Livestock genomics comes of age.

M Georges and L Andersson

Genome Res. 1996 6: 907-921
Access the most recent version at doi:10.1101/gr.6.10.907

REVIEW

Livestock Genomics Comes of Age

Michel Georges[1,3] and Leif Andersson[2]

[1]Department of Genetics, Faculty of Veterinary Medicine, University of Liège, 4000-Liège, Belgium; [2]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala Biomedical Centre, 24 Uppsala, Sweden

It is estimated that man[1] first domesticated animals as early as 10,000 BP. Since then, farmers have been unwittingly manipulating livestock genes by selective breeding. This early genetic engineering generated a wealth of variation for a myriad of traits in the different livestock species. The dramatic size difference between the Shire and Shetland pony or the plethora of dog breeds are just two illustrations of the diversification obtained by artificial selection. The resulting caricature of naturally occurring variation has played a major role in Darwin's formalization of his theory of the evolution of species by natural selection. The first chapter of his acclaimed *The Origin of Species* (1859) is devoted to "Variation Under Domestication."

In the beginning of this century, the coalescence of biometrics and Mendelian genetics pioneered by R.A. Fisher, J.B.S. Haldane, and S. Wright led to the foundation of quantitative genetics, which in turn allowed development of the theory of animal breeding that is still implemented to this date. This biometrical era of animal genetics has led to spectacular increases in productivity in all major livestock species during the second half of this century. As an example, in the United States milk yield has increased from approximately 4500 kg per cow per year to more than 6800 kg in less than 20 years (Pearson et al. 1990).

It is noteworthy that the vast majority of production traits undergoing selection are typical quantitative traits, that is, they exhibit a continuous rather than a discrete distribution and they are influenced by environmental factors as well as by an undefined number of polygenes or quantitative trait loci (QTL). Their heritabilities typically range from less than 5% to over 50%. Animal geneticists therefore have had a long-standing interest in the genetics of complex inheritance, the relevance of which is being recognized increasingly in medical genetics as well.

The implementation of breeding schemes that proved so efficient would have been impossible without the organization of extensive phenotypic record keeping. Particularly illustrative in this respect is the collection of individual records (milk yield and composition, type traits, health traits, etc.) that is performed on a monthly basis for millions of cows as part of dairy herd improvement programs in the United States, Western Europe, and several other parts of the world. Likewise, breeding companies carefully monitor their pig and poultry breeding stock for a whole range of phenotypic measurements.

Given the tradition of eagerly adopting modern technology that might increase genetic response as well as the availability of unique material, it is quite surprising that animal geneticists have been reluctant to invest in genomics when compared with plant breeders or human medical geneticists. This is likely due in part to the realization that the majority of economically important traits in livestock are typical multifactorial traits and are therefore the most difficult ones to tackle using genomic strategies. Moreover, given the spectacular genetic progress achieved by means of conventional breeding programs, some skepticism has prevailed regarding the cost-effectiveness of biotechnology in livestock production. It is only during the last 5 years that we have witnessed a growing interest of animal geneticists in genomics. This has undoubtedly been catalyzed by the successes of the Human Genome Initiative. This review will summarize the state of

[3]Corresponding author.
E-MAIL michel@stat.fmv.ulg.ac.be; FAX 32-41-66-41-22.

the art in livestock genomics, while emphasizing some specificities of animal studies, and will identify some of the major challenges for the future.

Current Status of Genome Analysis

Primary Microsatellite–based Maps Are Being Generated for Most Livestock Species

The description of microsatellites as an abundant source of highly polymorphic, well-dispersed, and conveniently typed markers has boosted the generation of primary maps in livestock species (for review, see Beattie 1994). Individual as well as internationally coordinated efforts have lead to the generation of a number of linkage maps in the different species: cattle (Barendse et al. 1994; Bishop et al. 1994; Ma et al. 1996), pig (Ellegren et al. 1994; Rohrer et al. 1994; Archibald et al. 1995; Marklund et al. 1996; Rohrer et al. 1996), sheep (Crawford et al. 1995), and poultry (Bumstead and Palyga 1992; Levin et al. 1994; Cheng et al. 1996; Crooijmans et al. 1996) in particular. When several parallel efforts have been undertaken in a given species, the number of common markers fortunately has usually been sufficiently high to allow for efficient cross-referencing (Eggen and Fries 1995; Marklund et al. 1996). At this point, the available maps number ~1100 markers for cattle and pig (C. Beattie, pers. comm.; Rohrer et al. 1996), 700 for sheep (A.M. Crawford, pers. comm.), and 450 for poultry (M.A.M. Groenen and N. Bumstead, pers. comm.). These maps provide very adequate genome coverage, especially for cattle and pig, where the average between marker interval is now ~2.5–5 cM.

