herd-year sizes, called overall herd size (oahsize). This was done due to the fact that herds could have a various number of first calving cows every year.

Multiple trait analysis

For the multiple trait analysis the observations were divided into low and high herd-year clusters with regard to LSCC, protein production, clinical mastitis, herd-year size and in herd clusters with regard to overall herd size, using the UNIVARIATE procedure in the SAS package (SAS Institute INC., 2000). Observations in low and high quartile of each environmental variable were chosen for analysis and are illustrated in Table 1.

Table 1. Number of cows in low and high quartiles of each production environment and the number of bulls with daughters in each quartile.

	Low quartile		High quartile	
Environment	**Cows**	**Bulls**	**Cows**	**Bulls**
Protein yield	52 513	836	52 512	835
Somatic cell count	52 514	835	52 504	838
Mastitis	52 503	836	52 499	834
Overall herd size	54 183	834	54 669	837
Herd-year size	53 036	835	45 612	837

The sires born before 1991 could not be considered as young test bulls because they had reached too high an age and only the selected bulls had daughters in the material. In an attempt to get more unbiased estimates of the variances, the older bulls (n=311 with 66 399 daughters) were considered as fixed and the young bulls (n=527 with 154 705 daughters) were considered as random in one of the models used. In the other all bulls were considered as random. The following bivariate multiple trait models were used for LSCC and clinical mastitis:

Model A: $y_{ijklm} = \mu + ym_i + age_j + hy_k + sire_l + b_1Het_m + b_2AmH_m + e_{ijklm}$ [5]

Model B: $y_{ijkmn} = \mu + ym_i + age_j + hy_k + sire_n + sire_o + b_1Het_m + b_2AmH_m + e_{ijkmno}$ [6]

where:

μ = overall mean
ym_i = fixed effect of i^{th} year by month of calving
age_j = fixed effect of j^{th} age in months at calving
hy_k = fixed effect of k^{th} herd by year of calving
$sire_l$ = random effect of sire *l* (Model A)
$sire_n$ = random effect of sire *n* in unselected batches (Model B)
$sire_o$ = fixed effect of sire *o* in selected batches (Model B)
b_1Het_m = fixed regression of coefficient of the proportion heterosis of animal *m*
b_2AmH_m = fixed regression of coefficient of the proportion American Holstein of animal *m*
$e_{ijklmn(o)}$ = random residual effect

Variance and covariance components were estimated with the DMU package, version 6, developed by Madsen & Jensen (2000). Both convergence criteria were set to 10^{-6} and in order to reach the convergence criteria faster the values from the previous run multiple trait analyses were used as starting values in subsequent analyses. The random effects were assumed to have zero means and the covariance structure was:

$$V\begin{bmatrix}\mathbf{s}_1\\ \mathbf{s}_2\\ \mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix}=\begin{bmatrix}\mathbf{A}\sigma^2_{s_1} & \mathbf{A}\sigma_{s_{1,2}} & 0 & 0\\ & \mathbf{A}\sigma^2_{s_2} & 0 & 0\\ & symm. & \mathbf{I}\sigma^2_{e_1} & \mathbf{I}\sigma_{e_{1,2}}\\ & & & \mathbf{I}\sigma^2_{e_2}\end{bmatrix}$$

where **A** is the additive relationship matrix and **I** is the identity matrix, and the indexes represent the two traits in the bivariate analysis.

Reaction norm model

In order to avoid dependence between the dependent and independent variables, new herd-year and herd mean values for LSCC (hylscc, hlscc), protein production (hyprot, hyprot) and clinical mastitis (hymast, hmast) were calculated for the reaction norm model. The value of the environmental scale for a particular individual was corrected for its own observation, to avoid including a cow´s observation both in the dependent and independent variable, by using this following formula:

$$\mu_2 = \frac{\mu_1 n - a}{n-1} \qquad [7]$$

where:
μ_2 = new herd-year or herd mean for the trait
μ_1 = previous calculated mean for the trait
n = number of observations μ_1 was based on
a = value of the trait for a particular cow

Two different sire models were used to study the data:

Model C: $y_{ijklm} = \mu + ym_i + age_j + hy_k + b_1 Het_m + b_2 AmH_m + s_{a_l} + s_{b_l} X_{ml} + e_{ijklm}$ [8]

Model D: $y_{ijlm} = \mu + ym_i + age_j + b_1 Het_m + b_2 AmH_m + b_{F_X} X_{ml} + s_{a_l} + s_{b_l} X_{ml} + e_{ijlm}$ [9]

where:
$y_{ij(k)lmn,}$ μ, $ym_{i,}$, age_j, hy_k, $b_1 Het_m$ and $b_2 AmH_m$ are as before and
s_{a_l} = random intercept or level of the random regression for sire l
s_{b_l} = random linear coefficient or slope of the random regression of y on $X_{ml,}$ for sire l
X_{ml} = the environment daughter m of sire l produced in
b_{F_X} = fixed coefficient of regression of y on X_{ml}
e_{ijklm} = random residual

The random effects were assumed to have zero means and the covariance structure was:

$$V\begin{bmatrix}\mathbf{s_a}\\ \mathbf{s_b}\\ \mathbf{e}\end{bmatrix}=\begin{bmatrix}\mathbf{A}\sigma^2_{s_a} & \mathbf{A}\sigma_{s_{a,b}} & 0\\ & \mathbf{A}\sigma^2_{s_b} & 0\\ symm. & & \mathbf{I}\sigma^2_e\end{bmatrix}$$

where **A** is the additive relationship matrix and **I** is the identity matrix, and the indexes represent the random intercept (level) and the random linear coefficient (slope). As for the reaction norm models, the DMU package was used to estimate variance and covariance components (Jensen and Madsen, 2000).

Model C and D have the random regression and the environmental variable, X_{ml}, added. For each sire the level and the slope of a linear reaction norm were estimated for the environments based on both herd-year and herd averages for protein, LSCC and mastitis and for overall herd size. In model D the fixed effect of herd year is replaced by a fixed regression on the herd-year or herd environment (X_{ml}).

POP, predicted offspring performance, depends on the environment the offspring will produce in. The formula used to calculate the POP for sire l in environment X is:

$$POP_{l|X} = \mu + s_{a_l} + s_{b_l}X \qquad [10]$$

The POPs were calculated from the herd year and herd means of protein yield, LSCC, mastitis and overall herd size. The predicted offspring performance was calculated in the range of ±3 standard deviations from the mean. The correlation between POP in the average environment and POP in deviating environment was calculated to illustrate the potential re-ranking of sires between environments. To have more accurate correlation curves these were based on only the sires with daughters in the data set. The range of environments in these curves contained 95 % of the observations.

