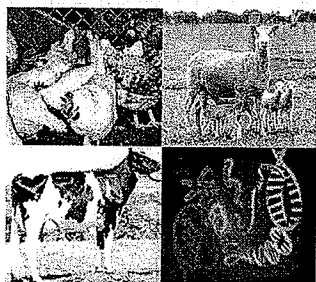# Use of molecular markers in breeding

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Use of molecular markers

Marker assisted selection / genotypic assisted selection

Marker assisted introgression

Parentage

Analysis of genetic diversity → new crossbreeds

Genomic selection

Other
  ❖ Tracing livestock domestication
  ❖ Traceability of animal products

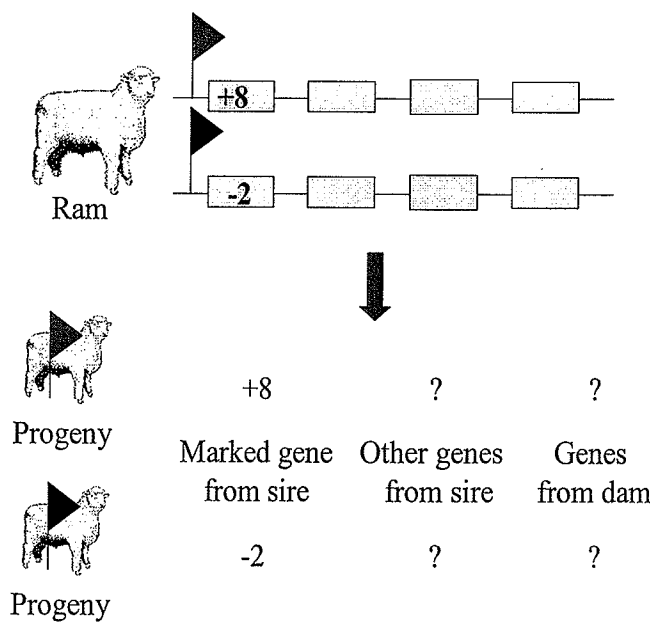Use of markers

MAS and GAS

Marker assisted selection (MAS)

❖ select on molecular marker(s) linked to the QTL of interest → indirect marker

❖ markers may be in linkage equilibrium (LE) with the QTL, or in linkage disequilibrium (LD) with the QTL
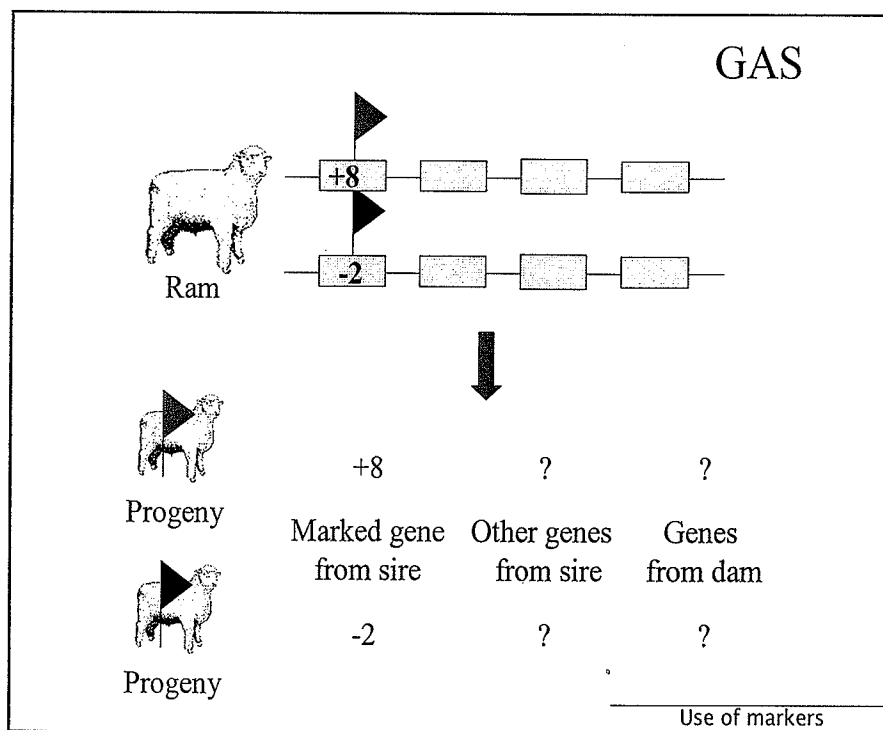
Genotypic assisted selection (GAS)

❖ select directly on the causative mutation(s) of interest →direct marker

Use of markers

MAS, markers in LE

Ram

+8

-2

Progeny

+8

Progeny

-2

Marked gene from sire	Other genes from sire	Genes from dam
+8	?	?
-2	?	?

