

## **STATISTICAL GENETICS TO IMPROVE ROBUSTNESS OF DAIRY COWS**

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### **SUMMARY**

Rapid changes in farm scale and pressures on costs and farm management together with the negative correlation between production and fitness traits have stimulated the demand for robust cows. We have defined a robust cow as: “a cow that is able to maintain homeostasis in the commonly accepted and sustainable dairy herds of the near future” which clearly contains an element of environmental sensitivity and genotype by environment interaction (GxE). Classical breeding solutions to breed for more robustness are i) avoiding inbreeding, ii) multi-trait selection and iii) allowing more natural selection. Statistical models allow more direct selection for robustness by estimating genetic correlations between environments (discrete macro scale), reaction norms describing genotypes as a function of a continuous environmental parameter (continuous macro scale) and genetic variation in residual variance which is environmental sensitivity for a large number of unidentifiable environmental aspects with a relatively small effect each (unknown micro scale). The application of these complex models is still under development, but first results indicate more GxE for fitness traits than yield traits, albeit evidence for strong re-ranking of animals is still limited, so it is mainly the magnitude of the variance which is affected by environment. These statistical models will contribute, together with classical tools and new phenotypic and genomic measurement tools, to the breeding of cows that also fit in future dairy systems.

### **INTRODUCTION**

Selection for more robust cows has been an important research topic in our group. The aim of this paper is to highlight some of the work we and others have done on robustness, especially in relation to the numerical methods used to select for more robust cows. But first we discuss conceptually why we think that robustness has become important and the definition of robustness Napel *et al.* (2009).

### **DEMAND FOR ROBUSTNESS**

Cannon (1932) first used the term homeostasis to indicate that a body continuously acts to maintain a stable internal environment by responding to external environmental stimuli. In the last two decades, there have been concerns that high-yielding dairy cows struggle to maintain homeostasis. Several studies reported unfavourable genetic correlations between milk yield and reproductive problems, locomotive problems and udder health problems (Pryce *et al.* 1997; Rauw *et al.* 1998; Royal *et al.* 2000), and there is general consensus that selection for milk fat and milk protein yield alone may give an unfavourable correlated response in these traits. The magnitude of these correlated responses is rather small, compared with direct effects of environmental disturbance, albeit when the effects of breeding are accumulated across years these might be substantial. The gradual reduction in genetic levels for fertility and health will put more pressure on management to maintain performance at acceptable levels. Such cows require more management attention. At the same time, the level of management is increasingly under pressure from other directions. For example, due to economic pressure, herd size is increasing and therefore the amount of labour available per animal is decreasing. The shortage of labour is aggravated by the fact that it is increasingly difficult to find suitably skilled labour. Also, pressure on

management increases because previously simple and effective management tools, such as the use of antibiotics, are now perceived as potential risks for human health and therefore regulated much stronger.

These two trends, i.e. negative effects from selection for yield and increasing pressure on management, have fuelled the demand for more robust cows. The demand for robustness in animals is a shift in emphasis between two paradigms both aiming to control the impact of disturbances on an animal (Ten Napel *et al.* 2006). The one approach is called the Control Model and is characterized by maintaining stability through keeping away disturbances. Typically a strategy is used of protecting animals from disturbances as much as possible, constantly monitoring animals, whether a disturbance occurs, and interventions targeted at the disturbance, when it does occur. When taken to the extreme, the homeostasis of the animal is dependent on proper and timely functioning of humans and technical equipment. The other approach is called the Adaptation Model. This approach is characterized by maintaining stability through minimizing the impact of disturbances in the presence of the disturbance. The design of such a production system seeks to utilize the intrinsic capacity of animals to adapt where possible, and use the Control Model approach where necessary (Ten Napel *et al.* 2006).

#### **DEFINITION OF ROBUSTNESS**

In the Netherlands, we gradually developed a concept of robustness of farm animals in the course of three to four years, based on discussions with many groups of stakeholders. This process resulted in the following definition of a robust dairy cow: "A robust dairy cow is a cow that is able to maintain homeostasis in the commonly accepted and sustainable dairy herds of the near future."

It is clear from this definition that robustness is not just a matter of the average level of management being suitable for the cow. Dairy herds are dynamic and fluctuations in temperature, air speed, humidity, disease pressure, fodder quality, stocking density, social interaction with other cows, aggression, interaction with stockmen, among other factors, occur. Over time or across herds, common fluctuations largely fit within a certain band width. A cow that is robust is able to maintain homeostasis in a range of production environments with a bandwidth that is wider than the common band width of fluctuations. It does not mean that a robust cow must be able to cope with anything. Some animals respond to a change in environment for some characteristics in a much stronger way than other animals. Such animals are more environmentally sensitive for these traits. In population biology terms, these animals are called 'specialists' as they have a very high fitness only in specific conditions. Less environmentally sensitive animals are called 'generalists'. These qualifications are not absolute, but relative to the range of environments considered. A cow may be a 'specialist' when considering all possible environments, but a 'generalist' when considering the range of acceptable production systems in a country. A robust cow is more of a 'generalist' as it has a reasonable fitness across relevant production environments (Bryant *et al.* 2006).