The sire variance was calculated as the variance of [10] since the POP, and also the heritability, varies with the environment. Also the range of environments in the heritability curves contained 95 % of the observations.

$$\sigma^2_{s|X} = \sigma^2_{s_a} + X^2\sigma^2_{s_b} + 2X\sigma_{a,b} \qquad [11]$$

$$h^2|X = \frac{4\sigma^2_{s|X}}{4\sigma^2_{s|X} + \sigma^2_E} \qquad [12]$$

where,

$$\sigma^2_E = \sigma^2_e - 3\sigma^2_{s|X=0} \qquad [13]$$

Results

Multiple trait analysis

Average LSCC and mastitis in low and high quartiles of the herd-year environments protein yield, LSCC, mastitis, herd-year size and overall herd size from the multi-trait analysis are shown in Table 2. Neither LSCC nor mastitis differed between low and high herd sizes and there was little difference between low and high production herds. As expected, there was a large difference in LSCC and mastitis, respectively, between low and high LSCC and mastitis herds, respectively. In fact, the mastitis mean and standard deviation are near zero in the low quartile of mastitis environment. When the environment variable was LSCC or mastitis, respectively, and the trait studied was mastitis or LSCC, respectively, the difference was less pronounced.

Table 2. Average and standard deviation (SD) of LSCC and mastitis in low and high quartiles of different environments.

	LSCC[1] mean ± SD		**Mastitis mean ± SD**	
Environment	**Low quartile**	**High quartile**	**Low quartile**	**High quartile**
Protein yield	0.824 ± 0.425	0.752 ± 0.421	0.098 ± 0.297	0.107 ± 0.309
LSCC	0.547 ± 0.316	1.039 ± 0.452	0.089 ± 0.285	0.123 ± 0.328
Mastitis	0.760 ± 0.413	0.814 ± 0.443	0.000 ± 0.000	0.285 ± 0.451
Overall herd size	0.788 ± 0.430	0.791 ± 0.421	0.103 ± 0.304	0.101 ± 0.301
Herd-year size	0.790 ± 0.429	0.797 ± 0.420	0.103 ± 0.304	0.103 ± 0.303

[1]LSCC = the average value of the monthly milk sampling results until 150 days after calving expressed in 10,000 cells per ml and transformed to a logarithmic scale to the base 10. For instance, the value 0.824 transforms into 66 681 cells/ml.

Genetic correlations for LSCC and mastitis in low and high herd-year protein yield, LSCC, mastitis and herd size environments from the multiple trait analysis are presented in Table 3. Genetic correlations between mastitis in low and high mastitis environment are missing in both models, A and B. These analyses were not carried through because the multiple trait analysis could not handle the fact that most of the observations of mastitis are zero in herds with low mastitis frequency. For both models analyzed in the environments protein yield and herd-year size, the correlation for both LSCC and mastitis was high. Genetic correlations for LSCC in low and high LSCC environments are lower for both models. The genetic correlation for LSCC in low and high mastitis environment for model B is also low but with a large standard error. The same is the case for the correlation for mastitis in model B in low and high environments of LSCC and overall herd size.

Table 3. Genetic correlations and standard errors from the multiple trait analysis for LSCC and mastitis in low and high quartiles of different environments for models A and B.

	Environment				
Model and trait	**Protein**	**LSCC**	**Mastitis**	**Overall herd size**	**Herd-year size**
A LSCC	0.986 ± 0.0167	0.840 ± 0.0434	0.993 ± 0.0138	0.993 ± 0.0111	1.00 ± 0.0143
B LSCC	0.980 ± 0.0627	0.803 ± 0.0829	0.887 ± 0.0689	1.00 ± 0.0588	1.00 ± 0.0588
A mastitis	0.997 ± 0.0573	1.00 ± 0.0695		1.00 ± 0.0638	1.00 ± 0.1113
B mastitis	0.950 ± 0.2535	0.891 ± 0.2265		0.857 ± 0.1869	1.00 ± 0.2118

The heritability of LSCC and mastitis in various environments is shown in Table 4. Model A gave a lower heritability for both LSCC and mastitis in nearly all environments except for LSCC in low LSCC environment and for mastitis in high protein environment. For LSCC in the environments LSCC, overall herd size and herd-year size the heritability tended to be higher in the high quartiles of the environment in model B.

Table 4. Heritability of LSCC and mastitis in low and high environment quartiles from the multiple trait analysis for models A and B.[1]

	Environment									
Model and	**Protein**		**LSCC**		**Mastitis**		**Overall herd size**		**Herd-year size**	
trait	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**
A LSCC	0.120	0.123	0.130	0.122	0.120	0.108	0.118	0.138	0.116	0.136
B LSCC	0.135	0.137	0.109	0.170	0.124	0.137	0.135	0.158	0.145	0.171
A mastitis	0.020	0.030	0.022	0.026			0.021	0.033	0.022	0.033
B mastitis	0.027	0.024	0.038	0.034			0.054	0.042	0.033	0.050

[1] The standard errors of the heritability estimates of LSCC in model A and B ranged from 0.12 to 0.14 and 0.11 to 0.17, respectively, and the standard errors of the mastitis heritability ranged from 0.005 to 0.007 and from 0.001 to 0.002 for model A and B, respectively.

Reaction norm model

The fixed regression coefficient of LSCC and mastitis on the environmental variable and its standard error from model D are presented in Table 5. The fixed regression is significant for both traits in all environments exept for LSCC in the environment hymast and for mastitis in the environments hylscc and hlscc.

Table 5. Fixed regressions and standard errors of LSCC and mastitis on the environmental variable from model D.

Trait	**Environment**	**Regression coefficient**	**Standard error**
LSCC	hyprot	-0.453	0.042
	hprot	-0.630	0.044
	hylscc	0.332	0.012
	hlscc	0.584	0.023
	hymast	11.478	6.725
	hmast	-23.421	11.483
	oahsize	0.551	0.263
Mastitis	hyprot	2.573E-04	2.649E-05
	hprot	3.324E-04	3.359E-05
	hylscc	-3.939E-06	26 723
	hlscc	-1.494E-05	17 786
	hymast	0.226	0.022
	hmast	0.491	0.036
	oahsize	-3.188E-04	1.254E-04

Variance components for the level and the slope, and the genetic correlation between the level and the slope of the reaction norm for various environments are shown in Table 6. The analysis of mastitis with model D in hylscc was problematic due to a problem in updating the parameter vector. When analyzing mastitis with model D in the environment hlscc the analysis never converged.

Table 6. Genetic variances and standard errors, correlations and standard errors for effects of level (a) and slope (b) of the reaction norm and residual variances and standard errors from models C and D.