Use of markers

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Although if recombination between the marker and gene occurs .....

Ram

+8

-2

Progeny

-2	?	?
Marked gene from sire	Other genes from sire	Genes from dam
+8	?	?

Progeny

Use of markers

GAS

Ram

+8

-2

Progeny

+8	?	?
Marked gene from sire	Other genes from sire	Genes from dam
-2	?	?

Progeny

Use of markers
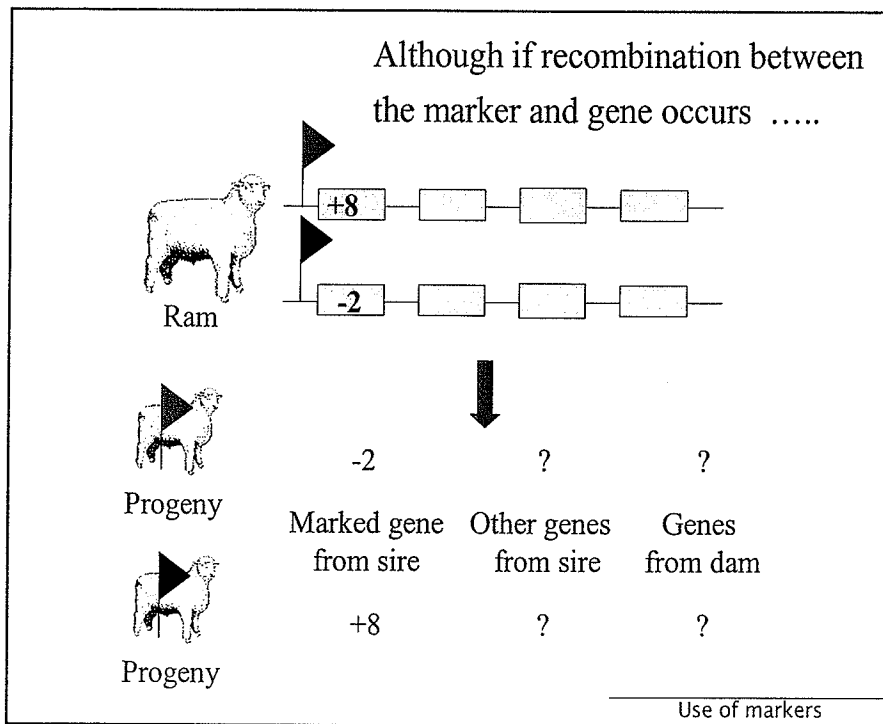
Some points about MAS

MAS is less accurate than GAS

- ❖ dependant on recombination frequency (linkage distance) between QTL and marker(s)

- ❖ results in *probabilities* of inheriting certain genotypes

- ❖ reduction in accuracy may be small if marker haplotypes are used

MAS with markers in linkage equilibrium requires progeny testing to determine linkage phase of QTL and marker in each family