#### **CLASSICAL TOOLS TO BREED FOR ROBUSTNESS**

Three obvious ways to account for robustness in animal breeding are (i) to avoid inbreeding, (ii) to include fitness traits in the breeding goal, breeding value estimation and the selection index and (iii) natural selection. Overcoming or avoiding inbreeding depression and maximizing heterosis is a relatively easy way to improve genetic fitness and is widely utilized in pig and poultry breeding programmes. This effect stems from the observation that characteristics associated with genetic fitness often reveal overdominance, that is the phenotype of heterozygotes is superior to the phenotype of any of the two types of homozygotes. Crossbreeding in a structured manner is less popular in dairy cattle breeding, because the average number of calves per calving is just over one. Furthermore, unfavourable recombination counteracts the favourable heterosis for

milk production traits (Pedersen and Christensen 1989; Dechow *et al.* 2007). Rotational crossing, may be suitable for this purpose (McAllister 2002), but within breed optimizing gain and inbreeding in a breeding program might be as effective (Meuwissen and Sonesson 1998).

Multi-trait selection has been practiced in many dairy countries (Miglior *et al.* 2005). Although multi-trait selection clearly works and is better than single trait selection, there are several reasons why multi-trait index might not be enough to maintain or improve robustness. Firstly, it is difficult for the many aspects of fitness to be clearly defined as a trait to be measured. Secondly, it has been suggested to include traits of the adaptive systems, e.g. immune system, in the multi-trait index (Wagter *et al.* 2000). These adaptive systems, however, are highly integrated life systems with a high degree of unpredictability and ambiguity observed (Tada 1997). Therefore, it is unlikely that genetic selection for changes in the immune system will improve robustness in the foreseeable future, and breeding is likely to be more successful when it concerns the result of adaptation (i.e. effective coping), rather than the adaptation process itself. Finally, fitness traits are expected to exhibit genotype by environment interaction, causing animals to rank differently in different environments. In this way, after any major change in living conditions, there is at least a part of the population that is able to survive in the new environment. Breeding value estimation does not take account of this variation, as generally the estimation models include a correction for heterogeneous variances. Therefore selection does not take into account differences in environmental sensitivity or genotype by environment interaction. When following the definition of robustness given above, robustness is about maintaining homeostasis across environments and environmental challenges, and not only about having a high fitness on average across environments. Clearly environmental sensitivity and genotype by environment interaction play an important role when breeding for robustness.

In natural populations without artificial selection, genetic fitness is maintained through a self-structuring force, called natural selection. If through a change in the environment, variation in genetic fitness arises, then the increase in fitness in the population is equal to the additive genetic variance of fitness at that time (Falconer and Mackay 1996). However natural selection cannot be utilized easily in breeding programmes, for practical reasons. Breeding animals are often kept under strict biosecurity control in order to be able to sell semen or breeding stock. Also, natural selection will be perceived as bad stockmanship or is not acceptable because of the welfare of the animals.

### **STATISTICAL TOOLS TO BREED FOR ROBUSTNESS**

Statistical models that enable to extract information on robustness and environmental sensitivity of animals might be important. In order to estimate genotype by environment interaction, models have developed from (1) environments falling into a limited number of categories (discrete macro scale), (2) production environments differing on a continuous environmental parameter (continuous macro scale), and (3) environments differing on a large number of aspects with a relatively small effect each, which are not identifiable (unknown micro scale).

**Disturbances by environments on a discrete macro scale.** The classical GxE model is when there are distinct discrete environments such as, for example, organic and non-organic dairy herds (Nauta *et al.* 2006). Grouping herds on prevailing soil type may yield five or six categories. Other examples are grouping by country, geographical region, type of production system or presence or absence of a major disease, and seasons. With these genetic parameters it is possible to carry out a multi-trait breeding value analysis for each trait measured in different groups of environments. In some cases distinct environmental groups, or character states of herds were formed within the environmental levels for yield, heat load index, herd size and altitude in relation to milk

production (Bryant *et al.* 2007). With these genetic parameters it is possible to carry out a multi-trait breeding value estimation for each trait measured in different groups of environments. If the genetic correlation between the two groups of environments is lower than 0.6-0.7 (Mulder *et al.* 2006), it suggests that in such a scenario most genetic progress is made for that trait, when breeding specialists rather than breeding for a generalist.