Trait and model	**Environment**	$\sigma^2_{s_a}$	$\sigma^2_{s_b}$	$r_{g_{a,b}}$	σ^2_e
LSCC					
C	hyprot	4.884E-03 ± 3.604E-04	1.310 ± 0.141	0.00 ± 0.300	0.148 ± 5.236E-04
	hprot	4.806E-03 ± 3.501E-04	0.332 ± 0.100	0.19 ± 0.460	0.149 ± 5.264E-04
	hylscc	1.723E-03 ± 1.454E-04	0.761 ± 0.040	0.15 ± 0.240	0.085 ± 2.894E-04
	hlscc	3.436E-03 ± 2.623E-04	3.511 ± 0.184	0.15 ± 0.230	0.127 ± 4.341E-04
	hymast	4.750E-03 ± 3.450E-04	72 323 ± 7 866	0.03 ± 0.290	0.158 ± 5.389E-04
	hmast	4.871E-03 ± 3.519E-04	207 586 ± 24 083	0.00 ± 0.300	0.159 ± 5.422E-04
	oahsize	4.742E-03 ± 4.137E-04	0.062 ± 0.167	0.44 ± 3.240	0.160 ± 5.440E-04
D	hyprot	5.330E-03 ± 3.694E-04	0.021 ± 0.019	0.60 ± 1.190	0.164 ± 5.351E-04
	hprot	5.427E-03 ± 3.728E-04	0.022 ± 0.020	0.85 ± 1.280	0.164 ± 5.328E-04
	hylscc	5.162E-03 ± 3.570E-04	0.003 ± 0.001	0.07 ± 0.510	0.170 ± 5.374E-04
	hlscc	5.270E-03 ± 3.604E-04	0.012 ± 0.003	0.11 ± 0.440	0.169 ± 5.301E-04
	hymast	5.540E-03 ± 3.793E-04	63.3 ± 422.5	-0.89± 11.528	0.175 ± 5.523E-04
	hmast	5.563E-03 ± 3.786E-04	103.9 ± 1 479.6	-0.94± 26.057	0.175 ± 5.489E-04
	oahsize	5.675E-03 ± 4.738E-04	1.338 ± 0.463	-0.14± 0.540	0.175 ± 5.490E-04
Mastitis					
C	hyprot	4.450E-04 ± 6.678E-05	2.897E-07± 4.585E-08	0.14 ± 0.494	0.082 ± 2.871E-04
	hprot	4.426E-04 ± 6.527E-05	2.059E-08 ± 1.715E-08	0.70 ± 1.225	0.082 ± 2.875E-04
	hylscc	4.226E-04 ± 6.304E-05	3.763E-08 ± 4.023E-09	-0.04± 0.390	0.083 ± 2.826E-04
	hlscc	4.935E-04 ± 6.780E-05	3.750E-08 ± 4-488E-09	-0.02± 0.420	0.083 ± 2.844E-04
	hymast	3.628E-04 ± 4.426E-05	1.000 ± 5.166E-02	0.78 ± 0.154	0.046 ± 1.520E-04
	hmast	1.126E-03 ± 1.085E-04	5.819 ± 0.301	0.85 ± 0.094	0.068 ± 2.257E-04
	oahsize	6.404E-04 ± 1.040E-04	3.667E-08 ± 8.961E-08	-0.16± 0.533	0.088 ± 2.929E-04
D	hyprot	5.548E-04 ± 7.047E-05	8.233E-09 ± 7.705E-09	1.00 ± 1.503	0.086 ± 2.871E-04
	hprot	5.730E-04 ± 7.129E-05	1.697E-08 ± 1.236E-08	1.00 ± 1.078	0.086 ± 2.788E-04
	hylscc	6.314E-04 ± 7.485E-05	3.693E-08 ± 4.909E-09	0.04 ± 0.338	0.088 ± 2.798E-04
	hlscc	6.563E-04 ± 7.556E-05	3.688E-08 ± 6.293E-09	-0.02± 0.382	0.088 ± 2.771E-04
	hymast	6.960E-04 ± 7.556E-05	1.760E-02 ± 2.647E-03	0.77 ± 0.259	0.092 ± 2.839E-04
	hmast	7.182E-04 ± 7.450E-05	4.668E-02 ± 6.813E-03	0.88 ± 0.186	0.091 ± 2.774E-04
	oahsize	8.005E-04 ± 1.119E-04	2.000E-07 ± 1.272E-07	-0.11± 1.010	0.093 ± 2.828E-04

The heritability of LSCC and mastitis in the average environment for each environment variable from models C and D are presented in Table 7. The heritability of LSCC is highest in the average herd-year and herd protein yield environments and lowest in average herd-year and herd LSCC environments for both models. For mastitis, on the other hand, the heritability is highest in average herd mastitis environments for model C and lowest in average herd-year LSCC environment for model C.

Table 7. Heritability of LSCC and mastitis in the average environment for each environment variable from models C and D.

Model	Environment	LSCC	Mastitis
C	hyprot	0.130	0.024
	hprot	0.126	0.022
	hylscc	0.083	0.020
	hlscc	0.106	0.024
	hymast	0.117	0.054
	hmast	0.119	0.083
	oahsize	0.120	0.028
D	hyprot	0.128	0.027
	hprot	0.129	0.027
	hylscc	0.118	0.029
	hlscc	0.121	0.029
	hymast	0.123	0.032
	hmast	0.123	0.032
	oahsize	0.123	0.034

The heritability of LSCC and mastitis as a function of the environmental scales protein yield, LSCC and mastitis in model C is shown in Figure 1 and Figure 2, respectively. The range of environments shown contains 95 % of the observations. The range in SD units around average for prothy, proth, lscchy, lscch, masthy and masth were -2 to +2, –2.2 to +1.9, –1.9 to +2.2, -1.9 to +2.1, -1 to +2.8 and –1.4 to +2.4, respectively. For LSCC in hylscc and hlscc and for mastitis in hymast and hmast the heritability is high (near 1) in most deviating environments. The heritability of LSCC and mastitis in model D are presented in appendix A.