Use of markers

Some points about GAS

Marker is the causative mutation
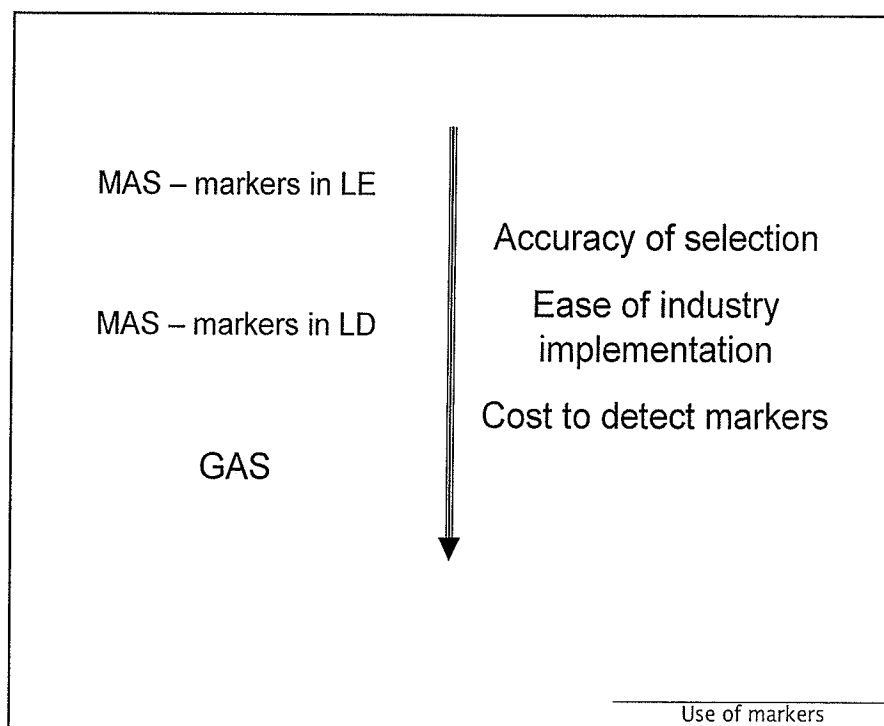- ❖ Thus *certainty* of inheriting a particular genotype

Identifying the gene and causative mutation can take many years
- ❖ More difficult for quantitative rather than discrete traits

Causative mutation is population wide
- ❖ Thus do not need to re-establish linkage phase in each family

Use of markers

MAS – markers in LE

Accuracy of selection

MAS – markers in LD

Ease of industry
implementation

Cost to detect markers

GAS

Use of markers

Traits for gene markers

*Gene markers are most beneficial for traits are difficult to
improve under traditional selection*

Require slaughter to measure
- Carcase traits
- e.g. meat pH, tenderness, colour

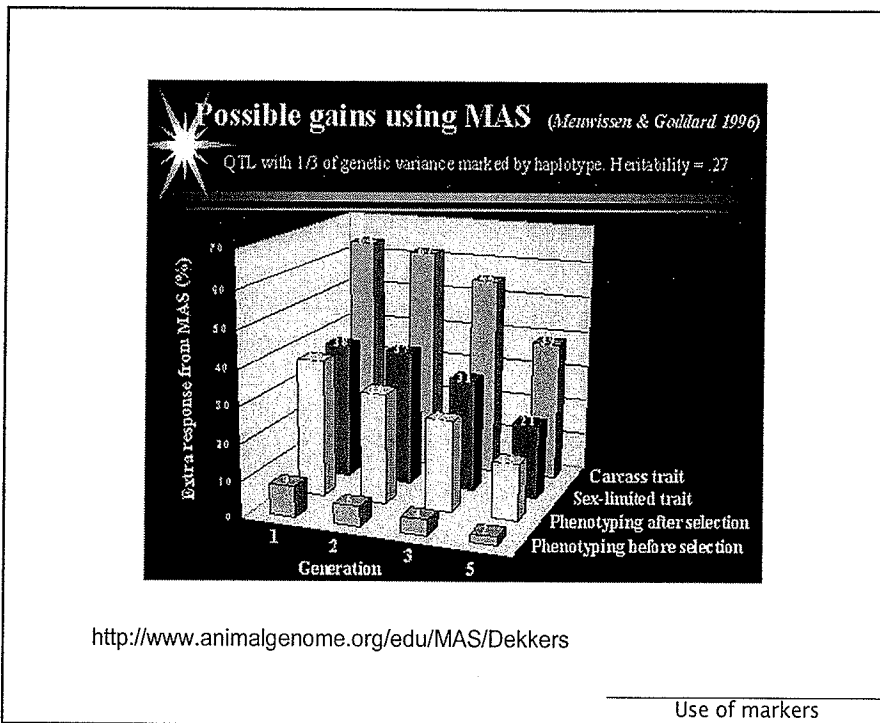Are measured on one sex only
- Milk Production

