Epigenetic Studies of the Ageing Process

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Abstract

The ageing organism goes through radical changes. Studies on the experimental populations show that there might be epigenetic mechanisms switching off homozygote loci and switching on heterozygote loci during the ageing process. This is assumed to be because of the higher metabolic demands from homozygote loci. A corollary to this hypothesis is that the loci with the largest effect on longevity must have a lower degree of homozygosity. We tested this hypothesis in an international dairy cattle population consisting of 7038 bulls genotyped with more than 50,000 SNPS. The results supported our hypotheses, however, only for loci whose effects were smaller than 4.0 standard deviations larger than the average.

Key words: epigenetic, ageing, longevity, genomic, homozygosity

Introduction

Pushing animal populations towards maximum production in the shortest possible time span, and slaughtering them afterwards, is not ethical. The ageing animal should also be provided the possibility of a long, healthy and vigorous life. However, our knowledge of the ageing process in farm animals is rather limited, because in the past the old animals have been culled from production system.

Experimental and farm animals, however, can provide valuable opportunities for understanding the ageing process, because they can be selected to have lifespans that exceed the normal lifespan by a large margin. As an example, in the Drosophila populations selected for increased lifespan, the generation interval has been increased from the ordinary 11 days to 80 days (e.g. Engström *et al.*, 1992a).

Length of the productive life, coined direct longevity (DLO) in the dairy cattle genetics, is nowadays a trait of interest for farmers all around the world. In different countries DLO is defined slightly differently, but it usually is defined as the total number of months that a cow produces milk. Interbull Centre has been working with international genetic evaluation of direct longevity in several major dairy breeds, from a large number of countries, since 2004 (http://www-interbull.slu.se/longevity/framesida-long.htm).

Studies of the senescence (ageing / lifespan / life history evolution / longevity) go far back in time (e.g. Darwin, 1874). However, the first genetic studies started in 1950's (Medawar, 1952; Williams, 1957; Fisher, 1958). There is little dispute as the ontogeny, up to the sexual maturity, being under genetic / evolutionary control (Hayflick, 2007). However, there has been much debate about the ageing process after that. Is post-maturity genetically controlled, or is it just an accumulation of damages at the cellular level?

Liljedahl (1968, 1974) could show age related changes in the expression of genetic variance. Later, Charlesworth (1980) could extend the lifespan of Drosophila populations, and show the existence of genetic variance and the success of genetic selection. Engström *et al.* (1992a) managed to repeat Charlesworth's experiment and extend the lifespan of experimental populations of Drosophila several fold. Discovery of specific mutations accounting for variation in longevity (Klass, 1983; Frieman & Johnson, 1988; Kenyon *et al.*, 1993) proved unequivocally that genetic factors have definitely a role in longevity. Establishing the connection of these mutations with the insulin-like globulin factors (IGF) was a breakthrough in understanding the mechanisms of ageing (Kenyon, 2010).

The next wave of breakthrough in this field came about with the understanding that environmental factors, like dietary restriction (Kaeberlein *et al.*, 2007; Piper & Partridge, 2007; Antebi, 2007) or viruses (Luo *et al.*, 2012) can affect longevity. The finding that epigenetic factors can have a role in the ageing process opens up more possibilities for understanding the senescence phenomenon. One interesting development is that there are functional gene groups that change their pattern of co-expression with age, i.e. there is a decline of expression as the age increases (Southwoth *et al.*, 2009).

In this study, we consider the following chain of arguments:

