

POULTRY BREEDING: PROGRESS AND PROSPECTS FOR GENETIC IMPROVEMENT OF EGG AND MEAT PRODUCTION

RW. Fairfull¹, L M^cMillan², and W.M. Muir³

¹Argo Breeding Co., Nacogdoches, TX 75963-1940, U.S.A.

²Department of Animal & Poultry Science, University of Guelph, Guelph, Ont. N1G 2W1, Canada

³Department of Animal Science, Purdue University, West Lafayette, IN 47907, U.S.A.

SUMMARY

Estimates of genetic gains and present performance of layers and broilers were reviewed. Current and developing technologies pertinent to the breeding of layers and broilers were discussed with some of their assumptions and limitations. Under present methods, contemporary theory and selection technologies are critiqued. Under developing technologies, selection for adaptation to production environments, multiple stage selection, and molecular techniques for poultry improvement are discussed.

Keywords: Chickens, broilers, layers, genetic improvement, molecular markers, selection.

INTRODUCTION

Webster's New **World** Dictionary defines proof as "anything serving or tending to establish the truth of something, or to convince one of its truth; conclusive evidence" (1968, Simon & Schuster, N.Y.). Presently, animal breeding **has an aura of unreality relying too heavily on** theoretical expectations without empirical verification. In a recent talk, **Lewontin** (personal communication) strongly criticized the tendency of population geneticists to devote too much interest to the development of statistical methods, and not enough on obtaining experimental **results** that could validate and expand the biological model. Animal breeders tend to accept models with unrealistic assumptions. Assumptions need to be validated and viewed with skepticism.

WHERE ARE WE?

Broilers. The performance of the modern broilers is vastly **different** from the broiler of 20 to 30 years ago (Havenstein et al. 1994a,b). The modern broiler grows more than three times faster than the vintage broiler (*circa* 1960) with greater meat yield and better feed conversion reaching market weight on less than ½ the feed required by the vintage broiler. Even when grown to a fixed age, the modern broiler has better feed conversion (30% better at 42 d) despite reaching a greater size (about 2130 vs 510 g at 42 d) than the vintage broiler (Havenstein et al. 1994a). The greater part of these changes have resulted from genetic improvement: 85.3% for growth rate, 9.1.3% for carcass yield and 62.5% for feed conversion; with the remainder being due to diet (Havenstein et al. 1994a,b).

Not all of the changes in broilers have been positive. The incidence of leg and other skeletal problems has increased. Mortality due to physiological changes has increased and is excessive under many circumstances such as at altitude, in high heat and humidity and under reduced air flow or any other extreme stress (Julian 1993). Reproductive fitness has been negatively affected by selection for increased body weight (Siegel & Dunnington 1985) although onset and severity vary and can be partially counteracted by selection. Reproductive

problems exacerbated by excess fatness are controlled in modern broiler parents by feed restriction programs. However, increased fat in modern broilers is exaggerated as vintage broilers and modern broilers of the same weight have a similar percent fat.

Layers. The modern layer considerably outperforms vintage layers (Anderson 1996). Egg numbers have increased from less than 270 to over 340 eggs between 1950 and 1993 (a 28.8% increase). This represents an average increase of about 1.8 eggs per year. Simultaneously, egg mass has been improved by **42.7%**, egg weight by 11.7% and feed efficiency by **32.4%**. Eggshell strength was unchanged and albumen quality has improved slightly. Fertility and hatchability have remained high or improved slightly. Since selection in layers is largely for fitness traits, most of the problems plaguing broilers have been avoided. Due to the nature of important traits in layers, change has been slower than in broilers.

Quantitative genetic theory in animal breeding, *The infinitesimal model.* Presently, one of the major approaches in quantitative genetic theory is the Infinitesimal Model. Some of the many Infinitesimal Model assumptions are: 1) an infinite number of loci; 2) unlinked loci; 3) only additive effects; base population in 4) Hardy-Weinberg equilibrium and 5) linkage equilibrium; 6) genetic and 7) environmental effects normally, independently distributed; 8) constant genetic and 9) phenotypic (co)variances; 10) genetic and phenotypic (co)variances of the base population are known without error; 11) selection is on a linear function of the records; 12) the relationship matrix is complete (no missing or incorrect records); 13) multivariate normality even after repeated selection, 14) genetic parameters are symmetrical; 15) no genotype-environment interactions; and 16) the underlying genetic model is known.