Are measured late in life
- Lifetime fecundity

Are difficult or expensive to measure
- Disease resistance

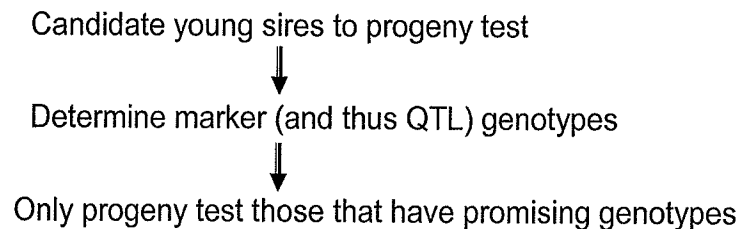Meuwissen and Goddard, 1996

Use of markers

http://www.animalgenome.org/edu/MAS/Dekkers

Use of markers

Breeding scheme structures can also be altered to accommodate markers

For example, progeny testing in dairy:

Candidate young sires to progeny test

⬇

Determine marker (and thus QTL) genotypes

⬇

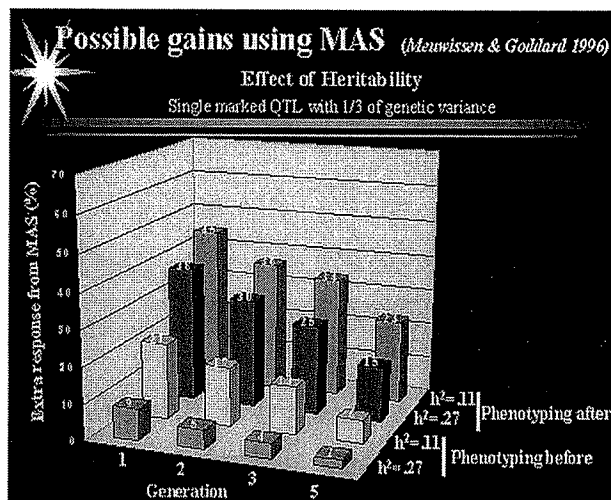Only progeny test those that have promising genotypes

Use of markers

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Response

Relative advantage of MAS/GAS over traditional selection
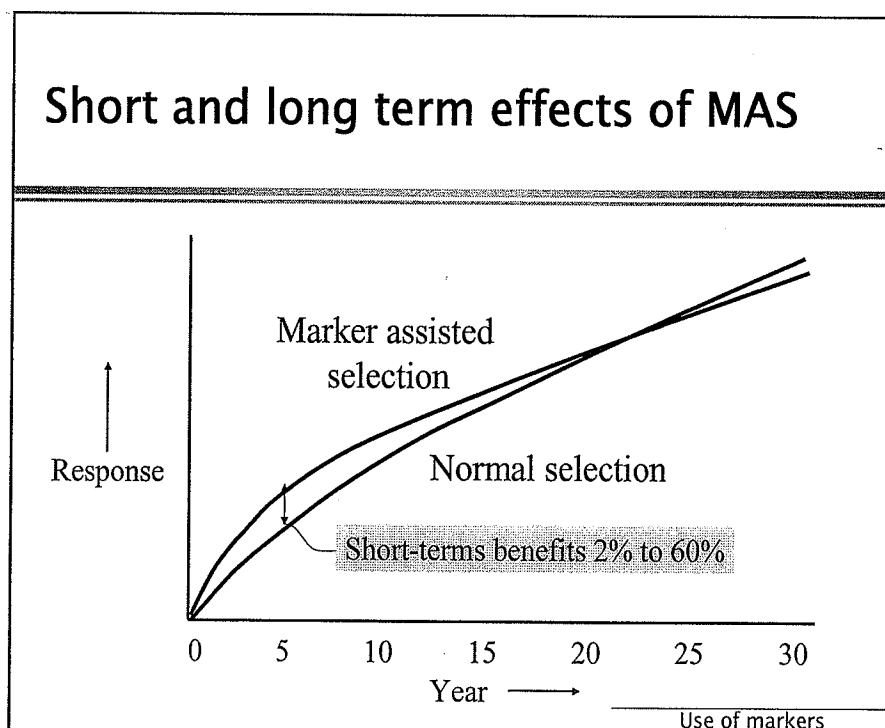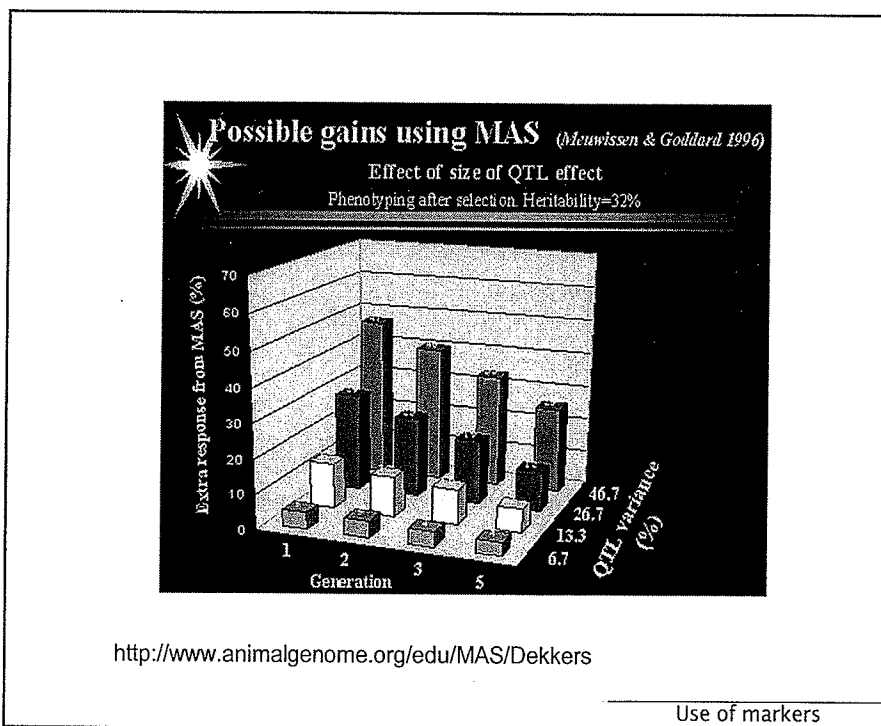 is higher if