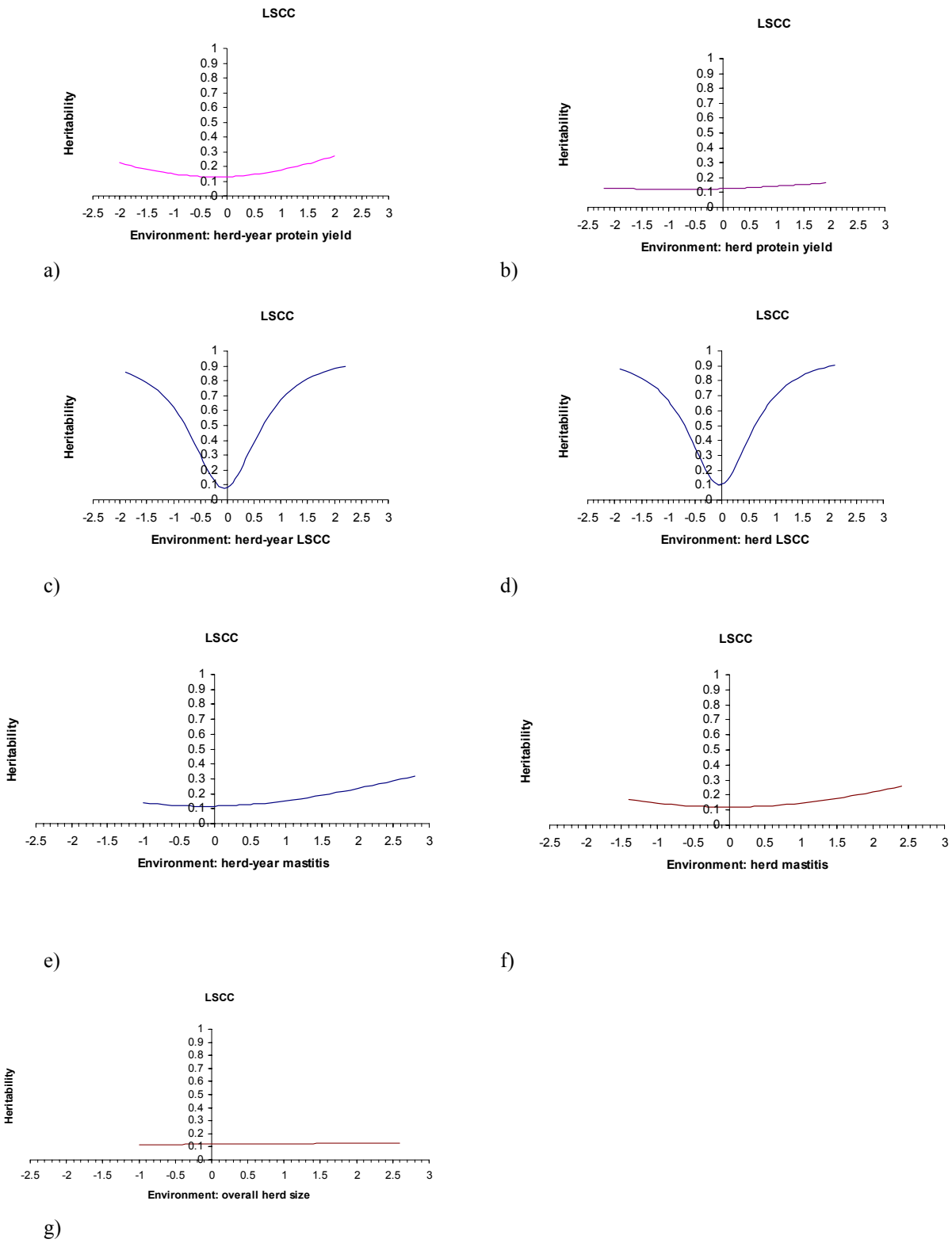
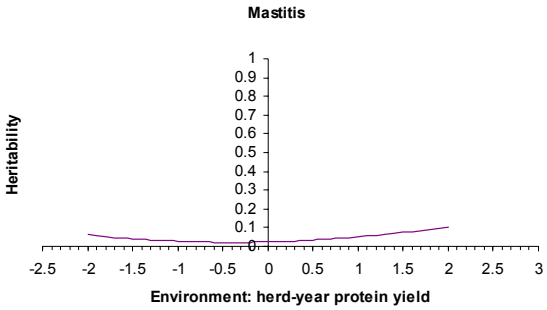


Figure 2. Heritability of LSCC in model C as a function of herd-year and herd protein yield (a and b), LSCC (c and d), mastitis (e and f) and overall herd size (g).



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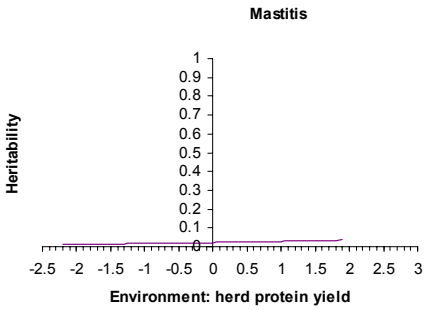

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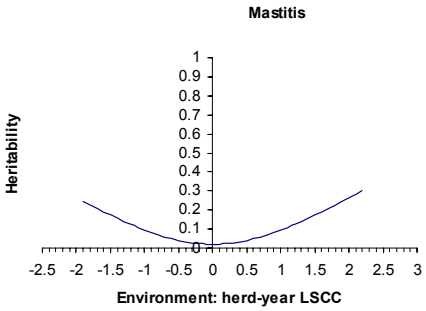

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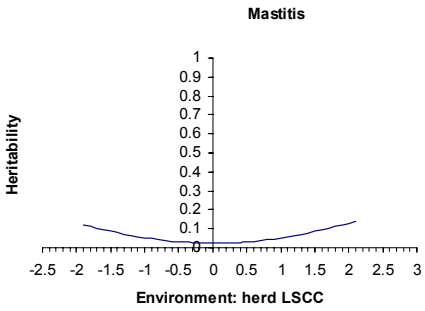

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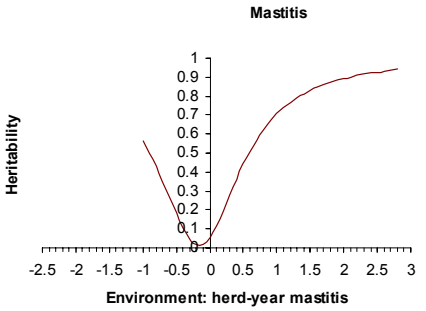
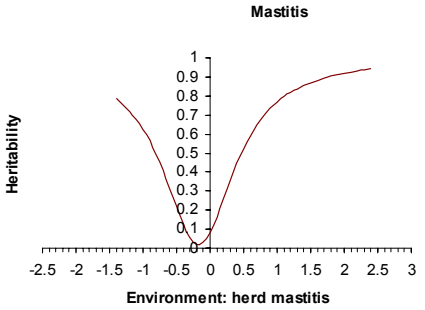


e)



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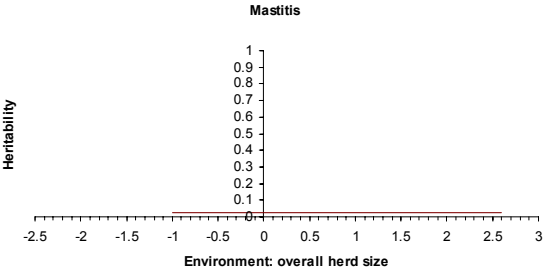

g)

Figure 3. Heritability of mastitis in model C as a function of herd-year and herd protein yield (a and b), LSCC (c and d), mastitis (e and f) and overall herd size (g).