Most Infinitesimal Model assumptions are known to be false with regard to the poultry genome. The number of loci in the poultry genome are finite. The assumption of normality may not be reasonable, especially after repeated selection. Directional dominance and linkage accentuate the problem. Economic traits in poultry have considerable genetic nonadditivity (Fairfull et al. 1987). Fitness characters have low, asymmetrical heritabilities (Frankham 1990) and asymmetrical correlations exist for several traits (Siegel et al. 1985). Low frequency genes with large negative effects have been observed for some fitness traits (Gowe et al. 1993) and many examples of major genes affecting economic traits exist (Mérat 1990; Haley et al. 1995). Simulation (McMillan et al. 1995) and experimentation (Gowe & Fairfull 1985) have shown that selection changes genetic parameters. Several studies reported substantial changes in genetic correlations (r_g) with selection (Fairfull & Gowe 1990). As so many Infinitesimal Model assumptions are incorrect, its value as a model for the assessment of genetic improvement is questionable.

Selection techniques. Several multiple trait, multiple source selection techniques exist: index selection (**IS**) or Best Linear Unbiased Prediction (**BLUP**); and several culling methods. Current theory heralds BLUP as the best selection method Fixed effects aside, as a generalization of selection index theory to unequal information on relatives (Lin 1978), BLUP embodies many aspects of the Infinitesimal Model. Thus, the efficiency of BLUP is suspect, especially in some cases.

In experimental comparisons with chickens, family and index selection was less effective while individual selection was more effective than expected (Kinney et al. 1970; Garwood &

Lowe 1979; Garwood *et al.* 1980). In more recent experiments with *Drosophila*, Perez and Toro (1992) compared three family selection indices for progeny number: dam record only, dam plus dam full-sibs, and dam plus dam and sire full sibs. Response was the reverse of that expected. The efficiency of family information may be affected by: weights placed on sources of information; inbreeding and the loss of allelic variation; incorrect parameter estimation; the use of theoretical genetic relationships; and assumptions of additivity.

The presence of nonadditive genetic effects (Johannsson *et al.* 1995) and common environmental (Harris *et al.* 1984) effects, such as congenital diseases like the lymphoid leukosis virus, may bias estimates of breeding values based on information from relatives. While adding **information** from relatives may potentially increase selection accuracy chiefly in traits with low h^2 , optimal weights of sources of information may deviate from theory. BLUP tends toward the selection of families resulting in an elevated rate of inbreeding. The loss of favorable alleles as a consequence of inbreeding may be more serious **by** lowering the limits of selection and the rate of genetic progress. Hypothetically, BLUP may optimize short-term response if the genetic model approximates that assumed, but even so, long-term response may be compromised. If the rate of inbreeding is restricted to the same level as that of phenotypic selection, then even theoretically BLUP has little advantage (Quinton & Smith 1995). Several investigators have examined the effect of parameter estimation errors on selection accuracy, concluding that errors in parameter estimation, notably in estimates of genetic correlations, could produce large biases in estimated selection criteria. In the use of IS and BLUP, additive genetic relationships are assumed to be known. In fact, they are theoretical estimates derived from the pedigree representing an expectation based on sampling parental genotypes. Even in direct descent where the assumed additive genetic relationship must hold, sampling of parental genotypes may be biased based on differential exclusion, **gametic** competition, or mortality. Also, the efficiency of IS and BLUP are greatest at constant allelic frequencies. As allelic frequencies change, IS and BLUP become increasingly less efficient. Since the only basis for genetic change is in fact genetic change (shifting allelic frequencies), the fiction of fixed allelic frequencies becomes foolish and the efficiency of IS and BLUP over generations of selection becomes increasingly questionable. BLUP and IS assume multivariate normality. In swine, Perez *et al.* (1994) reported selection results that deviated significantly from theoretical expectations due to the failure of the assumption of multivariate normality. For traits such as egg number which do not show multi-variate normality, IS may fail to perform as **predicted**.

