Genetic variability of egg quality and prospects for selection

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Practising selection for egg quality will continue to be one of the most important aspects of the breeding strategy for egg-laying hens. Genetics and genomics has identified new strategies to address egg quality including the use of very high density genotyping to allow genome wide selection which has potential benefits for measurements that can only be performed in one sex or late in life (Preisinger, 2010, Albers, 2010). What remains true, whether the method of selection is traditional or modern, is that improvements in the method of measurement will always be in demand if they better measure an existing trait. Similarly if new methods of assessing quality have clear benefits they will be adopted.

I will use this review to both cover recent progress in understanding variation of egg quality in its broadest sense and introduce some new measurements which we and others have been involved in developing for application. I will also use the review to consider where possibilities for the application of future research to address egg quality might lie.

Developments in trait measurement

Outside the shell: Cuticle and pigment

The cuticle is recognised as a structure which prevents the entry of microbes to the egg (Sparks and Board, 1984), In a review 8 years ago (Dunn, 2004) I identified that genetics was likely to be a major factor affecting the amount of cuticle as it had been noted anecdotally that within bird variance was less than between bird variance (Ball *et al*., 1975). We have now completed studies as part of an EU project (http://www.sabre-eu.eu/) that used reflectance spectrometry of eggs before and after the cuticle was stained with specific dye and estimated the heritability at 0.27 (Bain *et al*., 2009). Most importantly, clear evidence was found which suggests that selection of the hens laying eggs with the best cuticle coverage will have less bacterial penetration (Bain *et al*., 2009). This has important implications for consumer safety, reducing spoilage and reducing inter-generation transfer of micro-organisms if this or related measurements are implemented in selection.

The colour of eggs, although not considered important in the safety or the prevention of damage, does have a place in the marketing of the product and we know there are strong preferences in different markets (Johnston *et al*., 2011). The pathways that lead to the deposition of pigment on the shell are relatively simple (Lang and Wells, 1987) and it is somewhat surprising that players in this pathway have not been identified as candidates for variation in egg pigmentation or even the difference between brown and white eggs. The notable exception has been the identification of the 'O' loci in hens which lay blue eggs due to biliverdin secretion. This has been mapped to chromosome 1 and markers close to the loci have been identified (Wang *et al*., 2011b). However, as if we needed confirmation that biology is rarely simple heme oxidase expression, part of the biliverdin formation pathway, is highly expressed in hens that lay blue eggs but, although the heme oxidase gene is on chromosome 1, it is 13Mb away from the loci mapped to be responsible for the phenotype (Wang *et al*., 2011b). Nor is their association of shell colour with markers at the heme oxidase loci and it is assumed that a transcription factor at the identified loci alters expression of heme oxidase, but the gene responsible has not been identified (Wang *et al*., 2011a). So in future identifying the genes which alter colour might be expected to be relatively easy given the high heritability of the trait (Francesch *et al*., 1997, Zhang *et al*., 2005, Forster *et al*., 1996). This may be of utility to breeders in creating lines with different colour characteristics to allow the production of hybrids suited to different markets, however no progress has been reported and the

experience of the blue egg laying chickens suggests that it may be harder than we think.

Shell

The eggshell has been the subject of many studies and although our understanding is progressing (Nys *et al.*, 1999, Nys *et al.*, 2011) the shell and the shell gland are sophisticated structures and egg quality traits are complex. By which we mean many factors, including many genes influence the expression of the trait. The application of genomics to the shell gland has improved our knowledge of the repertoire of expressed genes (Mann *et al.*, 2006, Dunn *et al.*, 2009b, Jonchere *et al.*, 2010) but as yet we have not capitalised on that information to identify novel genes which are important to variation in egg quality. However it will be the combination of genetic and genomic resources along with genome wide association studies that will allow us to understand how the variance underlying shell quality is determined. Hopefully the results from genome wide association studies, many of which are being carried out by breeding companies, will be published to give researchers renewed impetus to understand the formation of the shell.

That said there has been progress recently in understanding the variation in egg shell quality without the fruits of genome wide association studies which I will discuss.

