USE OF MOLECULAR GENETICS IN POULTRY BREEDING

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INTRODUCTION
Poultry breeding prior to this decade was based mainly on what could be observed or measured at the phenotypic level such as egg number, body weight, and egg weight. Unfortunately those types of traits are also influenced by random environmental factors such as feed quality, peck order, temperature, and disease. Clearly random environmental factors are a hindrance to breeding superior genetic stock. However, even greater problems are sex limited traits which can only be measured in one sex, such as egg production, and traits which cannot be measured on either sex, such as disease resistance or meat quality. In those cases the breeder must rely entirely on information from relatives to make selection decisions. The desire of poultry breeders has always been to get directly at the underlying genetic worth of the bird, free from environmental effects, and on all animals regardless of sex or ability to measure the phenotype. Now biochemical techniques allow scientists to probe directly into the genetic code of life. These advances would seem to provide the answer to selecting superior animals without complications of environmental effects, but as Bulfield (1998) questions, will these advances cause animal breeding to become a biotechnology or will it just be a passing fad? The question most breeders are asking is how important are these advances to breeding and at what cost. In the following I will examine potential utility, implementation, and limitations of MAS in poultry breeding.

POTENTIAL UTILITY
The “Quest” of all animal breeding is to maximize long and short term response for all traits of economic importance: Long and short term response to selection depends on four factors: 1) Selection intensity, 2) Accuracy of selection, 3) Genetic variation, and 4) Effective population size. In what follows I will examine how molecular genetics can aid in each of these factors.

Selection intensity. For some traits, costs limit the number of animals that can be tested, such as egg production in the dam line of broilers. Molecular genetic in combination with multi-stage selection can increase the selection differential in such cases (Xu and Muir, 1992; Muir and Xu, 1992). If genetic markers linked to important quantitative trait loci (QTL) have been identified, then it is possible to do a first stage selection at a young age on a large population using MAS, then only test those in the later stage which pass the first culling.

Another example where multistage selection will be advantageous is lost selection intensity on males in poultry layer programs. Due to cost constraints, usually only one or two roosters are kept from each full sib family and those are chosen at random. By use of molecular genetics it would be possible to choose among those full-sib brothers in the first stage, then wait for the full record of sisters in the second stage for final selection. In both cases this added selection differential would be lost without molecular genetics and represents lost opportunity (Muir, 1997b; 1999).
Putative QTL’s have been identified in chickens for feed consumption and body weight at 48 days (Van Kaam et al., 1999), egg quality (Tuiskula-Haavisto et al., 1998), and egg production (Lamont et al., 1996). Szydłowski and Szwaczkowski (1998) used advanced statistical procedures to identify QTL’s associated with body weight, initial egg production, average egg weight and age at sexual maturity. Generally, the results showed mixed model inheritance of the traits studied indicating a high probability QTL’s with large effects for all traits studied.

**Accuracy of selection.** Accuracy is determined by two factors: 1) the heritability of the trait and 2) the amount of information available from the individual and relatives.

Combining information from relatives to maximize accuracy is the primary advantage of Best Linear Unbiased Prediction (BLUP) as a tool from quantitative genetics to improve response to selection. There are two ways that molecular genetics can aid in BLUP selection. The first is accuracy of the pedigree information. Fairfull et al. (1998) concluded that DNA-based technologies like DNA fingerprints could be powerful tools for preventing or correcting pedigree errors. The second way is to increasing the heritability of a trait. An increase in heritability results because molecular genetics allows direct assessment of the genotype without interference from the environment. As such, the molecular information can be combined with the phenotypic information to yield an index which has a higher heritability.

The use of molecular genetics for sex limited traits, or traits which cannot be measured on either sex, is perhaps one of the most compelling reasons to use molecular genetics (Lande and Thompson, 1990). For traits which cannot be measured directly in either sex, such as disease resistance, quantitative genetic techniques would require a sib or progeny test which would be costly and or increase the generation interval. Gavora (1998) concludes that disease resistance is particularly well suited for the application of marker assisted selection because most of the QTL identified are dominant for Marek’s disease (MD) resistance and should facilitate their use in practical breeding programs. Great strides toward establishing QTL’s for MD resistance has been made (Cheng, 2002).