- ❖ trait heritability is low

- ❖ the QTL is of large effect

- ❖ the favourable allele is initially rate

- ❖ markers trace QTL inheritance with a high level of accuracy

- ❖ mode of gene action is non-additive

Use of markers

Possible gains using MAS (*Meuwissen & Goddard 1996*)
Effect of Heritability
Single marked QTL with 1/3 of genetic variance

http://www.animalgenome.org/edu/MAS/Dekkers

Use of markers

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Possible gains using MAS (Meuwissen & Goddard 1996)
Effect of size of QTL effect
Phenotyping after selection. Heritability=32%

http://www.animalgenome.org/edu/MAS/Dekkers

Use of markers

Short and long term effects of MAS

Marker assisted selection

Normal selection

Response

Short-terms benefits 2% to 60%

Year ⟶

Use of markers

Current level of industry implementation

Industry implementation:

- ❖ Very difficult for MAS with markers in LE
- ❖ Some examples for MAS with markers in LD
- ❖ Some examples for GAS

Implementation often via breeding organisations

No clear signals in relation to whether markers are meeting expectations

Use of markers

Issues related to industry implementation

For MAS with LD markers:

- ❖ How many QTL?
- ❖ How many markers around each QTL?

For MAS / GAS:

- ❖ How well should markers be verified?
  - accuracy of effect estimate
  - population wide LD
  - frequency of favourable allele
  - epistatic (gene interaction) effects
- ❖ How to incorporate into the overall BO

Use of markers

Number of QTL

Any particular QTL explains a proportion of the
genetic variance

Estimated that between 10 and 20% of the largest
QTL would explain 50% of the genetic variance
for a quantitative trait (Hayes and Goddard, 2001)

Need to do a cost-benefit analysis to determine
how many QTL are optimal
❖ QTL of smaller effect have similar (or maybe greater)
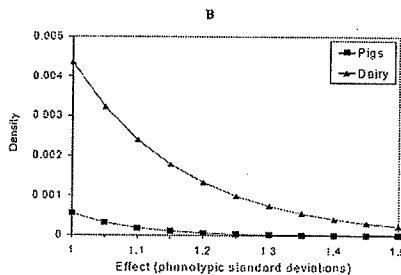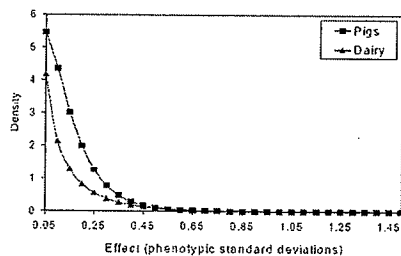detection costs than those of large effect,