#### **Disturbances by environments on a continuous macro scale**

In recent years the reaction norm model has become increasingly important. The performance of a genotype as a function of an environmental parameter is called the reaction norm. If a change in a certain environmental parameter affects some genotypes more than others, then there is genetic variation in environmental sensitivity. Reaction norm models have been applied to environments quantified by the mean performance of all genotypes, which then becomes the environmental parameter. Initially models were used using indirect estimation procedure (Veerkamp and Goddard 1998), but software developments allowing random regression models to be used (Schaeffer and Dekkers 1994; Gilmour *et al.* 2000), have taken over from the indirect approach. For example, (Kolmodin *et al.* 2002) used days open and average milk yield as environmental descriptors, others investigated a large number of environmental parameters describing differences between herds in for example production, management, climate and nutrition in relation to production (Calus and Veerkamp 2003; Fikse *et al.* 2003) or in relation to fertility and health (Windig *et al.* 2005b), or looked at a heat stress index in relation to days open (Oseni *et al.* 2004), fat% in relation to milk fat depression (Calus *et al.* 2005), or average herd life, yield and herd size in relation to length of productive life (Petersson *et al.* 2005).

Despite these relative complex models, for the yield traits genetic correlations across environments are all close to unity albeit variances almost doubled across environments (Calus and Veerkamp 2003; Kolmodin *et al.* 2004). For fitness traits, i.e. health and fertility, larger differences in genetic variances were observed across environments: genetic variances for fertility traits increased in some situations more than twofold, and a threefold increase for genetic variances of somatic cell scores was found (Windig *et al.* 2005a). Genetic correlations of a trait across environments were as low as 0.65 for survival (Windig *et al.* 2005a), while for somatic cell scores, on a test-day level, the lowest genetic correlation was as low as 0.72 (Calus *et al.* 2006). Within country, literature values of genetic correlations for health and fertility ranged from 0.74 (Petersson *et al.* 2005) to unity (Castillo-Juarez *et al.* 2000; Raffrenato *et al.* 2003; Carlen *et al.* 2005). Since most studies that reported environmental variances found changes in environmental variances with a change in environmental predictor, it is important to account for heterogeneous residual variances across environments as well as heterogeneous genetic variances.

The generally high genetic correlations found between environments are somewhat a surprise. One concern was that this might have been caused by the fact that environmental descriptors are often calculated from the same data. For example average milk yield as environmental predictor and milk yield as the trait analysed. Therefore there might be difficulty in disentangling the genetic level of a herd, the phenotypic level of a herd and the genetic level of animals within that environment. (Kolmodin *et al.* 2002) tried to solve this by taking more animals in the herd to calculate the environmental parameter. Another problem typical for animal breeding data is preferential treatment of animals with a high breeding value and selective use of sires in certain environments. Calus (Calus *et al.* 2004) investigated these questions with simulation. He found that there was little effect of the definition of the environmental descriptor on the estimated components. Non-random use of sires, little connectedness and small herd sizes had a large effect on the estimated covariance function. Even with random sire use the less favourable the data, the more the estimated genetic correlation was biased upwards towards unity, i.e. underestimating genotype by environment interaction.

From the initial models used, several authors have made advancements. For example, ASREML allowed further expansion of the single trait reaction norm model to a multi trait reaction norm model (Windig *et al.* 2006). The advantage of the multitrait model is that also the sensitivity of the genetic correlation between traits can be investigated. For example the genetic correlations between milk yield and number of inseminations ranged from 0.18 in large herds to 0.64 in high fertility herds. Also the genetic correlation between yield and SCC differed depending on the success of fertility management in a herd (from 0.25 to 0.47). Another expansion was implemented by including more than one environmental descriptor in the model (Calus *et al.* 2006), e.g. by combining herd bulk tanks SCC, days in milk and their interaction. This model was similar to the covariance function approach used by (Veerkamp and Goddard 1998), albeit Calus *et al.* (2006) were able to estimate the parameters of the covariance function directly from the data. Currently we are developing a model that combines the reaction norm approach with the multitrait approach, which for example, enables to analyse the effect of average milk production in different production systems. Su (2006) developed a Bayesian model that inferred the environmental values simultaneously with the other parameters of the model., which is theoretically more appealing than using a predefined environmental parameters. This model was applied to dairy cattle production data (Shariati *et al.* 2007) and further developed (Su *et al.* 2009) to allow for environmental sensitivity of heterosis. A semi-parametric non-linear longitudinal hierarchical model (Sanchez *et al.* 2009) was suggested by these authors to study longitudinal data, especially when the traits are subject to abrupt changes in due to the environment.

**Disturbances by environments on a unknown micro scale.** In practice, dairy herds differ in many ways and discrete or continuous environmental parameters describe these differences only in part. These unexplained micro-environmental differences may lead to genetic differences in micro-environmental sensitivity, which is observed as differences in residual variance. When bulls have at least 50-100 progeny, breeding values can be estimated for the size of the residual variance (Mulder *et al.* 2007). Bulls with progeny that exhibit a large residual variation across herds are the ones that are environmentally sensitive. Bulls with progeny that exhibit a small residual variation across herds are the ones that are not environmentally sensitive.