For LSCC and mastitis, correlations between POP in average and deviating environment of protein yield, LSCC and mastitis from model C are shown in Figure 3 and Figure 4, respectively. For these environments there is a tendency of re-ranking of sires in regard both to LSCC and mastitis. In the environment hylscc and hlscc there is most proof of GxE for the two traits. For mastitis correlation curves from model D are shown in appendix B. Correlations for LSCC from model D showed no GxE in any of the environments and therefore not presented. From both models, correlation in environment oahsize showed no GxE for neither of the two traits and is therefore not shown.

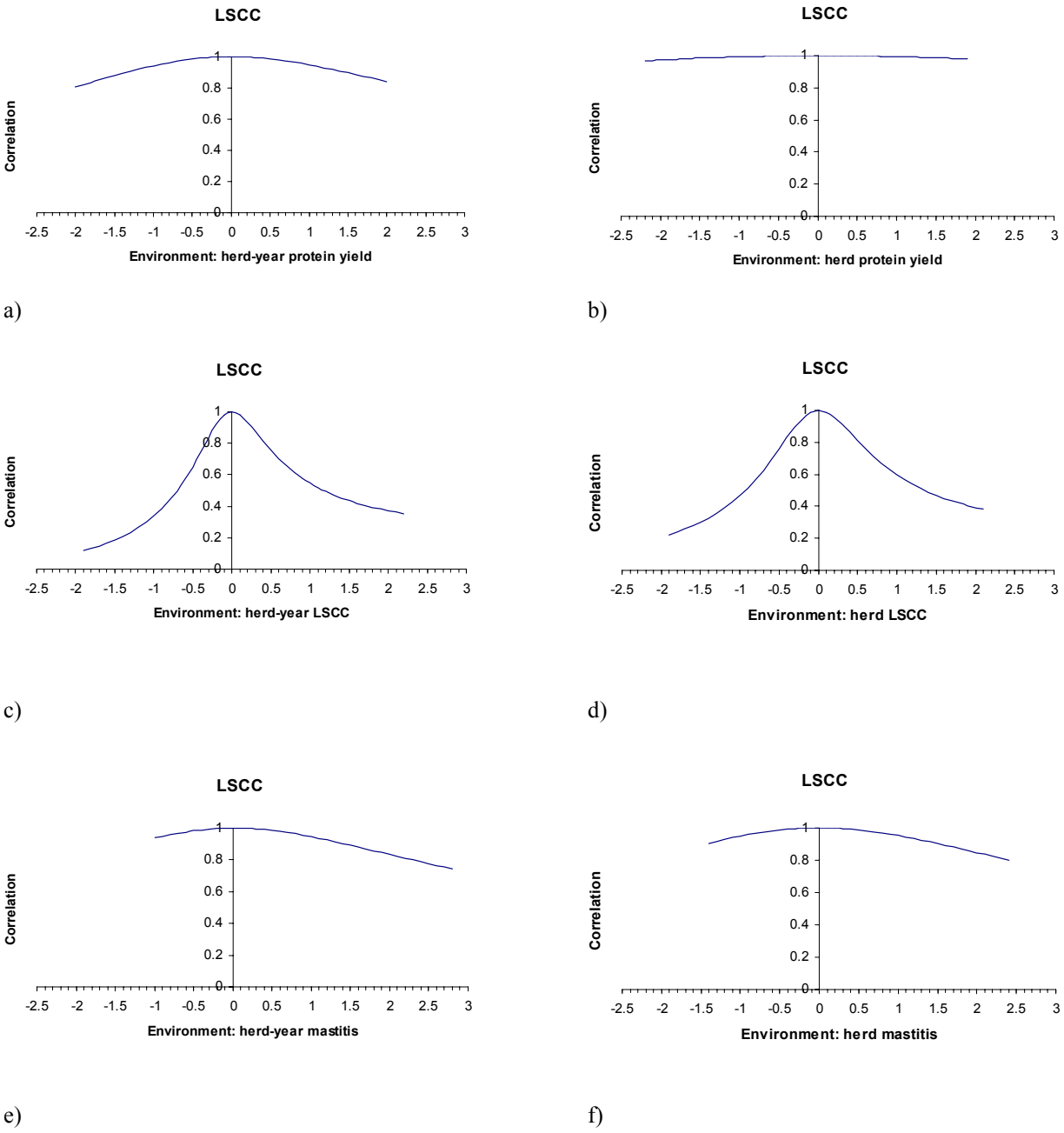

Figure 4. Correlations between POP in the average environment (0) and deviating environments for the environmental scales herd-year and herd protein yield (a and b), LSCC (c and d) and mastitis (e and f) in model C for LSCC.