Use of markers

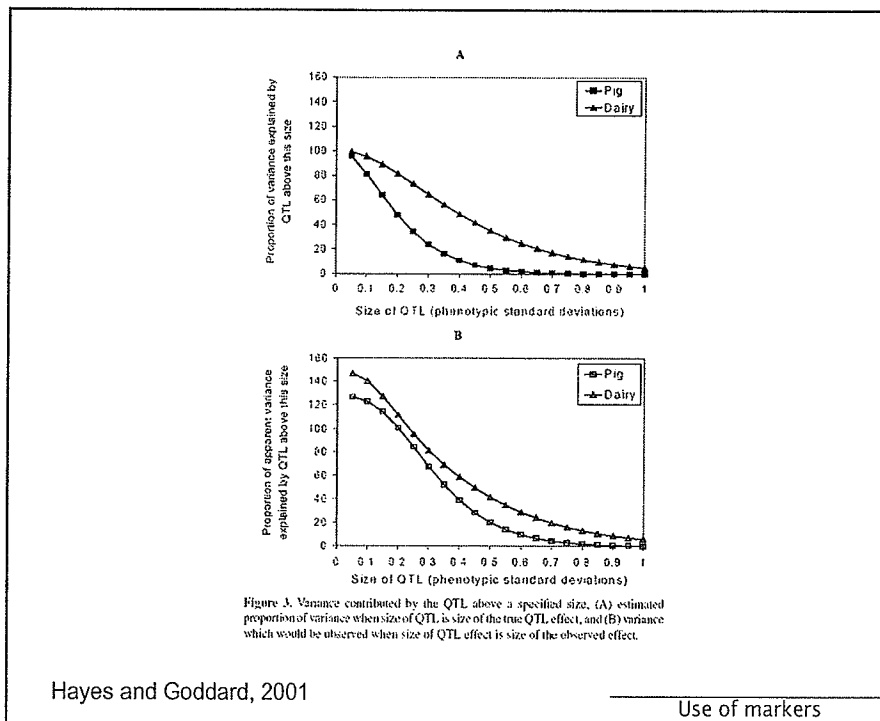

Figure 2. Distributions of QTL effect from pig and dairy experiments (A), and
magnification of distribution from effect = 1 to 1.5σₚ (B).

Hayes and Goddard, 2001

Use of markers

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Figure 3. Variance contributed by the QTL above a specified size. (A) estimated proportion of variance when size of QTL is size of the true QTL effect, and (B) variance which would be observed when size of QTL effect is size of the observed effect.

Hayes and Goddard, 2001

Use of markers

Number of markers for each QTL

Single marker versus marker haplotype

❖ How much additional information does a marker haplotype give over a single marker in LD?

Marker haplotypes

❖ Is a haplotype of a 5, 2, <1 cM required?

❖ How many markers within each haplotype?

Use of markers

Marker verification

Accuracy of effect estimate
- ❖ How well should effects be known before implementation MAS?
- ❖ For markers in LD, accuracy of effect estimate relates to the number of individuals with a particular haplotype
- ❖ Effects may depend on genetic background

Population wide LD
- ❖ How many populations / individuals from each population to test before claiming population wide LD?

Frequency of favourable allele
- ❖ Allele frequency is population dependant, thus additional gain from MAS is population dependant

Epistatic (gene interaction) effects
- ❖ Most difficult issue

Use of markers

Incorporation into a breeding objective

Markers provide another selection criteria

- ❖ Thus (following selection index theory) phenotypic and genotypic relationships to other traits in the selection criteria should be known

- ❖ Allele frequency will change with time – thus need to re-evaluate (as for other genetic parameters)

Use of markers

QTL in mixed models

As per earlier, but with additional term relating to QTL
effects

Distribution of the additive QTL effects can be defined as (0,
GV_{QTL})

  ❖ G is the genetic relationship matrix, and V_{QTL} the additive QTL
     variance
  ❖ G has dimensions $n \times n$, where n is the number of haplotypes
  ❖ elements in G represent the probability that haplotypes carry a
     QTL allele that is identical by descent (IBD)

Solving gives a polygenic effect and two allelic effects for
each animal

Use of markers

Examples of direct markers in sheep:
from Australian sheep gene mapping website

Fecundity

Inverdale fecundity (FecX)

The Inverdale mutation causes increased fecundity in heterozygous ewes and sterility in homozygous ewes. The causative mutation for Inverdale fecundity has been identified within the BMP15 gene on the X chromosome (Galloway et al., 2000).