Culling techniques such as multiple trait culling levels and index updating (Muir & Xu 1992) can be effective alternatives to BLUP and SI under some circumstances (Fairfull *et al.* unpublished). Where the genetic architecture of traits does not conform to BLUP and IS assumptions, culling might be more efficient than BLUP as might a base index ignoring genetic covariances. There might be a number of alternatives to BLUP, but we do not have any empirical evidence. Fitness traits which often have asymmetrical **heritabilities** (Frankham 1990) are good examples. In chickens, fertility and hatchability can easily be decreased and in some progenies decreases on the order of 30-40 % are not unusual: Corresponding increases cannot be found. Further, fertility and hatchability are at very high levels in most improved lines, such that they can be viewed as approaching a limit. Both fertility and hatchability can be maintained at high levels using a culling scheme that has little effect on the selection pressure available for other traits (Gowe *et al.* 1993). In chickens, most

mortality is due to disease. Each cause (pathogen and virulence) can be viewed as a separate trait. No effective method of selecting for general disease resistance exists (Gavora 1990). Selection for immune responses has been successful without corresponding improvements in disease resistance (Kean et al. 1994). Selection on general mortality in normal populations is selection primarily on exposure, which is futile. Although this is well known, many theorists still treat mortality as a single trait applying methods that will fail and even “prove”, using index in retrospect techniques, that genetic improvements have been made although challenge tests would prove to the contrary.

Treating traits or groups of traits differently makes good sense biologically. For characters with means approaching limits, negative major gene effects inherited in a Mendelian fashion and asymmetrical heritabilities, BLUP or IS may have little logic or value. Also, for compound traits like mortality, where each cause of mortality is a different trait, usually with very low incidence, and is highly dependent on exposure and virulence, intense BLUP selection will have little beneficial effect, wasting selection pressure that could be used elsewhere. Empirical evidence and accumulated knowledge of the genome support the current practice of using different methods for different traits as the biological nature of each dictates.

WHERE ARE WE GOING?

There are a number of techniques that are of potential value to future poultry improvement: 1) increasingly vocal animal welfare (and animal rights) concerns in addition to economic considerations may dictate that poultry be selected for adaptation to production environments; 2) animal breeding is becoming increasingly costly and multiple stage selection may allow more cost effective breeding programs and make better use of facilities; 3) molecular genetic techniques show promise for revolutionary changes in animal improvement techniques.

Adaptation to production environments. *The example of group selection.* The housing of laying hens in group battery cages is seen by many to reduce well-being and commercial performance. Muir and Craig (1996) reviewed international animal welfare issues, particularly with respect to beak trimming and battery housing of layers which are viewed as causing stress for extended periods of time. Such practices have been severely restricted in Europe. Muir and Craig (1996) suggest modifying the behavior of the bird so that beak trimming to prevent cannibalism is unnecessary and the cage environment is no longer stressful to the bird. Other types of housing designed to replace battery cages can also be very stressful to birds.

Muir (1996) tested group selection as a method to produce cooperative genotypes adapted to battery cages as opposed to the selfish, competitive genotypes maladapted to battery cages produced by conventional selection. Egg production and hen days increased while mortality decreased sharply. In a direct comparison with a commercial and genetic control line in multiple hens cages, the group selected line had much higher egg production, much lower mortality and much better feather scores than the commercial and control lines (Muir & Liggett 1995; Craig & Muir 1996). This clearly demonstrates that selection for adaptation can improve welfare as well as commercial performance.

Multiple stage selection. When traits are expressed for selection at different ages or there are large differences in costs of measuring traits, selection by independent culling levels may give

a higher aggregate economic return than IS or BLUP because not all traits need be measured on all individuals. However, general solutions for optimum independent culling levels are not possible and specific solutions require numerical integration. Also, independent culling for each trait ignores superiority in other traits. Xu and Muir (1991, 1992) developed a new independent culling levels procedure using orthogonal transformation of the original traits. This procedure furnishes solutions for optimum truncation points and has similar properties to multiple stage selection index so that resulting genetic gains may exceed conventional independent culling levels selection. For most situations, a multiple stage selection scheme, either conventional or transformed culling, can have an efficiency close to that of index selection (Muir & Xu 1991). Further, their procedure can optimize aggregate economic return per unit of cost associated with obtaining measures on each trait.