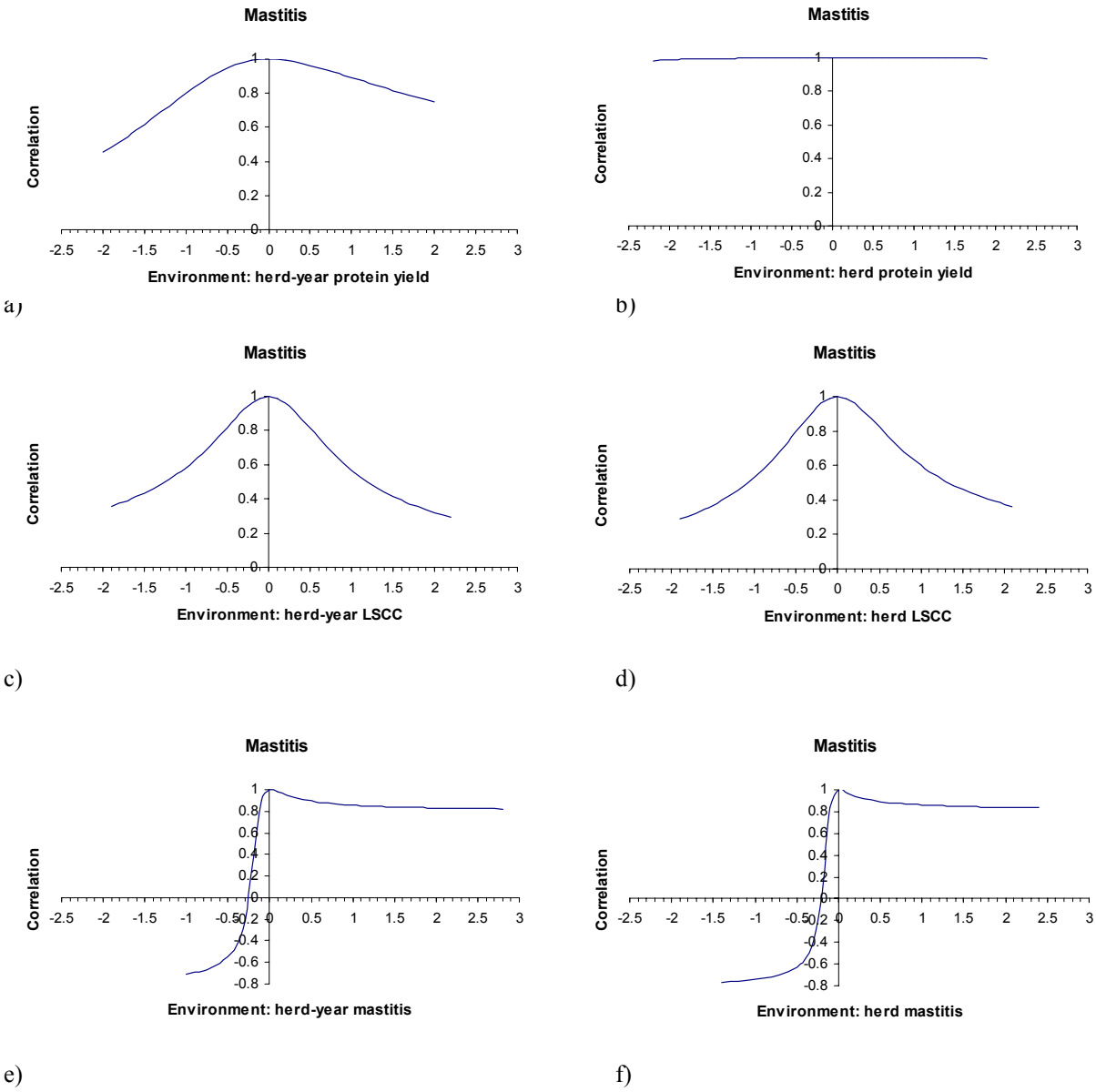

Figure 5. Correlations between POP in the average environment (0) and deviating environments for the environmental scales herd-year and herd protein yield (a and b), LSCC (c and d) and mastitis (e and f) in model C for mastitis.

Discussion

The genetic correlations between LSCC and mastitis in low and high quartile herd environments estimated in the multiple trait analysis indicated some re-ranking in a few environments. This was the case for LSCC in the environment LSCC for both model A and B (0.80-0.84). For LSCC in mastitis environment for model B (0.89) and for mastitis in LSCC environment (0.89) and overall herd size environments (0.86) for model B, correlations were also below 1, however, not significantly so. To set these correlations in perspective, they can be compared with the correlations used by Interbull for production traits between countries in the

Northern hemisphere (e.g. USA and Europe) on one hand and New Zealand/Australia on the other (around 0.75-0.85) (Interbull, 2004).

The fact that this analysis did not provide us with the genetic correlation between mastitis in low and high mastitis environment, is not surprising considering the all-or–none character of mastitis. This means that in a low mastitis environment the mastitis frequency is zero and consequently there is a lack of phenotypic variation. Even if this becomes very obvious for the mastitis environment the same problem does occur also for other traits as was pointed out by Kolmodin et al. (2002) for the trait days open. This phenomenon is most problematic when the environmental scale is based on the same trait as the dependent variable. In the current study we avoided the obvious dependency by excluding the individual's own observation from the herd-year average, however, the does not solve the problem of low variation in herds with low mastitis treatment incidence. This phenomenon highlights a crucial point in the study of GxE, the definition of the environmental scale. This applies to all methods of analyzing GxE. There is a need for a better and hopefully generally accepted approach for defining the environment.

Two different models were used in the multiple trait analysis, one that did not account for the fact that only some sires of a batch had daughters in the data (so-called selected sires), model A, and one that did, model B. Model B provided a few more genetic correlations below unity and also tended to give higher heritability of LSCC and mastitis in most environments. This latter result may be due to that the sire variance in model B was estimated only from complete bull batches of young bulls. However, the standard errors of both heritabilities and genetic correlations were higher from model B, probably owing to less information used (fewer sires and fewer records of cows).

There was a tendency to higher heritability of LSCC and mastitis in high protein yield and in large herd size environments. Previous studies (Castillo-Juarez *et al,* 2000; Castillo-Juares *et al,* 2002) have reported the opposite relationship between the heritability of LSCC and yield environment.

The reaction norm model was analyzed using two models, one with the fixed effect of herd-year, model C, and one with this effect replaced by a fixed regression on the herd-year environment, model D. There were some problems updating the parameter vector using the AI-algorithm when running mastitis in the environment hylscc with both models and in the environment hlscc with model C, and the EM-algorithm was used instead. When mastitis was analyzed with the environment hlscc in model D the analysis did not converge properly.

The fixed regression coefficients of LSCC and mastitis on the environmental variables from model D were not significant for LSCC in hymast environment. The same was true for mastitis in hylscc and hlscc environments, but here the standard errors were incorrectly calculated, most likely due to the problems with convergence and the AI-algorithm mentioned above.

The genetic variance components of level of reaction norms from model C and D were always significantly different from zero for LSCC and mastitis, regardless of the environmental scale. Model C (with herd-year effect) detected genetic variance in slope, indicating GxE, for LSCC for all environments except overall herd size, whereas in model D we only detected GxE when the environment was LSCC or overall herd size. For mastitis, the sire variance for slope was significantly different from zero for both mastitis and LSCC environments in both models C and D, and also for the environment hyprot for model C.

When studying the correlation curves between POP in average and deviating environments, it becomes clear that the largest re-ranking would be expected for LSCC between average and low and high LSCC environments (Figure 4). These results were in agreement with the multiple trait analysis (Table 3) where the genetic correlations between low and high quartiles were 0.80-0.84. For mastitis there were also low correlations between POP in average and low or high LSCC environments, indicating potential re-ranking (Figure 5). The genetic correlation for mastitis between low and high LSCC quartile herds in the multiple trait analysis also indicated re-ranking (Table 3), however, the correlation (0.89) was not significantly different from unity. Previous performed studies have shown evidence of GxE due to scaling in dairy cattle but little proof of GxE due to re-ranking (Cromie *et al.*, 1998).