Artificial breeding schemes often result in a reduction in effective population size. Assuming selective neutrality of microsatellite alleles, one could therefore expect reduced allele numbers and heterozygosity when compared with the human. In addition, because fewer markers have been developed in animal species, less selection has taken place based on information content in putting together marker panels for genome scans. As an example, the battery of markers used to scan the bovine genome in a search for QTL affecting milk production had an average heterozygosity of 56% in the studied Holstein-Friesian population (Georges et al. 1995), compared with heterozygosities typically >70% in human microsatellite-based studies. Part of the segregation information can be recovered by performing multipoint linkage analyses in which the information provided by linked markers is considered jointly. Moreover, animal geneticists may have the option to arrange matings between breeds and even subspecies in a manner reminiscent of the *Mus musculus domesticus* × *Mus spretus* or *castaneus* crosses, enhancing the information content at the marker as much as trait loci (see below). Nevertheless, continued marker development seems advisable in most domestic species to ensure adequate information content across the respective genomes.

Sequence conservation has proven to be sufficiently high between cattle and sheep to allow for ~50% of primer sequences developed in one species to work in the other (Moore et al. 1991; A. Crawford and N. Cockett, pers. comm.). This percentage is considerably higher than what has been observed for the mouse and rat, which are thought to be as closely related evolutionarily. As expected, this cross-species use of microsatellite markers has proven inefficient for more distantly related domestic species despite the occasional demonstration of remarkable conservation of microsatellite position (Moore et al. 1991; Ellegren et al. 1993; Sun and Kirkpatrick 1996).

Most of the linkage groups have been anchored and oriented to specific chromosomes in cattle and pig using fluorescence in situ hybridization (FISH) mapping. In poultry, establishing connections between the linkage and cytogenetic maps is complicated by nonidentifiable minichromosomes, which might represent as much as 30% of the genome.

These mapping data are being compiled in a number of data bases conveniently accessed via the internet (e.g., http://www.ri.bbsrc.ac.uk/genome_mapping.html; http://dirk.invermay.cri.nz; http://locus.jouy.inra.fr/cgi-bin/bovmap/Bovmap/intro.pl; http://sol.marc.usda.gov/marc/html/gene1.html; http://probe.nalusda.gov:8000/index.html).

Thus, with the available genetic maps, we have reached the point where the marker resources are no longer the limiting factor when attempting to map trait loci by exploiting within-family linkage disequilibrium. It can be argued, however, that in a number of instances the mapping method of choice would be based on linkage disequilibrium existing across the population (such as in identity-by-descent mapping methods). Efficient implementation of such strategies will require further development of a substantial number of additional markers in the different livestock species.

Comparative Mapping Confirms the Remarkable Conservation of Synteny Amongst Mammalian Species

Although microsatellites undoubtedly form the scaffold of all livestock linkage maps, a sufficient number of evolutionarily conserved Type I markers (O'Brien 1991; O'Brien et al. 1993) are interspersed in these maps to confirm the extensive conservation of synteny among distantly related mammals that was predicted from early mapping data using somatic cell hybrids (Womack and Moll 1986). Comparative chromosome painting (Zoo-FISH) using individual human chromosome-specific libraries has further defined the boundaries of conserved chromosome segments between human, pig, and cattle. It seems well established now that not more than 56 and 47 blocks of conserved synteny emerge from the comparison of the human genome with the bovine and porcine genomes, respectively (Rettenberger et al. 1995; Solinas-Toldo et al. 1995). Interestingly, this number is substantially higher, of the order of 85, when confronting the mouse and human genomes (Nadeau and Taylor 1984; Peters and Searle 1996). Despite this remarkable conservation of synteny, there is growing evidence that the linear order of genes within homology blocks has often been altered by intrachromosomal rearrangements (Johansson et al. 1995). Therefore, efficient cross-talk between mammalian maps requires the urgent development of high-resolution comparative maps for domestic species.