Booroola fecundity (FecB)

The Booroola mutation causes increased fecundity in heterozygous ewes with a further increase in fecundity in homozygous ewes. The causative mutation for Booroola fecundity has been identified within the BMPR1B gene on chromosome 6 (Wilson et al., 2001; Mulsant et al., 2001; Souza et al., 2001). Commercial testing for Booroola fecundity is available from Genomnz.

Woodlands fecundity (FecX2)

The Woodlands fecundity trait is maternally imprinted and has a complex inheritance pattern. This trait has been mapped to a region on chromosome X (Davis et al., 2001).

Meat Traits

Callipyge
"beautiful buttocks"

The *callipyge* locus causes muscular hypertrophy of buttock muscles in sheep with the hypertrophied muscles being less tender than those in normal sheep. This trait has a complex mode of inheritance (Cockett et al., 1996) and has been mapped to a 400 kb region on chromosome 18 (Berghmans et al., 2001; Charlier et al., 2001). This region influences the expression of the GTL2 gene in hypertrophic muscles (Bidwell et al., 2001).

Carwell

The *Carwell* locus causes a milder form of muscular hypertrophy than *callipyge* and maps to a similar region on chromosome 18 (Nicoll et al., 1998, Proc VI World Conf. Genet. Appl. Livest. Prod. 26:529-532). It is likely that Carwell is allelic to *callipyge*.

Diseases

Spider Lamb Syndrome

Spider Lamb Syndrome is a skeletal disorder that has a recessive mode of inheritance. The causative mutation for Spider Lamb Syndrome has been identified within the FGFR3 gene on chromosome 6 (Cockett et al., 1999). Contact Dr Cynthia Bottema for details about testing for Spider Lamb Syndrome in Australia.

Other

Horns

The Horns locus of Merino sheep has been mapped to a region on chromosome 10 (Montgomery et al., 1996).

Black wool

The recessive self-colour phenotype of Australian Merino sheep has been mapped to a region on chromosome 13 (Parsons et al., 1999, Australian Journal of Agricultural Research 50:1099-1103). The agouti gene is a candidate for the self colour phenotype.

Use of markers

1ˢᵗ Australian marker on the market

http://www.geneticsolutions.com.au

GeneSTAR Marbling is a DNA diagnostic test for a major gene associated with marbling. It is the first gene marker for a production trait in beef cattle. The test enables cattle breeders to select individuals that carry one or two copies of the favouable allele.
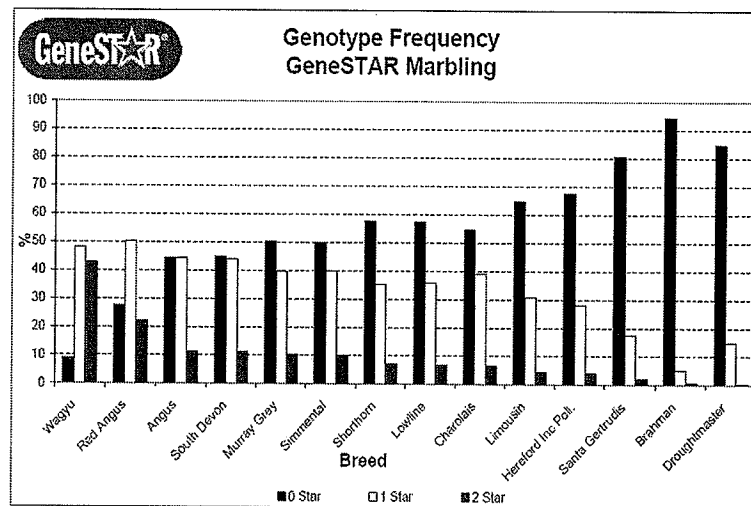
GeneSTAR® Results Explanation

GeneSTAR Marbling

0 This animal carries zero copies of the favourable form of the GeneSTAR Marbling gene

This animal carries one copy of the favourable form of the GeneSTAR Marbling gene

This animal carries two copies of the favourable form of the GeneSTAR Marbling gene