For both LSCC and mastitis, there was some indication of re-ranking also when the environment was herd-year average protein yield, and for LSCC also when the environmental scale was mastitis average. The latter indication if GxE was found also in the multiple trait analysis, however again, the correlation (0.89) was nor significantly different from unity. For mastitis, the correlation dropped sharply when the environment changed from average to low herd(-year) average mastitis and went to negative values (Figure 5). This was not corroborated from the multiple trait analysis, in fact, that analysis did not converge owing to too low variation in the trait mastitis in low mastitis herds. It is likely that the same phenomenon has influenced the estimates of the reaction norms. If there is no or little (genetic) variation at a certain point on the environmental scale, all reaction norms would tend to cross at that point. This would automatically lead to a change of sign of the correlation of POP across this point. This negative correlation should probably be interpreted with caution.

The genetic correlation for LSCC between the reaction norm level and slope against hprot environment was high with model D as well as for mastitis against mastitis environments for both models. This means that animals with high breeding values for level for LSCC are sensitive to changes in the herd production environment. Similarly, animals with high breeding values for level of mastitis are sensitive to changes in the production environment as well as in the mastitis frequency environment. This is an example of the scaling effect of GxE (Kolmodin *et al,* 2002).

The genetic correlation for LSCC between the reaction norm level and slope against herd-year protein yield and mastitis environments with model C and against LSCC, mastitis and overall herd size environments with model D are low or even negative. The low genetic correlation between level and slope means that the animals can be sensitive to environmental changes regardless of their breeding value for the level (Kolmodin *et al,* 2002).

The heritability estimates in average environment of LSCC and mastitis vary from 0.083 to 0.130 and from 0.020 to 0.083, respectively, for models C and D (Table 7). When estimating the heritability as a function of the environment the lowest heritability of LSCC is found in average environment. With increased distance from the average the heritability also increased, especially for LSCC in LSCC environment. The heritability of mastitis as a function of the environment was projected in the same way except in mastitis environments where the lowest heritability was found below the average environment. In environments over the average environment the heritability increased more then in low environment.

The results from the multiple trait analysis are not easily comparable to the results from the reaction norm model since the models used in the analyses differ and the multiple trait analysis uses only half of the herds.

Conclusions

GxE was detected with both the multiple trait analysis, as a low genetic correlation between the trait in low and high environment, and the reaction norm model, as a significant variation in the slopes of the reaction norms. The two analyses used to detect GxE for LSCC and mastitis were possible to use, even if there were some problems with the trait mastitis in mastitis environment.

The genetic correlation estimated in the multiple trait analysis indicated some re-ranking for LSCC in low and high LSCC environments. There was also indication of re-ranking for LSCC in mastitis environment and for mastitis in LSCC and overall herd size environments, but they were not significant. The results from the multiple trait analysis corroborates the results from the reaction norm model as the genetic variance in slope indicated re-ranking for LSCC in LSCC environments. The correlation between POP in average and deviating environments also show that the largest re-ranking would be expected for LSCC between low and high LSCC environments.

In practice, the detected GxE for LSCC in LSCC environment, could affect the choice of bulls when selecting for udder health for the next generation of dairy cows within a dairy herd.

Acknowledgements

First I would like to thank my supervisors Erling Strandberg and Emma Carlén for their everlasting patience and pedagogic explanations.

Then I would like to thank my family for their love and support**.**

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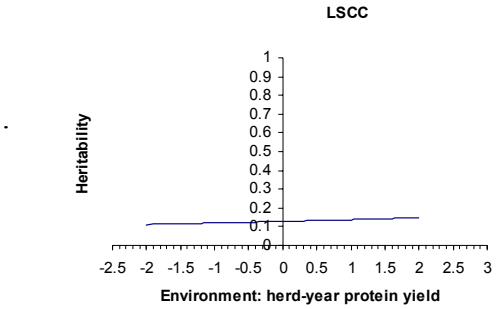
Appendix A. Heritability of LSCC and mastitis as a function of different environments from model D.



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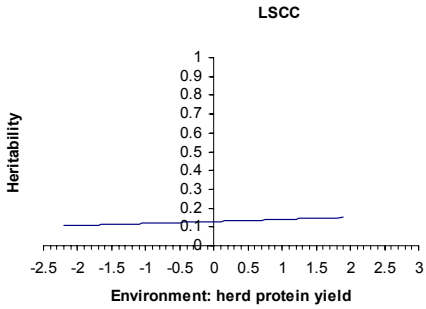

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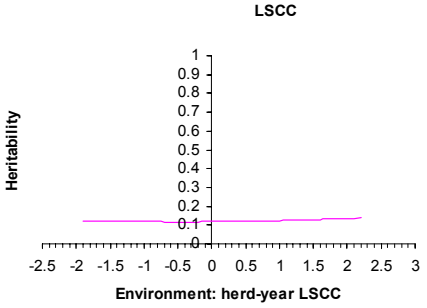

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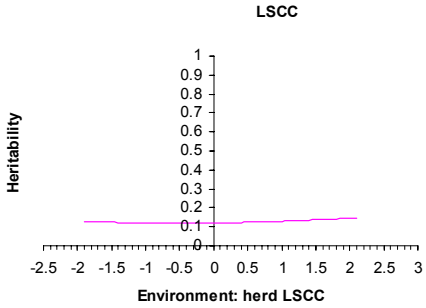

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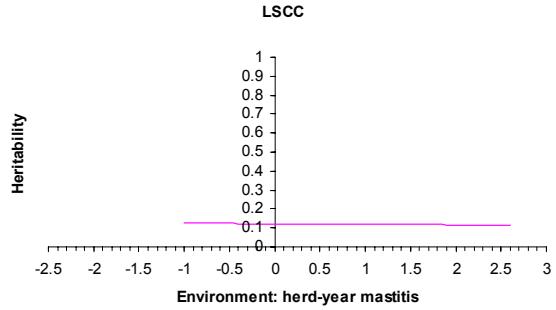

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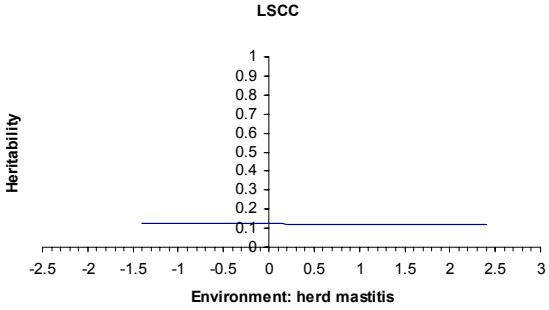
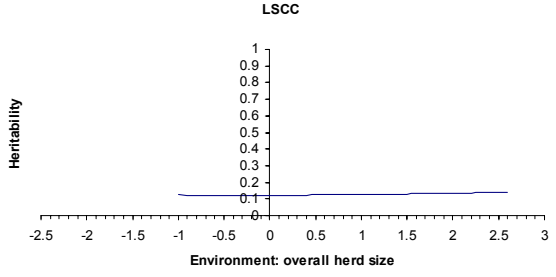


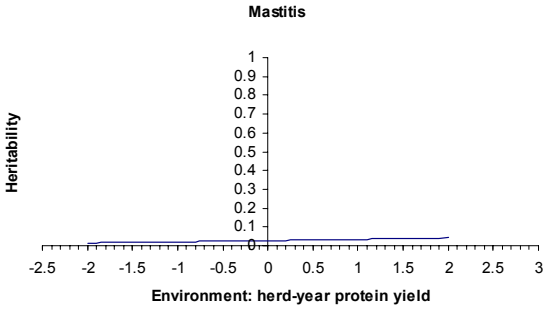
f)



g)

Figure A1 will be continued.