Chromosome Sorting and Microdissection Emerge as the Methods of Choice for Chromosome– and Region–specific Mapping Efforts

Animal geneticists are increasingly focusing their attention on specific chromosomal regions in which genes of interest have been located. The major limiting factor in their efforts to produce fine maps of these regions is rapidly becoming the paucity of genetic markers. Efforts to increase the number of markers by a factor of five to ten on a genome-wide basis have been difficult to justify in livestock species, and several groups have tried to adapt methods for efficiently targeting marker development to specific chromosomes or even chromosome bands. In the absence of chromosome-specific somatic cell hybrids, two methods are emerging. The first is chromosome flow sorting, which has been successfully applied to the bovine and porcine karyotypes, allowing for the majority of chromosomes to be sorted as pure fractions (Schmitz et al. 1992, 1995; Langford et al. 1993; Yerle et al. 1993). These advances should facilitate the development of chromosome-specific libraries and markers in the near future. Likewise, a number of successful applications of chromosome or chromosome-band microdissection for the development of region-specific markers are being reported (Schmutz et al. 1994; Ponce de Leon et al. 1996).

Large–Insert Libraries Are Being Constructed for Most Livestock Species

In anticipation of future fine-mapping and positional cloning efforts, large-insert libraries are being produced for most livestock species. To be able to capitalize on their complementary merits, both yeast artificial chromosome (YAC) as well as bacterial artificial chromosome (BAC)/P1/PAC libraries are being generated.

YAC libraries have been constructed for cattle (Libert et al. 1993; Smith et al. 1996; A. Schoeberlein, unpubl.), sheep (Broom and Hill 1994), pig (Leeb et al. 1995; S. Meier–Ewert, unpubl.; C. Beattie, pers. comm.; P. Chardon, pers. comm.), and poultry (A.A. Toye, unpubl.). When comparing the libraries of different species, sufficient depth (5–10 genome equivalents) is achieved at least for cattle, sheep, and pig, which should compensate for the high level of chimerism and rearrangements typical of this cloning system.

Efforts are also being devoted to the construction of large insert libraries using prokaryotic (BAC, P1, or PAC) cloning systems. A bovine BAC library has been constructed in pBeloBAC11; at this point, the BAC library numbers 60,000 clones with average insert size of 146 kb for a total of approximately three genome equivalents (Cai et al. 1995). Similar progress is being reported in pig (A.L. Archibald, pers. comm.) and poultry (M.A.M. Groenen, pers. comm.).

Genetic Analysis of Single-gene Traits in Livestock

As expected, the first successful implementations of the new mapping tools in livestock dealt with single-gene traits. Table 1 lists loci controlling monogenic traits that have been positioned on the corresponding maps using linkage strategies.

Table 1. Monogenic Traits Mapped by Linkage Analysis in Livestock Species

Species	Locus	Trait	Position	Gene	Reference
Pig	*MH*	Malignant hyperthermia	6	*CRC*	Fuji et al. 1991
	I	Dominant White coat color	8	*KIT*	Johansson et al. 1992; Johansson Moller et al. 1996
	E	Extension coat color locus	6	?	Mariani et al. 1996
	Rn	Muscle glycogen content	15	?	Milan et al. 1995, 1996; Mariani et al. 1996
	ECK88a	Intestinal receptor for *E. coli*	13	?	Guérin et al. 1993; Edfors-Lilja et al. 1995
	b,acR	K88ab,ac fimbriae			
	ECF107	Intestinal receptor for E. coli F107	6	?	Voegeli et al. 1994
	R	fimbriae			
	CPS	Campus tremor syndrome	7	?	I. Tammen and B. Harlizius, pers. comm.
Cattle	*PDME*	Weaver	4	?	Georges et al. 1993a
	Polled	Presence/Absence of horns	1	?	Georges et al. 1993b
	Roan	Roan coat color locus	5	?	Charlier et al. 1996a
	MH	Double muscling	2	?	Charlier et al. 1995
	E	Extension coat color locus	18	*MC1R*	Klungland et al. 1995
	Sy	Syndactyly	15	?	Charlier et al. 1996b
Sheep	*FecB*	Booroola fecundity gene	6	?	Montgomery et al. 1994
	CLPG	Callipyge muscular hypertrophy	18	?	Cockett et al. 1994, 1996
Goat	*Polled*	Presence/Absence of horns	1	?	Vaiman et al. 1996
Horse	*E*	Extension coat color locus	LGII	*MC1R*	Johansson et al. 1994; L. Marklund, M. Johansson Moller, K. Sandberg, and L. Andersson, pers. comm.
Poultry	DW	dominant white	LG22	?	Ruyter-Spira et al. 1996
	SLD	sex linked dwarfism	Z	*GHR*	Ning et al. 1994