1- Experimental populations of drosophila, mice and chickens show that the ageing animal expresses a higher level of heterosis (hybrid vigor), especially for the fitness related traits (e.g. health / disease related traits) (e.g. Liljedahl *et al*, 1984; Engström *et al.*, 1992b);
2- Heterosis can arise when the dominance / recessive relationship exists between the two alleles of a locus in an individual, i.e. when the animal is heterozygote (e.g. Falconer & Mackay, 1996; Goff, 2011);
3- This is a paradox! An animal's genotype is fixed at birth and cannot change during the lifetime (ontogeny), and yet increasing heterosis during the lifetime means that the animal becomes more heterozygous during the ageing process, i.e. changes the genotype. This cannot be;
4- One way to solve this paradox is to assume that the fitness traits are under the control of many loci, where in the early life more homozygote loci are expressed, and in the late life more heterozygote loci are expressed. For this assumption to be plausible there must be mechanisms that activate / inactivate different loci during the lifetime (ontogeny), i.e. there should be some epigenetic mechanisms to switch on / off loci (e.g. Cheverud *et al.*, 1996; Atchley & Zhu, 1997);
5- One possible explanation (or working hypothesis) for the expression of homozygote loci in the early life, and their gradual inactivation during lifetime could be the postulate that the homozygote loci are more resource demanding (and consequently the heterozygote loci less resource demanding) (e.g. van der Waaij, 2004; Ginn, 2010);
6- A corollary to the above argument is that any locus with a large effect on the trait should be considered more resource demanding, and consequently should show, on average, less homozygosity.

In this study we address the question of the role of epigenetic factors in the ageing process. Specifically, we would like to examine if loci with large effects show a lower degree of homozygosity.

Materials and Methods

In a recent study, we used the information on 44826 single nucleotide polymorphism (SNP), in an international population of 7038 Brown Swiss dairy bulls (Jorjani *et al.*, 2012) and performed extensive genomic evaluations. The bulls had been genotyped with the Illumina's Bovine 50K BeadChip. The phenotype studied was direct longevity (DLO) measured on daughters of these bulls, i.e. total number of month in milk production. The bulls originated from Austria-Germany (DEA), France (FRA), Italy (ITA), Switzerland (CHE), and the United States of America (USA).

Results & Discussion

Distribution of SNP effects in different country scales are shown in Figure 1. Two points are emphasized here. First, there are a large number of SNP effects that stand out from the rest of the SNPs. Our genomic evaluation method does not produce any p-values. However, we assumed that there is a high positive correlation between the size of SNP effects and their p-values. Second, there are noticeable differences among countries as to which chromosome harbors the SNPs with the largest effects.

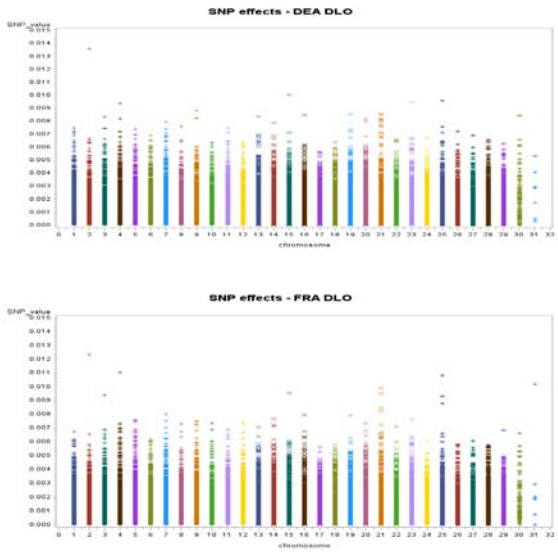

Figure 1. Manhattan plots of SNP effects for cow longevity in the GWAS, based on 44826 SNPs. Bovine species have 30 chromosomes. We have assigned the SNPs on the pseudo-autosomal part of the X-chromosome as chromosome 30, and the rest of the X-chromosome as chromosome 31.

Consequently, it can be concluded that longevity is partially under control of different sets of loci in different countries. Are there any systematic environmental differences that cause these differences? Or are these differences related to different breeding goals in different countries?

Based on the point 5 in the chain of arguments mentioned in the "Introduction", we hypothesized that if the homozygote loci are more resource demanding, then the frequency of the homozygote loci must go down as the SNP effect (size of the locus effect) goes up. In other words, loci with the largest effect must have lower homozygosity.