Multiple stage selection is a common procedure used by all commercial broiler breeders. In dam lines, birds are first culled on weight with the superior pullets further tested for adult performance. Culling levels are commonly derived empirically over time through feedback of results and within generation by iteration to achieve specific goals rather than based on theory. Too intense early culling can erode genetic gain for later traits. Too feeble an intensity at early culling can incur significant additional costs. Industry culling procedures may be close to optimum, but this new theory may make additional gains or profits possible. Also, profitability of alternative breeding schemes can be assessed using index updating (Muir & Xu 1992).

Molecular markers (or genes) in poultry breeding. Markers have many important and useful applications in poultry improvement. Some of the many applications will be briefly discussed: Establishing genetic relationships; Predicting heterosis; Genomic selection; and Marker Assisted Selection (MAS). A more comprehensive discussion of some of these areas can be found in Muir (1994; 1996), Fairfull (1996), and Fairfull and Muir (1996, 1997).

Establishing genetic relationships. DNA-based technologies like DNA fingerprints (DFP) are powerful tools for identification and pedigree determination. Identification applications have many mundane but useful functions: preventing or correcting pedigree errors; recovering pedigrees; retrospective genetic analysis. In the study of inbreeding, genetic drift or mutation, DNA-based methods provide an unparalleled tool to follow changes at the DNA level.

Predicting Heterosis. Heterosis is substantial for most commercial traits in chickens (Fairfull 1990). Almost all commercial male and female parent lines and virtually all commercial broilers or layers are crosses. There could be a substantial advantage to predicting the heterosis expected from crosses at all levels. Preliminary results in laying hens show promise for the prediction of heterosis using DNA fingerprint information (Gavora et al. 1996).

Genomic Selection. Occasionally, genes must quickly and economically be introduced into poultry populations (introgression). Undesirable genes in the donor genome must be excluded as far as possible. Theoretically, DNA-based markers can enhance the efficiency of introgression (Groen & 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**. However, suitable markers must be found and unless they can be located in a database with suitable cloned DNA, this may be no trivial problem.

Marker assisted selection (MAS). Use of MAS in any form requires linkage disequilibrium, either at the family or population level (Muir 1994). In the case of a randomly mating population, **different** individuals will tend to be in equilibrium with QTL alleles segregating in proportion to the relative frequencies of the alleles. Alternative marker genotypes will include both positive and negative alleles at any linked QTL, and the mean **quantitative** value of the alternate marker genotypes will not **differ** even when a linked segregating QTL is present in the population. Therefore, specific linkage arrangements must be determined for each individual by progeny testing numerous offspring (Beckmann and Soller 1986).

Theoretically, generalized mixed model (**BLUP**) approaches for incorporating markers into breeding programs (e.g. Goddard 1992) reduce the erosion of marker information from one generation to the next and maximizes expected response of MAS, but require abundant QTL's with large effects to be effective assuming all alleles are traceable. However, finding loci with large effects in a population which has undergone long term selection would be unexpected unless the gene has a negative pleiotropic effect on fitness (Lin et al. 1992). Using a Monte Carlo simulation program, Zhang and Smith (1993) simulated the effectiveness of **MAS**. Selection on the phenotype always gave greater response than selection on markers only. As expected, combined selection gave greater response than selection on the phenotype or marker. However, the improvement was considerably below the 100% to 200% improvement predicted by Lande and Thompson (1990). However, MAS assumes an additive model. In wide crosses of tomatoes, De vicente and Tanksley (1993) found at least one QTL had an effect opposite of that expected due to overdominance. In inbred line crosses, Edwards et al. (1987) found that overdominance occurred frequently for yield traits. Evaluating expression of QTL's in **different** dams, Dunnington *et al.* (1993) concluded that associations between DFP band patterns and quantitative traits may not be consistent in different genetic backgrounds. **Fairfull et al.** (1987) found that epistatic effects (**AxA**, **AxD**, and **DxD**) were significant for egg production traits. All of this suggests problems in the application of MAS.

In an economic study of MAS, De Gatori and Muir (unpublished) using the **efficiencies** of Zhang and Smith (1993) and costs from **Beckmann** and **Soller** (1983) with an assumed 100 fold decrease due to technology advances by 1994, concluded that in most realistic animal breeding operations use of MAS will not be profitable. Zhang and Smith (1993) concluded that MAS will have limited value until close linkages or the QTL's themselves are identified. From numerous other theoretical evaluations of MAS (see **Fairfull** and Muir 1997) and the results discussed above, it can be generally concluded that MAS will only be advantageous for traits which cannot be measured on the individual and in species which allow for large family sizes. Thus, MAS appears to be of little importance in genetic improvement of poultry.