The mapping of the malignant hyperthermia (*MH*) locus in pigs in fact predated the discovery of microsatellite markers and relied on the use of biochemical polymorphisms. For five of these traits, the actual gene and causal mutation have been identified. In all cases, the gene identification relied on the known location of a candidate gene as deduced from comparative mapping data. Several of the single genes mapped so far control coat color. There is in fact considerable interest in the breeding industry to use DNA tests for coat color since by tradition coat color is often used as a breed trademark.

A number of distinctive features characterizing livestock populations emerge from these studies and are worth noting, because they might suggest novel mapping strategies.

Founder Effects and Reduced Effective Population Size Allows for Within-Breed Identical-by-Descent Mapping

Historical records show that livestock breeds often trace back to a very small number of founder individuals. A popular example is thoroughbred horses, which trace back to three famous stal-

lions: Darley Arabian, Byerley Turk, and Godolphin. In addition and as already mentioned, the extensive use of modern reproductive technologies leads to a considerable reduction in the effective population size. While the American Holstein Friesian cow population exceeds 10 million, its effective size is estimated to be <1000, primarily as a result of the systematic use of artificial insemination, allowing for a few elite males to have tens of thousands of offspring. As expected, the proportion of inbred individuals has a tendency to increase within this highly selected population, with average inbreeding coefficients among inbred individuals of ~2–5%. Interestingly, it has been shown that the nucleotide diversity π is ~0.0007 in the American Holstein Friesian, which is approximately three times lower than values typically measured in human populations (Steele and Georges 1991).

Given the history and structure of most domestic breeds, it is reasonable to predict that most inherited disorders will be genetically homogeneous in a given breed. In other words, affected individuals are expected to share identical-by-descent (IBD) causal mutations flanked by IBD chromosomal segments, the size of which is determined by the number of generations from the common carrier founder. Therefore, domestic animal populations share similarities with human "isolates," which have proven to be particularly useful for disease gene mapping (Hastbacka et al. 1992). Indeed, the identification of such shared chromosomal segments amongst affected individuals allows one to determine very efficiently the location of the disease-causing genes.

This prediction has been verified in a study aimed at locating the gene causing *syndactyly* in Holstein Friesian cattle (Charlier et al. 1996b). Examination of the pedigree records of 12 affected individuals showed that they all traced back to a common known carrier individual not more than seven to nine generations back. A shared segment on chromosome 15 was detected using a primary DNA marker map comprising 213 markers with average intervals of ~15 cM and a multipoint maximum likelihood method.

The important implication for animal geneticists is that this strategy allows for the mapping of genes underlying inherited disorders using a limited number of affected offspring, even if these do not constitute a nuclear pedigree typically considered to be required for linkage analysis. Thus, the time-consuming and expensive step of breeding such a segregating pedigree can be avoided. As available marker maps improve, the power to detect shared IBD chromosomal segments will increase as well.

Between-Breed Admixture May Allow for IBD Fine Mapping

While in the previous paragraph we stressed the likely IBD of alleles underlying a phenotype of interest within a given breed, the same IBD status may apply to between-breed comparison as well, especially because mutations causing a desirable phenotype may have spread across populations by migration and selection. The occurrence of the same C→T transition in the calcium release channel (CRC) gene in all populations in which porcine malignant hyperthermia (MH) has been described, and undoubtedly propagated as a result of its association with enhanced muscularity, illustrates this assertion. Likewise, historical records suggest that migration of a single mutation might account for the "double-muscling" trait observed in several continental cattle breeds (Ménissier 1982). A similar scenario can be proposed easily for the spread in several breeds of the *Polled* allele causing hornless cattle, as well as the mutation causing dominant white coat color in pigs. Another striking case of between-breed admixture is the recent strong gene flow from the American Holstein Friesian breed to several dairy cattle breeds around the world. Breeds showing phenotypic resemblance due to migration of an IBD mutation are expected to share an IBD chromosome segment flanking these mutations. Again, the size of this shared chromosome segment will reflect the number of generations to coalescence. This number, however, may be substantially larger in these cases when compared with the situation observed within the Holstein breed for syndactyly. Dissemination of the double-muscling trait in several European breeds, for instance, might coincide with the extensive use of Shorthorn animals to improve these breeds during the early 19th century. This would trace the migration back to perhaps as many as 50 generations, allowing one to predict an IBD chromosome segment of the order of 1 cM.