Figure A1 continued.



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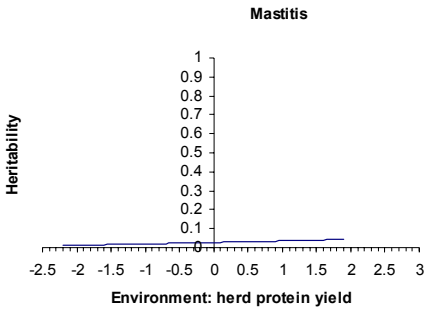

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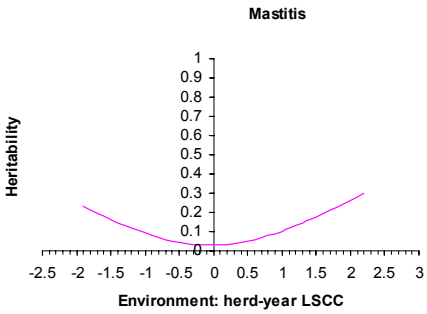

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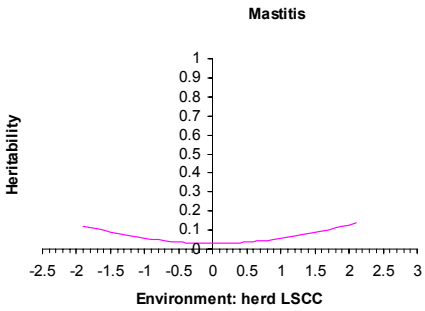

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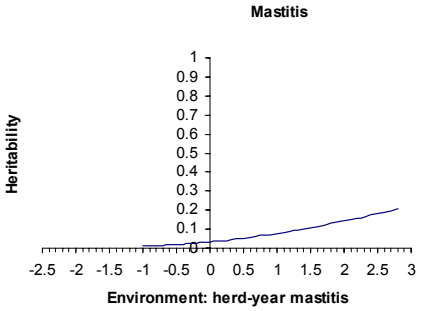

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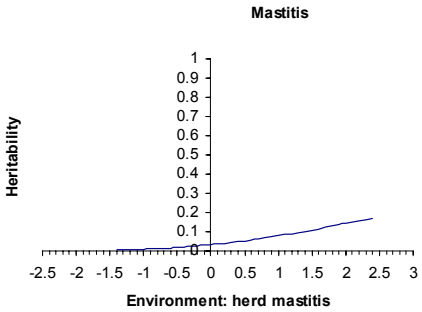

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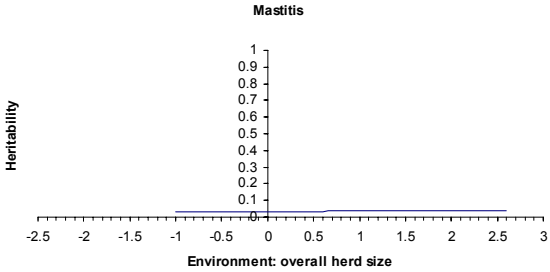
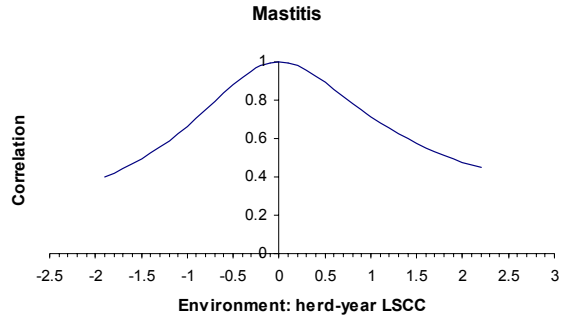


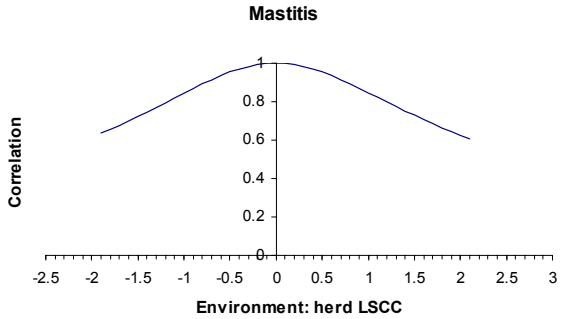
n)

Figure A1. Heritability for somatic cell count (a-g) and mastitis (g-n) from model D as a function of different environments.

Appendix B. Correlations between POP in average and deviating environments for mastitis from model D.



a)



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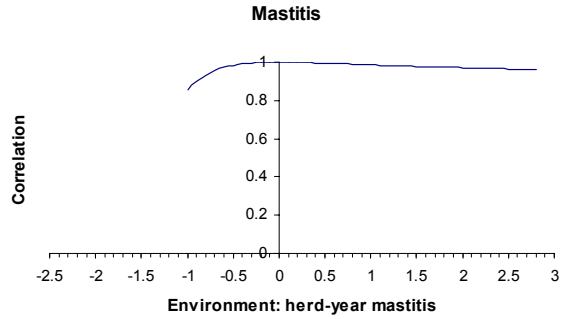

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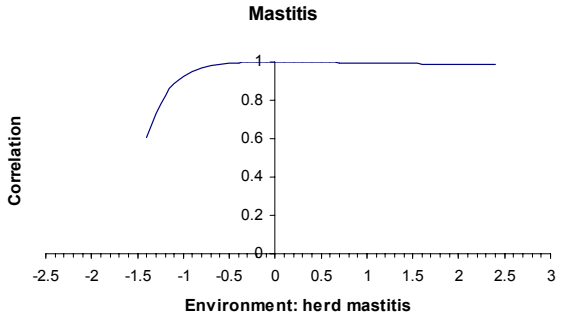

d)

Figure B1. Correlations between POP in the average environment (0) and deviating environments for the environmental scales herd-year and herd LSCC (a and b) and mastitis (c and d) in model D for mastitis.