To maintain eggshell quality as the quantity of egg production was improved has been for the principle goal of genetic selection. By eggshell quality I mean preventing damage during packaging and on the journey to the consumer. The success of this approach can be witnessed in experiments which observed no differences comparing egg quality across traditional breeds which are relatively poor egg layers and lines of commercial hens with the highest production levels (Hocking *et al.*, 2003). The methods that have been employed to achieve this are shell weight, specific gravity which estimates shell weight, quasi static compression such as deformation or currently breaking or puncture strength (Hunton, 1982, Wells, 1967, Dunn, 2004) which are assumed but rarely tested to be analogous to the trait which is sought, resistance to breakage Few instances are reported of having used the number of cracks observed in eggs layed by individual hens directly, presumably because there tends to be large environmental components to the variation in the collected data because the insults an egg receives is not controlled. However

with heritabilities between 0.50 and 0.62 observed when a threshold method for crack frequency was implemented it may well offer a viable method of capturing this information if it could be validated in commercial populations (Wolc *et al.*, 2005).

Although most of the other measurements are proxies for breakage, in a mixture of commercial and experimental populations heritabilities of around 0.2-0.37 can still be estimated for breaking strength and thickness (Zhang *et al.*, 2005, Besbes and Gibson, 1999, Chen *et al.*, 1993, Dunn *et al.*, 2005). This is presumably why selection has remained successful in maintaining quality. A recent addition to the collection of measurements available to maintain egg shell quality was dynamic stiffness which had a larger value for its heritability than breaking strength or shell thickness in the same population (Dunn *et al.*, 2005, Dunn *et al.*, 2009a). The measurement was developed at the University of Leuven using measurement of the eggs resonant frequency (Coucke *et al.*, 1999) and had reasonable genetic correlation with breaking strength (0.49). Because the measurement was non-destructive it offered the possibility to conduct an experiment which it has been impossible to do with other methods of egg shell quality. It was possible to measure the dynamic stiffness of an individual egg and trace if that egg survived the journey through the machinery of a modern packing plant. This is after all the trait that is really the target of breeding for shell quality. This demonstrated that eggs with small dynamic stiffness values were increasingly unlikely to reach the packing cartons destined for the consumer (Bain *et al.*, 2006). The relative simplicity of the measurement means this has found a place in the breeders selection tools (Icken *et al.*, 2006). The report of a method to determine thickness non-destructively by ultrasound may have some potential for breeders but there may be a need to overcome the need for immersion in a liquid (Sun *et al.*, 2011).

If we look at the principle component analysis **(Figure 1)** of shell quality data taken from publications using a line of Rhode island red hens (Dunn *et al.*, 2011, Dunn *et al.*, 2009a, Dunn *et al.*, 2005) we can see that measurements of crystal size, shell thickness, breaking strength, dynamic stiffness and stiffness form the first component explaining 29% of the data variance. Shape and weight traits dominate the second component. This suggests that although the measurements discussed above are different, there are underlying features which are common, information which we know from the genetic correlations between the traits.. Using more than one determinant of

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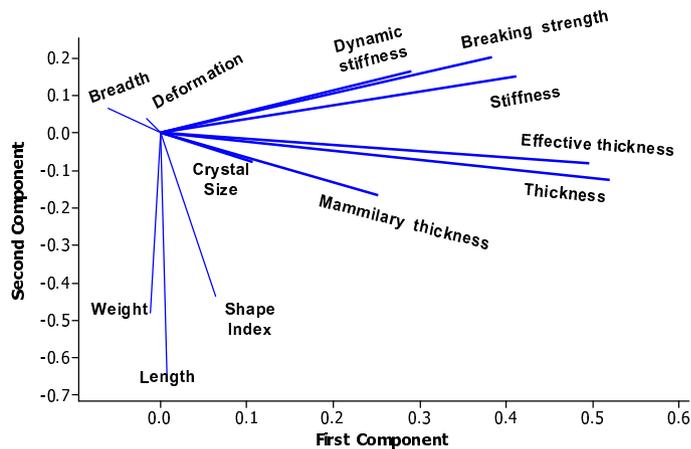
Loading plot showing the the 1st and 2nd principal components of egg quality measurements derived from Dunn 2005, 2009a, 2011 (n=898). The full description of the trait measurements can be found in these publications.