As pointed out by Boehnke (1994), the resolution of linkage mapping is limited as much by the availability of recombinants as by the density of available markers in the chromosomal region of interest. As a matter of fact, positional cloning efforts in domestic animals are likely to be ham-

pered in many cases by the scarcity of recombinants and the ensuing difficulty of defining unambiguously an interval containing the gene that is small enough to be compatible with positional cloning. Exploiting linkage disequilibrium across breeds might help to increase the resolution of fine-mapping efforts prior to the actual positional cloning.

Selection for Complex Production Traits May Affect Monogenic Traits by Pleiotropy or Hitchhiking

Associations observed between monogenic characteristics and quantitative production traits may result from population stratification due to the use of unique breeding strategies and reproductive technologies in livestock. Alternatively, however, such associations might be due either to the pleiotropic effect of a single gene on both a monogenic trait and the production character undergoing selection or to a hitchhiking effect affecting the monogenic trait because of its tight linkage with a QTL. The latter phenomenon might be exploited to map some QTL underlying the production traits of interest.

The best-known example of such an association is the one between MH or the porcine stress syndrome (PSS) and lean meat content in pigs. MH develops in susceptible pigs after exposure to stress or halothane anesthesia and is due to homozygosity for a recessive allele. There is strong evidence showing that MH-affected animals as well as MH carriers give a higher yield of lean meat and that selection for meatier carcasses resulted in a high prevalence of MH in several breeds including Piétrain, Poland China, and Landrace. The MH phenotype and its simple inheritance allowed for the fine-mapping of the corresponding chromosomal region and led to the identification of the C–T transition in the *CRC* gene (Fuji et al. 1991), which apparently is the causative mutation for MH in all pig breeds tested so far. MacLennan and Phillips (1993) hypothesized that this mutation may have pleiotropic effects on muscularity and lean content as well. They suggested that the mutant form of *CRC* is hypersensitive, causing spontaneous muscle contractions and a continual toning of the muscle that, in turn, may lead to muscular hypertrophy and reduced fat content. However, one cannot formally exclude the possibility that independent mutations in closely linked genes underlie the different phenotypic effects associated with MH.

Another possible example of a monogenic trait affected by the selection for a quantitative trait by pleiotropy is hyperkalaemic periodic paralysis (HYPP) in horses. It has indeed been suggested that the high incidence of this disorder in quarter horses might be due a pleiotropic effect of the causative mutation in the α subunit of the adult skeletal muscle sodium channel, causing muscular hypertrophy (Rudolph et al. 1992).

There is some evidence in favor of hitchhiking effects on single gene traits due to selection. The most publicized example is the linkage association found between *Weaver* or progressive degenerative encephalopathy and milk production traits in the American Brown Swiss cattle population (Hoeschele and Meinert 1990). So far, however, it has not been possible to confirm this association using genetic markers originating from the chromosome 4 region to which *Weaver* has been mapped (Georges et al. 1993b; M. Georges, pers. comm.).

Non–Mendelian Inheritance Patterns of Monogenic Traits in Livestock May Help in Understanding Genetically Complex Traits

In 1983, a new mutation was described in a sheep flock causing an exceptional muscular development of the hindquarters primarily and was therefore referred to as "*callipyge*." Matings between callipygous males and normal females allowed the unambiguous mapping of the *callipyge* locus to ovine chromosome 18 (Cockett et al. 1994). A genetic model assuming a dominant *CLPG* mutation accounted for all the variance within these crosses. Surprisingly, further crosses involving callipygous animals clearly indicated a non-Mendelian behavior of the callipyge phenotype. Analysis of the segregation of the *callipyge* locus in these crosses using linked microsatellite markers allowed Cockett et al. (1996) to propose a genetic model that would account for the vast majority of the observations. The model assumes that the *callipyge* locus is subject to parental imprinting and that the dominant *CLPG* allele does not lead to expression of the muscular hypertrophy when inherited from the mother. Moreover, the inactivation of the maternal *CLPG* mutation dominates the active paternal *CLPG* allele, meaning that homozygous *CLPG/CLPG* animals were paradoxically found not to express the trait. This non-Mendelian segregation pattern has been referred to by the authors as "polar overdominance." Further controlled matings are necessary

to test and refine the model. Already, however, these results clearly point to the existence of non-Mendelian inheritance patterns whose understanding may help in the dissection of other complex traits in the areas of human, plant, and animal genetics. Moreover, the understanding of the genetics underlying traits such as the callipyge phenotype not only suggests specific breeding schemes, but has implications in population genetics theory as well. Indeed, the proposed model would be a cause of balanced polymorphism if individuals expressing phenotypes like callipyge benefited from a selective advantage.