Improvement of animal well-being could greatly benefit from molecular genetics. Animal welfare issues are becoming a major concern that breeders will need to addressed (Arthur and Albers, 2002; Kjaer and Mench, 2002; Muir and Craig, 1998; Preisinger, 1998). Selection to improve animal well-being is difficult and requires either direct measurement for traits related to well-being (Faure et al., 2002) or indirect measurement using group selection (Muir and Craig, 1998). However, direct selection on either behavior or physiological objectives should be viewed with caution, the intended results may not be as expected (Muir and Craig, 1998). For example, Webster and Hurnik (1991) showed that traits associated with non-aggression, such as sitting and resting, were negatively correlated with productivity. Furthermore, the link between behavior and stress can be misinterpreted. For example, Duncan and Filshie (1979) showed that a flighty strain of birds which exhibited avoidance and panic behavior following stimulation returned to a normal heart beat sooner than a line of more docile birds, implying that docile birds may be too frightened to move. Therefore, is flightiness good or bad for well-being? Markers linked to QTL’s which improve well being while at least not compromising productivity would allow genotypic selection of hard to measure phenotypes.
Group selection has been very effective in eliminating cannibalism in non beak trimmed birds (Muir and Craig, 1998), however group selection requires that families be housed together and selected as a group. As a result, the rate of inbreeding would increase rapidly, which would be unacceptable for commercial breeding. Further, because birds in breeding programs are housed in single bird cages to obtain individual egg production records, aggression cannot be measured in that environment. Thus measurement of animal well-being in group cages would conflict with current selection programs, such as BLUP, which requires individual records. As such, alternatives such as molecular genetics might offer a better solution if markers associated with well-being can be found. Because only females are housed and some traits are not measurable in males, i.e. vent picking, group selection is primarily limited to females. Males are chosen based on their sisters performance. Again, within family selection for behavior in males represents a lost opportunity that molecular genetics can address.

Arthur and Albers (2002) conclude that Research is urgently needed to better understand the biological basis of the consequences of the unbalancedness of the modern broiler compared to its wild ancestor. Understanding this biological basis should direct researchers and breeders to design selection approaches aimed at preventing this unbalancedness from progressing further. Genomics could well play a key role in this, both in unraveling the biological mechanisms and in supporting the breeders in selection programs.

**Genetic variation.** Initial response to selection for any trait is dependent on polymorphic loci which influence the trait. Quantitative and molecular genetics is limited to changing frequencies of existing alleles (except for transgenics). A beneficial use of molecular genetics is to search for alleles in wild ancestors of domesticated species which have been lost during the selection process. In every instance where this technique was used, new alleles that outperformed the elite parents by as much as 20% were found ( Tanksley, 1997).

While it would be possible to cross unimproved populations segregating for desirable alleles with elite lines and start selecting from the new synthetic line, the frequency of undesirable alleles would also be dramatically increased and require long term selection to restore the population to high productivity. Introgression has traditionally been used when genes must be quickly and economically introduced into poultry populations. However, undesirable genes in the donor genome must be excluded as far as possible. DNA-based markers can enhance the efficiency of introgression (Groen and Timmermans, 1992; Hospital et al., 1992). Ideal introgression would employ equally spaced markers in the host genome and tightly linked flanking markers for the donor gene. The gene of interest could then be introgressed with the highest recovery of the host genome Fairfull et al. (1998). An example of successful application of this technique is the naked neck gene desirable for production in hot environments (Yancovich et al., 1996).

Preserving genetic variation is essential for finding genes which may have value in the future to combat a new disease or to address a new selection objective, or simply to recover alleles lost during the selection process. Molecular genetic tools can help maintain this diversity (see Delany, 2002 for review)

**Effective Population Size.** Optimal long term response to selection is achieved by minimizing loss of favorable alleles, which occurs as a result of random genetic drift and associated
inbreeding depression, while maximizing frequency of desirable alleles (Robertson, 1960; 1961). On the one hand selection increases frequency of favorable alleles and opposes loss of alleles through drift. On the other hand, increasing selection intensity also reduces the effective population size which increases rate of loss of favorable alleles. Similarly, for a given selection intensity, selection programs which increase the accuracy of selection, such as Best Linear Unbiased Prediction (BLUP), reduces the effective population size because relatives tended to be selected. Thus, selection program which optimize short vs. long term response are usually not the same (Muir, 1997a; 2000; Quinton, 2002). However, molecular tools can help in this regard too. Van Arendonk et al (1998) and Van der Beek (1996) suggested using mixed semen and determining the sire from parentage testing based on information from genetic markers. As a result, a factorial mating design can be implemented which leads to a higher selection response without increasing the rate of inbreeding.