To date, **MAS** has not been used or tested on a wide scale. The only reported attempt to actually use MAS was that of Dunnington *et al.* (1992) who used within family MAS by tail analysis. Birds were **bi-directionally** selected based on two markers which had a significant effect on body weight (**BW**). **Offspring** of those matings were not significantly **different**. The authors attributed the lack of response on BW to false positives. Nevertheless, the authors examined the most favorable case for MAS, i.e. intermediate gene frequencies for the **QTLs**

by crossing bidirectionally selected populations, and it failed.

All theoretical studies of MAS assume an existing gene pool. MAS only changes frequencies of existing alleles (Tanksley 1997). A more beneficial use of this technology is to search for alleles in wild ancestors of domesticated species which have become lost. In every instance where this was used new alleles that outperformed the elite parents by as much as 20% were found. Thus, the real value of MAS in most species may be to look for lost alleles in distant ancestors (Muir 1994).

REFERENCES

- Anderson, K.E. (1996) In "Proc. 45th Ann. National Br. Roundtable", p. 123, St. Louis, Mo.
- Beckmann, J.S., and Soller, M. (1986). *Oxford Surv. Plant Molecul. Cell Biol.* 3: 196-251.
- Bulfield, G. (1992) In "Proc. 19th Wld Poul. Congr." 1:479-484.
- Craig, J.V., and Muir, W.M. (1996) *Poult. Sci.* 75:294-302.
- De vicente, M.C., and Tanksley, S.D. (1993) *Genetics* 134:585-596.
- Dunnington, E.A., Haberfeld, A., Stallard, L.C., Siegel, P. and Hillel, J. (1992) *Poult. Sci.* 71: 1251-1258.
- Dunnington, E.A., Stallard, L.C., Siegel, P.B., and Hillel, J. (1993) *Poultry Sci.* 72: 1000-1004.
- Edwards, M.D., Stuber, C.W., and Wendel, J.F. (1987) *Genetics* 116:113-125.
- Fairfull, R.W. (1990) In "Poultry Breeding and Genetics", p. 9-13, Editor R.D. Crawford, Elsevier Science Publishers, Amsterdam.
- Fairfull, R.W., 1996. In "Proc. XX* Beltsville Symp.", p. 289, Editors R.H. Miller, V.G. Pursel and H.D. Norman, Beltsville, Md.
- Fairfull, R. W. and Gowe, R.S. (1990) In "Poultry Breeding and Genetics", p. 705, Editor R.D. Crawford, Elsevier Science Publishers, Amsterdam.
- Fairfull, R. W., Gowe, R.S., and Nagai, J. (1987) *Can. J. Anim. Sci.* 67:663-680.
- Fairfull, R. W., and Muir, W.M. (1996) In "Proc. of the XX* Wld Poul. Cong.", 1:395-415.
- Fairfull, R.W., and Muir, W.M. (1997) In: "Proc. 34th Ann. Meet. Brazilian Soc. Anim. Sci.", (IN PRESS), Juiz de Fora, M.G.
- Frankham, R. (1990) *Genet. Res.* 56:35-42.
- Garwood, V.A., and Lowe, P.C. (1979) *Poult. Sci.* 58:751-753.
- Garwood, V.A., Lowe, P.C., and Bohren, B.B. (1980) *Theoret. Appl. Genet.* 56:5-9.
- Gavora, J.S. (1990) In "Poultry Breeding and Genetics", p. 805, Editor R.D. Crawford, Elsevier Science Publishers, Amsterdam.
- Gavora, J.S., Fairfull, R. W., Benkel, B.F., Cantwell, W., and Chambers, J. (1996) *Genetics* 144:777-784.
- Goddard, M.E. (1992) *Theor. Appl. Genet.* 83:878-886.
- Gowe, R.S., and Fairfull, R.W. (1985) In "Poultry Genetics and Breeding", p. 125, Editors W.G. Hill, J.H. Manson and D. Hewitt, Longman, British Poultry Science.
- Gowe, R. S., and Fairfull, R. W. (1994) In "Poultry Production in Hot Climates", p. 11, Editor N.J. Dagher, CAB International, Wallingford, Oxon.
- Gowe, R.S., Fairfull, R.W., McMillan, I., and Schmidt, G.S. (1993) *Poult. Sci.* 72:1433-1448.
- Groen, A.F., and Timmermans, M.M.J. (1992) In "Proc. 19th Wld Poul. Congr." 2:523-527.
- Haley, C., Knott, S., and Andersson, L. (1995) In "Proc. 44th Ann. Nat. Br. Roundt.", p. 1, St. Louis, Mo.