Genetic Analysis of Complex Production Traits in Livestock: Mapping QTL

As previously mentioned, the majority of economically important traits in livestock are typically multifactorial, influenced by environmental factors as well as an undefined number of polygenes or QTL. Dissecting these traits into their Mendelian components is undoubtedly the major driving force behind ongoing mapping efforts in livestock. Identifying the QTL underlying the genetic variation for such traits might indeed pave the way toward the use of marker-assisted selection (MAS) schemes in animal breeding.

Animal geneticists have opted for two possible experimental designs in their quest for QTL. One method is to map QTL segregating in crosses based on parental populations that are highly divergent for the traits of interest. This approach, which has typically been exploited in plant genetics as well as when using rodent models, provides a maximum of information at both QTL and marker loci. Such QTL mapping experiments are primarily intended to shed light on the genetic background of complex traits. It is still an open question whether QTL segregating in crosses between divergent lines also contribute to the genetic variance for the trait of interest within commercial elite populations. Because this genetic variance is the usual substrate for selection programs, this may limit the practical use of the identified genes. The alternative approach, therefore, consists in directly mapping QTL that are still segregating within elite populations. Obviously, this approach is more demanding because more individuals have to be studied to compensate for the reduced information and because QTL alleles with large effects are likely to have reached fixation or near-fixation in highly selected populations. If genes are identified using this approach, however, their exploitation by MAS is likely to be easier.

A number of studies aimed at mapping QTL in livestock were performed as early as in the 1960s using blood group and biochemical polymorphisms (e.g., Neimann-Sorensen and Robertson 1961; Geldermann et al. 1985). The scope of these experiments was primarily limited, however, by the paucity of available markers. More recently, the effect of candidate gene variants on quantitative traits has been applied successfully as a way to identify the molecular basis of production traits (e.g., Bovenhuis et al. 1992; Rothschild et al. 1996). Two recent studies, however, describe results of the first whole-genome scans made possible by the availability of microsatellite maps in pigs and cattle. Both experiments yielded convincing evidence that genes underlying complex production traits can indeed be genetically mapped.

Mapping QTL for Growth and Fatness Using a Wild Boar × Domestic Pig Intercross

The short generation interval and the large litter size make it feasible to generate experimental pedigrees in the pig. Intercross pedigrees between Chinese and European domestic pigs as well as between the wild pig and domestic pigs have been constructed for the purpose of gene mapping (for review, see Archibald 1994). The wild and domestic pigs are referred to as subspecies (*Sus scrofa scrofa* and *S.s. domesticus*, respectively), but domestic pigs have been developed from wild pigs within the last 10,000 years, which means that the two groups are closely related from an evolutionary perspective. Nevertheless, they show remarkable phenotypic differences for a number of important traits.

A three-generation pedigree comprising 200 F_2 animals has been generated in Sweden after crossing two wild pig boars with eight Large White females (Andersson et al. 1994). The design of this experiment was intended to generate segregation at the major loci that have responded to artificial selection during the development of the modern, fast-growing domestic pig. A number of single-gene traits, such as coat color phenotypes, and complex polygenic traits, such as growth and fatness, were recorded on all F_2 animals. Interval mapping using a least-squares method (Haley et al. 1994) revealed QTL with large effects for both growth and fatness (Anders-

son et al. 1994). A major QTL affecting fatness was located on chromosome 4, and the data suggested that the same locus affected both average back-fat depth and abdominal fat. The locus explained as much as 20% of the phenotypic variation for these traits. QTL for growth were found on chromosomes 4 and 13 and explained 7–12% of the phenotypic variation in growth. These data were consistent with more than one QTL for growth on chromosome 4 and the pleiotropic effect on both fatness and growth of the same major QTL on that chromosome. Future studies will reveal whether the QTL with large effects identified in the wild-pig cross will explain genetic variance in other experimental crosses as well as commercial populations. Interestingly, Yu et al. (1995) have recently reported a significant association between growth and polymorphism in a candidate gene (PIT1, encoding a pituitary-specific transcription factor) in a cross between Chinese and European breeds. PIT1 maps to the same region of chromosome 13 as one of the QTL reported in Andersson et al. (1994).