Figure 1 - 1st and 2nd principal components of egg quality measurements.

shell strength or quality allows ultimately more of the variation to be detected despite the fact that the underlying trait may be common. It was with this in mind that we made an effort to understand the underlying biological factors that determine the eggshell's strength and structure and to compare with measurements of the basic components of the eggshell. One of the most striking discoveries has arisen from the measurement of $CaCO_3$ crystal size using X ray diffraction (Dunn *et al.*, 2011). Crystal size was found to have very high heritability (0.6) which means that the measurement is very reproducible, but we also think it indicates that we are getting close to measuring the basic biological factors which underlie variation in egg shell structure. $CaCO_3$ crystal size is genetically correlated principally with the thickness of the mammillary layer of the shell which has major implications for the way the crystal columns that form the shell are initiated and their role in embryo development (Dunn *et al.*, 2011). Interestingly genetic markers in ovocalyxin 32 and ovocleidin-116, genes known to be involved in the formation of the eggshell protein matrix were highly associated with the direction of crystal growth in this study (Dunn *et al.*, 2011). This suggests that, as we have observed previously, for ovocleidin-116 with the thickness of the shell and ovocalyxin 32 with relative thickness of the mammillary layer (Dunn *et al.*, 2009a) that matrix proteins and variations in their expression or structure influence the properties of the shell. In this case we have been able to capture the variation with genetic markers and it can be used in selection.

There have been a number of reports of QTL for egg shell quality traits (Wardecka *et al.*, 2002, Wright *et al.*, 2006, Tuiskula-Haavisto *et al.*, 2002) one of which has used a relatively dense SNP approach (Abasht *et al.*, 2009). One of the most comprehensive approaches was published recently (Tuiskula-Haavisto *et al.*, 2011). This used commercially relevant lines and relatively large numbers of animals to detect QTL with high confidence on chromosome 2, 6, 14 and Z for the traits of shell weight deformation and breaking strength (Tuiskula-Haavisto *et al.*, 2011). Further studies will be required to understand the genes which are responsible but combined with the transcriptomic information and genomic markers now available it is an achievable prospect.

Albumen

Just like shell strength it seems likely that selection for increased egg numbers would come at the expense of egg white quality since reducing protein concentration would be a metabolically efficient way for hens to deal with increased output. There is some contradictory evidence as to whether this has occurred (Silversides and Budgell, 2004, Tharrington *et al.*, 1999). It is likely that constraints imposed on the health of hatched chicks will impose lower limits on these factors anyway. Currently quality is principally estimated using albumin height or Haugh units which have relatively high heritabilities between 0.2 and 0.5 when measured recently (Zhang *et al.*, 2005, Ledur *et al.*, 2002, Chen *et al.*, 1993). However, protein content would seem to be of more practical use in the processing industry and Haugh units or albumin height have a relatively poor genetic correlation with protein content (Hammershoj *et al.*, 2001). Haugh units or albumin height seems to pertain more to the appearance of a fresh egg than as a reference to its nutritional composition. A method to estimate protein content remotely and non-destructively would be a big step in improving not only the appearance of fresh eggs but their nutritional and processing qualities. So far attempts to do this using near-infrared transmission spectroscopy and low-resolution proton nuclear magnetic resonance have been relatively disappointing (Kemps *et al.*, 2007). It would appear that although there are many proteins in egg white (Mann, 2007) those making the major contribution are not large and that aspects of quality such as gelling ability might be well suited

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to a candidate gene approach since studies have identified ovotransferrin as a good starting point (Hammershoj *et al*., 2001). A combination of candidate gene and QTL approach centred on a loci on chromosome 2 did not confirm vimentin as the loci responsible for egg white thickness (Honkatukia *et al*., 2005b). Other efforts to locate genetic loci for egg white properties have met some success (Abasht *et al*., 2009, Hansen *et al*., 2005).