IMPLEMENTATION
Markers fall into two categories: 1) direct, those in causal mutations (QTLs), and 2) indirect, either anonymous polymorphisms, usually in non-functional genes, or in functional genes, closely linked to the causal mutation, sometimes called candidate genes (Dekkers and Hospital, 2002). Use of linked markers to improve traits through marker assisted selection (MAS) requires linkage disequilibrium (LD), either within family or at the population level. If the population has been selected for many generations without crossing, most loci will be in equilibrium at the population level unless they are very closely linked (Visscher and Haley, 1998). However, LD always exists within families, even between loosely linked loci. Hence, most animal breeding applications will have to utilize LD within families. A mixed model approach which utilizes within family disequilibrium was developed by Fernando and Grossman (1989). Meuwissen and Goddard (1996) generalized the procedure to take advantage of flanking markers. Although all LD will eventually degrade or breakdown, with this method and the others that followed, with regard to the use of within-family LD, two things are happening: the LD established within a given family in a given generation, e.g. among half-sib progeny of an heterozygous sire, will erode over generations but each generation, new LD will be established within the newly generated families. Because the Fernando and Grossman (1989) model utilizes the within-family LD jointly from all families, as it arises; although LD established within a family in a given generation will erode over generations, the joint effect will not. Thus, the efficiency of this method will not erode over generations (Dekkers, J. Personal Communication). In fact Fernando (2002) concludes that as data accumulate over the generations, the marked QTL effects will be evaluated more accurately.

Several computer based simulations have been completed comparing MAS with phenotypic selection for populations in linkage equilibrium utilizing within family LD. Meuwissen and Goddard (1996) showed that if the marker QTL explain 33 % of the genetic variance, and selection is before or after recording the trait MAS increased genetic gain by 9 % to 38 % and for sex limited traits 38 % and for carcass traits 64 %.

LIMITATIONS
Biological. The chicken has some unique aspects that limits the application of MAS. Arthur and Albers (2002) conclude “As the economic value of individual chickens is relatively low, DNA based genotyping of individual breeding candidates must be done at low cost per bird.
Therefore commercial application of genotyping at the DNA level will largely be through
direct genotyping for critical genes and not through Marker Assisted Selection approaches *per se* that are being designed for larger species.”

Several problems have been observed with implementation of MAS, some of these are most
likely due to other QTL that were lost, genotype-environment interactions, negative epistasis
between QTL or epistasis between QTL and the genetic background. Also, QTLs that are
detected by crossing divergent lines identify QTL that differ between breeds, but have limited
direct application for within breed improvement (Dekkers and Hospital, 2002).

**Statistical.** There remain several statistical issues for MAS: The effect of the QTL must be
established empirically on the basis of statistical associations between markers and phenotype,
and hence suffer from the same limitations as quantitative genetic selection. Thus, although
combined selection is most effective with traits of low heritability or traits difficult to measure
(Lande and Thompson, 1990), the ability to detect QTL also requires phenotypic data and is
similarly limited in such cases. Thus, the “greatest opportunities for MAS might exist for traits
with moderate rather than low heritability” (Dekkers and Hospital, 2002).

Another statistical issue that remains is determining which of the many QTL-marker associates
to include in the marker score. Both false positives and false negatives in QTL selection are an
issue, but false negatives have a greater impact on efficiency of MAS (Moreau *et al.*, 1998 ;
(2001) were able to obtain a molecular score with high predictive ability based on high-density
marker genotyping data by using all estimated marker effects, regardless of their statistical
significance.

In experiment with low power, QTL effects are overestimated (Georges *et al.*, 1995). Incorrect
estimation of QTL effect can lead to the wrong weights used in selection programs (Spelman
and Van Arendonk, 1997) with corresponding reduced genetic gain.

**Theoretical.** Optimizing long term selection response is much more difficult than short term.
Existing quantitative genetic theory is only adequate for strategies which maximize short term
response to selection and is much less developed for long term response. Selection on an index
of molecular score and phenotype will only optimize short term selection but for the long term,
phenotypic selection alone is superior because it better distributes pressure over all loci
(Dekkers and Hospital, 2002).

**Costs.** The major detriment to molecular genetics at this time is expense and speed of analysis.
However, as the technology progresses, costs can decrease by orders of magnitude in a few
years. Colleau (1998) found that if typing costs were included into the comparison with MAS,
a decrease in the overall superiority of MAS resulted and even became negative when the
heritability was large. Thus Visscher and Haley (1998) conclude that it may only be feasible to
use microsatellite markers to select on limited areas of the genome (e.g. to introgress a QTL
from one breed to another) but using many such markers at the same time in a breeding
program may be impossible until costs are reduced.