Mapping QTL Controlling Milk Production in Elite Dairy Cattle by Exploiting Progeny Testing

The second study was aimed at identifying QTL influencing milk yield and composition in elite dairy cattle (Georges et al. 1995). These traits are typically characterized in the corresponding populations by heritabilities around 30% and approximately normally distributed. The study exploited two specific features of dairy cattle populations. (1) Extensive use of artificial insemination makes it easy to sample very large half-sib families. By concentrating on the segregation of the genes originating from the common founder sire in a within-family analysis one can efficiently reduce the genetic heterogeneity for the traits of interest in a given family. (2) Rather than use individual production records as phenotypic measurements, one can use male "breeding values" (BV) (Falconer and Mackay 1996) estimated from the production records of their daughters using a procedure referred to as "progeny-test." As bulls typically have 50–100 daughters, the accuracy of these BV estimates is such that their heritabilities are ~80%, compared with 30% for the original phenotype, leading to a considerable reduction in environmental noise. It was estimated in this specific case that the use of estimated BV rather than production records allowed for a 3.5- to 4-fold reduction in the required sample size.

The analysis of 14 such half-sib families totaling more than 1500 progeny-tested sons led to the identification of five chromosome regions (on chromosomes 1, 6, 9, 10, and 20) affecting milk yield and composition. The magnitude of the identified QTL effects was on the order of one additive genetic standard deviation. Although it was realized that these estimates were likely biased upwardly [given the limited power of the experimental design and the maximum likelihood (ML) statistical method chosen], these results nevertheless indicate that alleles with substantial effects are still segregating in these populations despite the intense selection. Because the analyzed milk yield and composition phenotypes are known to be highly correlated, the observation that the identified QTL were affecting several of these traits was not surprising. Although the inherent imprecision in the estimation of the effects calls for prudence in the interpretation, the mapped genes seemed to have fairly distinct effects on the different traits. As an example, while the QTL on chromosome 9 increased milk yield without significantly altering its composition, the QTL identified on chromosome 6 obviously leads to an increase in milk volume accompanied by a dilution of its solid components. These results therefore illustrate how QTL mapping should allow for a dissection of not only individual traits but the correlations between them as well. Several independent studies are presently being performed that will test the validity of the identified loci and hopefully reveal additional QTL.

IBD Fine-mapping of QTL in Outbred Populations?

Already, the QTL for milk production mapped to chromosome 6 in this initial study (Georges et al. 1995) has been confirmed in an independent analysis performed in the same breed (Spelman et al. 1996; W. Coppieters, pers. comm.). Indeed, lod scores superior to 2.7 were obtained in one sire family, at a virtually identical chromosome location and with effects of comparable magnitude on the different milk production traits when compared with the initial study.

Interestingly, analysis of the pedigree records indicates that the two informative sire families connect via a common ancestor, respectively, two and three generations back (Fig. 1). These results suggest that both sires might be carriers of an IBD relatively rare QTL allele. This assumption implies that the two sires will also share an IBD