- Harris, D.L., Gavora, J.S., and Spencer, J.L. (1984) *Theoret. Appl. Genet.* **68**:397-413.
- Havenstein, G.B., Ferket, P.R., Scheideler, S.E., and Larson, B.T. (1994a) *Poult. Sci.* **73**: 1785-1794.
- Havenstein, G.B., Ferket, P.R., Scheideler, S.E., and Rives, D.V. (1994b) *Poult. Sci.* **73**: 1795-1804.
- Hospital, F., Chevalet, C., and Mulsant, M. (1992) *Genetics* **132**: 1199-1210.
- Johannsson, K., **Kennedy, B.**, and Wilhelmson, M. (1995) In "Proc. 5th WCGALP" 1&386-389.
- Julian, R.J. (1993) *Avian Path.* **22**: 419-454.
- Kean, R.P., Kahaner, A., Freeman, A.E., Lamont, S. J. (1994) *Poult. Sci.* **73**: 18-32.
- Kinney, T.B. Jr., **Bohren, B.B.**, Craig, J.V., and **Lowe, P.C.** (1970) *Poult. Sci.* **49**:1052-1064.
- Lande, R., and Thompson, R. (1990) *Genetics* **124**:743-756.
- Lin, C.Y. (1978) *Theor. Appl. Genet.* **52**: 49-56.
- Lin, C.Y., **Sabour, M.P.**, and **Lee, A.J.** (1992) *Anim. Breed. Abs.* **60**:1-10.
- M&eat, P. (1990) In "Poultry Breeding and Genetics", p. 429, Editor R.D. Crawford, Elsevier Science Publishers, Amsterdam.
- M^cMillan, I.**, **Fairfull, R. W.**, Friars, G.W., and **Quinton, M.** (1995) *Theor. Appl. Genet.* **91**:776-779.
- Muir, VVM, 1994. In "Proc. 5th Wld Cong. Genet. Appl. Livestock Prod.", **20**:5-12.
- Muir, W.M. (1996) *Poult. Sci.* **75**:447-458.
- Muir, WM, 1997. In: "Proc. 34th Ann. Meet. Brazilian Soc. Anim. Sci.", (IN PRESS), Juiz de Fora, M.G.
- Muir, W.M.**, and **Craig, J.V.** (1997) In "Proc. 1st N. Amer. Symp. Poult. Welfare", (IN PRESS), Edmonton, Alb.
- Muir, W.M. and Liggett, D. (1995) *Poult. Sci.* **74s1**:101.
- Muir, W.M., and **Xu, S.** (1991) *Theoret. Appl. Genet.* **82**:457-465.
- Muir, W.M. and **Xu, S.** (1992) In "Proc. of the 19th Wld Poultry Cong.", p. 707, Amsterdam.
- Perez, M., Foulley, J.L., Bodin, L., and Poivey, J.P. (1994) *J. Anim. Sci.* **72**:2775-2786.
- Perez, M., and Toro, M. (1992) *J. Anim. Sci.* **70**:2673-268 1.
- Quinton, M.** and **Smith, C.** (1995) *J. Anim. Sci.* **73**:2208-22 12.
- Siegel, P.B., and Dunnington, E.A. (1985) In "Poultry Genetics and Breeding", p. 59, Editors W.G. Hill, J.H. **Manson**, and D. Hewitt, **Longman**, Br. Poul. Sci.
- Tanksley, S. (1997) In "Proc. Plant Anim. Genome V", p18, San Diego, Ca.
- Xu, S.**, and **Muir, W.M.** (1992) *Theor. Appl. Genet.* **83**:451-458.
- Xu, S.**, and **Muir, W.M.** (1991) *Genetics* **129**:936-974.
- Zhang, W., and **Smith, C.** (1993). *Them Appl. Genet.* **86**:492-496.