Dekkers and Hospital (2002) conclude that MAS will be most cost effective when molecular
costs are less than that of phenotypic observations, such as genotype building and population
wide LD, or the ability to select early. But with combined selection, costs are a greater issue because molecular information is in addition to, rather than in place of, phenotypic information, in which case the cost:benefit of MAS may not be more effective than simple phenotypic selection.

**Diversion of Resources.** Use of MAS, either for introgression or recurrent selection programs, diverts some selection pressure away from traits of economics importance. Thus in introgression the benefit of the target gene must be greater than that which could be achieved by regular selection over the same time period (Dekkers and Hospital, 2002). In recurrent selection programs, monetary resources devoted to MAS could also be allocated to phenotypic selection programs. Alternatively, conventional selection programs can also be enhanced by increasing the number of individuals tested and/or the effective population size, this would allow either the selection intensity to increase or the rate of inbreeding to decrease thus increasing both short and long term responses (Muir, 1997a; 2000; Quinton, 2002).

**FUTURE DIRECTIONS.**
With recurrent animal breeding programs, based primarily on within line or breed selection, the primary limitations are 1) the inability to utilize population wide LD and 2) the ability to utilize the power of molecular genetics at the cellular level.

**Population wide LD.** Utilization of population wide LD would allow less expensive MAS programs with potentially greater relative efficiency than that possible with within family LD. Increasing flanking marker density to 1-2 cM would uncover substantial population wide LD (Dekkers and Hospital, 2002; Smith and Smith, 1993).

**Utilizing the power of molecular genetics.** The real power of molecular genetics is the ability to sort genotypes by some criteria at any age post fertilization. More specifically, the process of animal breeding has been to increase the frequency of favorable alleles into one genotype. The problem is these alleles are scattered in the population and possibly linked to undesirable loci. Even assuming we knew the locations and functions of all the genes in the genome of an animal, and assuming these all acted additively, there would still be a formidable task of trying to get all the best alleles into one genotype and would require several generations of selection on the genotype to bring about the desired combinations.

To address this problem, Visscher and Haley (1998) and Meuwissen et al. (2001) suggested breeding strategies to select on molecular score at an early age. For example, with selection only on molecular score the only limitation is the reproductive cycle. Some technologies may be able to break this limitation by recovery of oocytes before puberty or even unborn fetus. This is then combined with in vitro fertilization and embryo transfer. Further reductions in generation interval are possible if meiosis could be conducted in vitro. Such advances would allow several generations of selection to occur in the laboratory in what has been termed velogenetics (Georges and Massey, 1991). However, as with most new technologies, there is a downside. With each meiotic event and union of gametes (or whatever process is used to recombine the haploid cells), random loss of alleles will occur and inbreeding will increase. This loss of alleles will primarily affect traits not under selection but will also impact to some degree those under selection because not all QTL’s for a given trait will be marked. Thus 4 or 5 quick cycles of velogenetics could increase short term response but limit long term response.
and result in other fitness problems associated with inbreeding depression. This problem can only be addressed by utilizing a large effective population size of selected cells or embryos. A large effective population size of selected cells would require an even larger number of cells to be genotyped so as to allow selection among them.

There are clear advantages for such technologies for species which have longer reproductive cycles, such as cattle, but for chickens, which reach sexual maturity at 18 weeks, the advantages may not offset the added costs. Arthur and Albers (2002) conclude that the ability to reproduce at an earlier age would support the increase of genetic progress by a reduction of the generation interval. But, the technical problems associated with this, the acceptability of the technologies to be used, and the relatively small impact of this make such a new development unlikely in poultry. Nevertheless, 5 or 10 generations of genotypic selection within possibly a few weeks would give such a company a definite competitive advantage.

CONCLUSION

Dekkers and Hospital (2002) concluded “as the theoretical and experimental results of QTL detection have accumulated, the initial enthusiasm for the potential genetic gains allowed by molecular genetics has been tempered by evidence for limits to the precision of the estimates of QTL effects. The present mood is one of cautious optimism”. Dodgson (2002) offers an alternative view: he asks “The real question is whether continued progress in quantitative genetic theory will be made. Certainly computer technology will make it possible to collect and process more data and to employ more sophisticated models, but how far can one go with a technology based on NOT KNOWING what the genes/alleles are actually doing? Meanwhile, molecular genetics (genomics) technology continues to move forward at breathtaking speed.”

REFERENCES