Another trait related to aesthetics is meat and blood spots which clearly are under selectable genetic control (Lerner *et al*., 1951). However estimates of heritability are low (0-0.18) especially in brown egg laying hens (Noda *et al*., 2007) due to the similarity of the pigments in the shell and blood and meat spots. Despite this measurement of the size of spots in relatively small samples of eggs from individual hens met with some success in reducing the overall incidence and the larger spots (Noda *et al*., 2007). Because of the problem in brown egg layers searches for genetic markers or loci have been undertaken and a QTL was located for the trait on the Z chromosome and a putative candidate gene ZO-2 suggested (Honkatukia *et al*., 2011).

An aspect of the egg white which is not likely to be in the mind of the consumer when buying eggs, but which has important implications for reducing risk to consumers or to reducing transmission of microorganisms between the generations, is the antimicrobial potential of egg white. Against salmonella the trait has modest but useful heritability (Sellier *et al*., 2007) and this has proven to be reproducible in subsequent generations 0.27 (Nys *et al*., 2010).

Yolk

The vitelline membrane is important to the egg breakout industry because of the requirement for clean separation of yolk and white. To date however there has been no method which seems to be sufficiently reliable or simple to have been adopted by breeders. Strength measurement using hydrostatic pressure (Moran, 1936) or rupture strength (Kirunda and McKee, 2000, Berardinelli *et al*., 2008, Fromm and Matrone, 1962) have been tried and the former looks potentially promising but is not amenable to scaling up for multiple measurements. Achieving a reliable and repeatable measurement for vitelline membrane strength would seem to be a useful research objective.

The sensory quality of egg yolk was the target

of a programme to eradicate a defect in a gene responsible for fishy taint. The gene was identified as a flavin-containing mono-oxygenase isoform, FMO3 using a marker approach from a segregating F2 cross and by using knowledge of the trimethylamine metabolism pathway (Honkatukia *et al*., 2005a).

Conclusions and the future

The search for new measurements that more reliably reflect the traits which are the ultimate target of selection, whether it be reduction in bacterial contamination, resistance of the shell to damage or processing and nutritional qualities, will be a major focus of continued research. There should however be some thought given to new aspects of the effect of egg quality. One area which perhaps deserves more attention is the effect of egg quality on chick quality. Some of these maternal effects may be obvious in terms of the supply of nutrients but there are other ways that selection could be applied to alter the development of embryos such as the deposition of maternal hormones in the egg which has heritabilities of 0.4 (Okuliarova *et al*., 2011) and may affect behaviour and post hatch performance.

Whether for established or novel traits increased focus on trying to define the biology underlying the traits is critical as improved measurements will only come from understanding. Integrated approaches to egg quality are likely to deliver that understanding but it requires the joining of multiple disciplines; engineers, physical chemists, geneticists and physiologists.

After a large amount of effort has been put into genomic technologies and a number of false dawns we appear to be closer than ever to the reality of marker assisted selection in poultry which would truly allow measurements that are hard to carry out to be included in selection programmes by using genome wide selection (Meuwissen *et al*., 2001). One of its attractions to breeders is that it does not require crossing experiments to locate markers which explain variation in traits but it can utilise single generations and takes a relatively black box approach to produce breeding values based on many markers. The approach requires at least in part very high density SNP panels of informative markers and efforts are underway to supply these for commercial populations (Solberg *et al*., 2008). Of course as we have already emphasised the accurate measurement of the trait is as important for this approach as it is for standard selection and this is to be reflected in good heritability values for

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the trait (Luan *et al*., 2009, Hassen *et al*., 2009). Modelling has estimated that a marker about every 0.2Mb would give acceptable accuracy of selection for a highly heritable trait (Solberg *et al*., 2008). It is expected that the next generation of genotyping chips will deliver this.

Breeding values estimated in laying hens using genetic markers trained on a preceding generations phenotypic information rather than its own phenotype was shown to be effective but as expected the accuracy falls with successive generations and therefore will require revalidation of the marker breeding value relatively frequently depending on the traits heritability (Wolc *et al*., 2011).

Although these findings show that genome wide selection may not be as simple as initially sold, if it can increase genetic progress by reducing generation intervals and improving selection of males for using expensive to measure traits it is likely that we will see its implementation for layers (Preisinger, 2010, Albers, 2010). It is likely that the development of denser and cheaper SNP genotyping technologies will assist this but the improvement of measurements for the traits that are proven to improve egg quality will be even more important to make the most of this power.

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