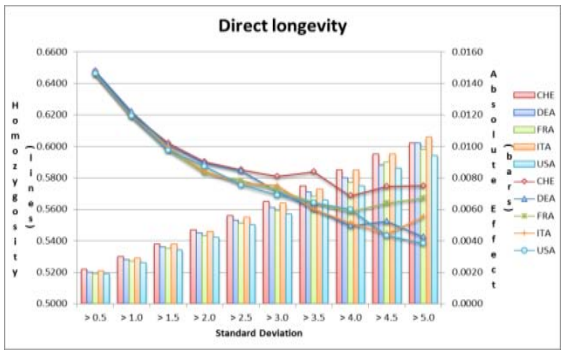

Figure 2. The level of homozygosity (lines) and the SNP effects (bars), for SNPs with 0.5 to 5.0 standard deviation larger than the average, for cow longevity in the five countries included in the genomic study.

The graphs in Figure 2 generally support our hypothesis, i.e. as the size of SNP effects go up, the degree of homozygosity show a general decline. However, as the size of the SNP effects get larger than 4.0 standard deviation of the average, the decrease in homozygosity is not observed anymore. It seems that when the SNP effect gets too large, it becomes so necessary for the body cannot afford to inactivate them. It should also be said that the number of SNPs with such large effects is around 5-15 (results not shown). One interesting question is if these loci overload the body, that is, if they have a negative effect on the lifespan, for example, through taking away resources from other loci.

The choice of longevity for our study was a necessity, and not the first choice. Ideally, a trait that can be measured several times during life should be used in our study, e.g. milk yield or fertility for different lactations. Preferably the time span between the first and the last measurement should cover a large period of natural life. However, the data used in our study did not contain such a trait. The closest trait to an ideal trait among available traits would be female fertility traits. The five existing fertility traits conceptually can be assumed to be measured during different periods of life, though in practice there is much overlap among them.

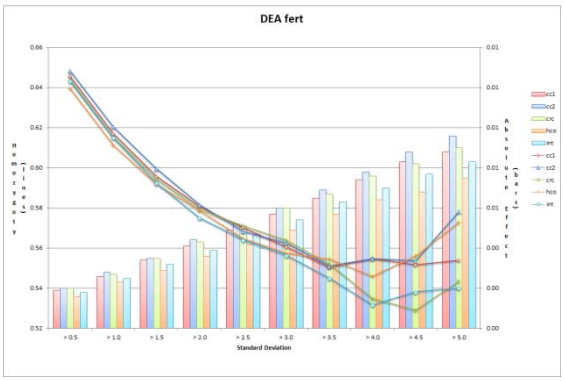

Figure 3. The level of homozygosity (lines) and the SNP effects (bars), for SNPs with 0.5 to 5.0 standard deviations larger than the average, for fertility traits in the DEA scale.

Heifer conception (HCO) is definitely an early measured trait, followed by cow's ability to return to cycle (CRC). The two traits, cow conception measured as a rate trait (CC1), and as an interval (CC2) are the traits measured after CRC. The pure interval trait (INT) for days open and calving interval is measured last. It should be mentioned that given the structure of dairy cattle populations, these traits cover a short period with much overlap.

The pattern observed in Figure 3 is not very clear, and we have not tried to perform any statistical test to examine the differences in the present preliminary report. However, it seems that the trait INT has lower homozygosity compared to the other traits.

Conclusions

This study showed that the international predicted genetic merit (IPGM) estimated through the MACE methodology (Schaeffer, 1994) can be used in genome wide association studies (GWAS). Results also support our general hypothesis about the decreasing pattern of homozygosity during ageing. The general conclusion is that this is a subject worth

pursuing. Currently applied methodology seems to be suitable for our purpose. However, access to traits distinctly measured during lifetime would be highly desirable.

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References

Antebi, A. 2007. Genetics of aging in Caenorhabditis elegans. *PLoS Genet 3 (9): e129* doi:10.1371/journal.pgen.0030129.

Atchley, W.R. & Zhu, J. 1997. Developmental quantitative genetics, conditional epigenetic variability and growth in mice. *Genetics 147(2),* 765-776.

Charlesworth, B. 1980. *Evolution in age-structured populations.* Cambridge Univ. Press, Cambridge.

Cheverud, J.M., Routman, E.J., Duarte, F.A.M., van Swinderen, B., Cothran, K. & Perel, C. 1996. Quantitative trait loci for murine growth. *Genetics 142,* 1305-1319.

Darwin, C. 1874. *The descent of man in relation to sex.* John Murray. London.