One of the main problems with analysis of genetic heterogeneity of residual variance is its estimability. Rowe *et al.* (2006) used a two-step approach, in which the first stage used a model to analyze body weight with allowance for differences in residual variance among sire families, and in the second stage the variation among these estimates of the residual variance were analyzed with least squares. In some studies log-transformed squared residuals have been analyzed, also using a two-step approach (Larzul *et al.* 2006; Bolet *et al.* 2007), which gives the flexibility to account for environmental effects on the residual variance at the level of the record that is not possible in the least squares analysis of Rowe *et al.* (2006). Ideally, one would simultaneously fit a model for the mean of the trait and for the residual variance using a structural model. (Sorensen and Waagepetersen 2003; Ros *et al.* 2004; Gutierrez *et al.* 2006) applied these structural models in a Bayesian context, implemented using Markov Chain Monte Carlo sampling (MCMC). Mulder *et al.* (2009) extended the two-step approach of Larzul *et al.* (2006) and Bolet *et al.* (2007) to a bivariate analyses using ASREML to get an estimate of the genetic correlation between the additive genetic effects for the mean and the residual variance. Wolc *et al.* (2009) used a similar approach combined with a generalized linear model for the log-transformed squared residuals, but they were not able to run a bivariate analysis.

There is some empirical evidence that genetic variation in residual variance exists. (SanCristobal-Gaudy *et al.* 2001; Sorensen and Waagepetersen 2003; Ros *et al.* 2004; Gutierrez *et al.* 2006; Ibanez-Escriche *et al.* 2008a,b) used a structural model for heterogeneous residual variance and found substantial genetic variation in residual variance for litter size in sheep, litter

size in pigs, body weight in snails, litter size and weight in mice, body weight traits in mice, and slaughter weight in pigs, respectively. Rowe *et al.* (2006) found substantial genetic heterogeneity of residual variance between sire families in body weight of broilers. Mulder *et al.* (2009) found low heritability for residual variance of body weight in broilers (0.03 - 0.05), but a relatively high genetic coefficient of variation (0.35 - 0.57) was estimated. Probably the clearest example is by Mackay and Lyman (2005), who derived 300 isofemale lines of *Drosophila melanogaster* and found substantial highly significant genetic variance in residual variance between lines under controlled laboratory conditions. Other evidence comes from the selection experiment by Garreau *et al.* (2008) who obtained in a divergent selection experiment with rabbits a clear selection response when selecting on high or low within-litter birth weight. All these results indicate that environmental sensitivity to unknown environmental disturbances can be improved by means of genetic selection. No estimates or selection experiments have been published for dairy cattle yet. However, if we suppose that also in dairy cattle this genetic variation in residual variance exists, then these genetic differences may be utilized to breed more robust cows, cows that are insensitive to unknown disturbances e.g. for fertility and health traits.

## **DISCUSSION**

A robust dairy cow is able to maintain homeostasis in the commonly accepted and sustainable dairy herds of the near future. Robustness is largely an acquired characteristic through building up experience from exposure to a very large number of minor and major environmental signals. Breeding may give animals an advantage in acquiring robustness. Several methods have been discussed to improve robustness through breeding in practice. Finding re ranking of animals across environments, even with the sophisticated reaction norm models, is difficult, in the scenarios where enough data is available. For production traits it is even questionable if substantial GxE exists, whilst re-ranking for fitness trait has been reported in some studies. The more diverse the systems will be, the more important this GxE becomes for fitness traits. An important strategy will always be to collect data in the same environment we are selecting our breeding animals for. However, this is a practical challenge for dairy cattle breeders, when the speed of change, in for example farm size and scale, continues with the same rate that has been observed over the past 15 years and at the same time the generation interval remains 5 years. Therefore, within the RobustMilk project ([www.RobustMilk.eu](http://www.RobustMilk.eu)), there is also attention for measuring robustness traits with mid-infrared spectrometry using a spectra, as part of routine milk analysis. Preliminary analysis indicates that equations developed using the spectra can be used to predict milk fatty acid content (Soyeurt *et al.* 2006) and the question is whether they can be used to also predict dairy cow robustness. i.e. energy balance and immune parameters. Furthermore, in this project rare detailed phenotypes on 2000 cows from four research herds have been brought together, and in the past months all these animals have been genotyped with the 50k SNP array. Statistical analyses of these spectra and genomic data form a challenge on their own, but together with the statistical models describing the genetic variation in environmental insensitivity, they are an essential part in developing a strategy to breed more robust cows suitable for future farming systems.

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