Use of markers

**Genotype Frequency
GeneSTAR Marbling**

Breed

■ 0 Star   □ 1 Star   ■ 2 Star

http://www.lowline.com.au/LowlineFrq.M1.pdf

Use of markers

Examples of tests on the market

Name	Trait	Desired genotype	Company
GeneSTAR®	Marbling in beef	**	Genetic Solutions (Aus)
Igenity-L™	Marbling in beef	TT	Select Sires (USA)
GeneSTAR®	Tenderness in beef	**	Genetic Solutions (Aus)
TenderGENE	Tenderness in beef	SNP316CC SNP530GG	Select Sires (USA)
Igenity-OptiYIELD™	Milk production in dairy		Select Sires (USA)
Igenity-ComponentMaker™	Milk composition in dairy		Select Sires (USA)

Use of markers

Marker assisted introgression

Introgression:

❖ e.g. introgress allele from Breed A into Breed B

• A x B → rounds of [identify animals with favourable allele and backcross to Breed B] → 99% Breed B with favourable allele from Breed A

In relation to MAI, markers can be used to

❖ Identify animals that have inherited the allele being introgressed

❖ quantify % of original breed

Use of markers

Parentage

Parentage can be determined using a marker panel

❖ Typically 10-20 markers

❖ More markers if population is inbred / markers are uninformative

Parentage analysis for a number of livestock species is commercially available

Parentage software includes CERVUS

❖ Practical difficulty is discriminating genotyping errors from parentage errors

Use of markers

CERVUS

CERVUS is a Windows 95-based program designed for large-scale parentage analysis using co-dominant loci.

What analyses can CERVUS do?
Allele frequency analysis
Simulation of parentage analysis
Parentage analysis
File conversion
Identity check

http://helios.bto.ed.ac.uk/evolgen/cervus/cervus.html

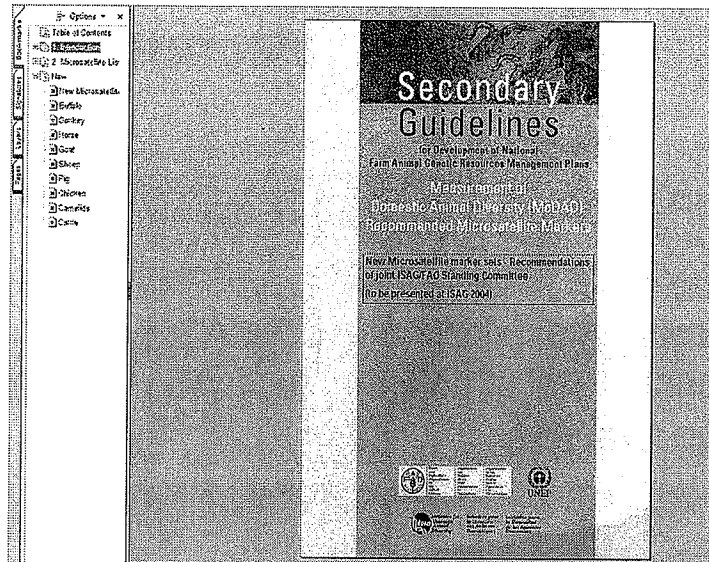Use of markers

Analysis of genetic diversity

Genetic diversity of microsatellite loci in fifty-six Chinese native pig breeds.

Yi Chuan Xue Bao et al. 2003 Mar;30(3):225-33

The genetic diversity of fifty-six indigenous pig breeds in China, and three introduced pig breeds (Duroc, Landrace and Large White) was surveyed using twenty-seven microsatellites recommended by the International Society for Animal Genetics (IS-AG) and Food and Agriculture Organization (FAO).

By means of the **allele frequencies, mean heterozygosity, effective number of alleles, estimator of gene differentiation, polymorphism information content, genetic distance and dendrogram analysis,** the genetic variability and population structure of native pig breeds were estimated. ....

Use of markers

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http://dad.fao.org/en/refer/library/guidelin/marker.pdf

Use of markers

New crossbred populations

Isolated populations evolve independently

❖ Evolution of different adaptive alleles to a selection pressure (e.g. disease)

❖ Some experimental support of this for trypanosomosis tolerance (Hanotte et al., 2003)

Can test for difference adaptive alleles via QTL mapping crossing animals from the isolated populations (e.g. diverse breeds)

Outcome may be a cross with adaptive alleles from two sources and e.g. greater disease resistance (Gibson, 2004)

Use of markers

Genomic Selection

SNP genotyping of 10,000+ markers

Allows for application of LD-MAS, however some level of pedigree recording will still be required

Use of markers