Engström, G., L.iljedahl, L.-E. & Björklund. T. 1992a. Expression of genetic and environmental variation during ageing. 2. Selection for increase lifespan in Drosophila melanogaster. *Theor. Appli. Genet. 85,* 26-32.

Engström, G., Liljedahl, L.-E., Wilhelmson, M. & Johansson, K. 1992b. The pattern of of genetic and environmental variation in relation to ageing in laying hens. *Genet. Sel. Evol. 24,* 265-275.

Falconer, D.S. & Mackay, T.F.C. 1996. *Introduction to quantitative genetics.* 4th ed. Longman. Essex.

Fisher, R.A. 1958 (1930). *The genetical theory of natural selection.* Dover, New York.

Friedman, D.B. & Johnson, T. 1988. A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility. *Genetics 118,* 75–86.

Ginn, B.R. 2010. The implications of gene heterozygosity for protein folding and protein turnover. *J. Theor. Biol. 265,* 554-564.

Goff, S.A. 2011. A unifying theory for general multigenic heterosis: energy efficiency, protein metabolism, and implications for molecular breeding. *New phytologist 189,* 923-937.

Heyflick, L. 2007. Entropy explains aging, genetic deteriminism explains longevity, and undefined terminology explains misunderstanding both. *PLoS genet. 3(12) e220.* doi:10:1371/journal.pgen.0030220.

Jorjani, H., Jakobsen, J., Nilforooshan, M.A., Hjerpe, E., Zumbach, B., Palucci, V. & Dürr, J. 2012. InterGenomics project: Genomic evaluation of BSW populations. *Interbull Bulletin 43,* 5-8.

Kaeberlein, M., Burtner, C.R. & Kennedy, B.K. 2007. Recent developments in yeast aging. *PLoS Genet 3 (5): e84.* doi:10.1371/journal.pgen.0030084

Kenyon, C. 2010. The genetics of ageing. *Nature 464,* 504–512.

Kenyon, C., Chang, J., Gensch, E., Rudner, A., & Tabtiang, R. 1993. A C. elegans mutant that lives twice as long as wild type. *Nature 366,* 461–464.

Klass, M.R. 1983. A method for the isolation of longevity mutants in the nematode Caenorhabditis elegans and initial results. Mech. *Ageing Dev. 22,* 279–286.

Liljedahl, L.-E. 1968. Studies of inbreeding and ageining in female mice. Akademisk avhandling--Lantbrukshögskolan, Uppsala. Reprint from: *Lantbrukshögskolans annaler 1968.* Published 1968 by Almqvist and Wiksell in Uppsala.

Liljedahl, L.-E. 1974. Effects of heterozygosity in relation to ageining in female mouse. Proc working symposium Breed evaluation and crossing experiments. *Zeist, 1974.*

Liljedahl, L.-E., Gavoura J.S., Fairfull R.W. & Gowe, R.S. 1984. Age changes in genetic and environmental variation in laying hens. *Theor Appl Genet 67,* 391-401.

Luo, J., Yu, Y., Tian, F., Zhang, H. & Song, J. 2012. DNA methylation fluctuation induced by virus infection differs between Md-resistand and –susceptible chickens. *Frontiers in Genetics.* doi: 10.3389/fgene.2012.00020.

Medawar, P.B. 1952. *An unresolved problem in biology.* H.K. Lewis. London.

Piper, M.D.W. & Partridge, L. 2007. Dietary restriction in drosophila: Delayed aging or experimental artifact? *PLoS Genet 3 (4): e57* doi:10.1371/journal.pgen.0030057.

Schaeffer, L.R. 1994. Multiple-country comparison of dairy sires. *J. Dairy Sci. 77:9,* 2671-2678.

Southworth, L.K., Owen, A.B. & Kim, S.K. 2009. *PLoS Genetics. E1000776.*

van der Waaij, E.H. 2004. A resource allocation model describing consequences of artificial selection under metabolic stress. *J. Anim. Sci. 82,* 973-981.

Williams, G.C. 1957. Pleiotropy, natural selection, and the evolution of senescence. *Evolution 11,* 398-411.