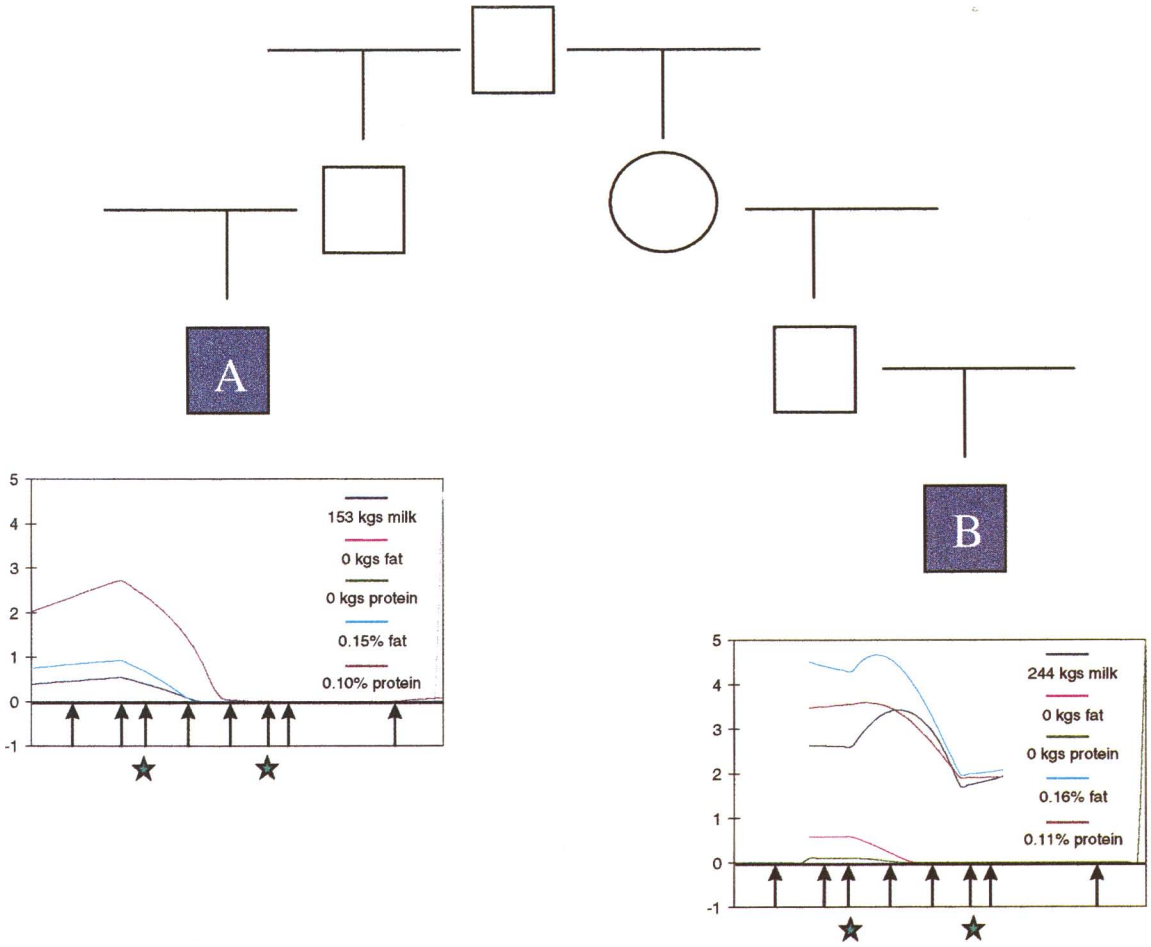

Figure 1 Pedigree relationships between two sires showing evidence for the segregation of the same chromosome 6 QTL effect on milk production. The lod score curves obtained with the ANIMAP programs for different milk production traits (Georges et al. 1995) are shown for each sire family. The associated most likely effect of half an allele substitution is given. The arrows along the *X* axis point toward the position of the microsatellite markers used in the latest study (Spelman et al. 1996; W. Coppieters, pers. comm.), whereas those used in the Georges et al. (1995) study are marked by a star.

chromosome 6 segment flanking the QTL. Identifying the limits of such IBD segment would unambiguously map the QTL. Assuming that the rare QTL allele indeed originates from the nearest common ancestor shown in Figure 1, this IBD segment is expected to be ≅35 cM in size. Using QTL segregation in the offspring as a single-gene trait amenable to straightforward IBD mapping might be an alternative strategy to fine-map QTL with a resolution difficult to achieve using conventional approaches to quantitative phenotypes.

Interestingly, Maki-Tanila and colleagues (H.J. Vilkki, K. Elo, M. Honkatukia, J. Jonkinen, and A. Maki-Tanila, pers. comm.) recently reported evidence for the segregation of what might be the same QTL effect in another breed, the Finnish Ayrshire population. If an IBD QTL allele were to underlie the same effect in both breeds, real QTL fine-mapping could be achieved.

Future Challenges

From Mapping to Cloning Trait Loci

The first successful outcomes of mapping efforts, targeting both single-gene and multifactorial traits, demonstrate that the tools required for the initial localization of the corresponding genes are available in livestock. These first results pave the way toward the implementation of MAS schemes. Indeed, one of the features of positional cloning appealing to animal geneticists is the prospect of exploiting markers linked to QTL as

diagnostic tools in breeding programs prior to the actual cloning of the underlying genes. Nevertheless, efficient use of these mapped genes in breeding programs would greatly benefit from more refined mapping data if not the cloning of the actual genes.

At present, animal geneticists are poorly prepared to address this second phase efficiently. Flow sorting and chromosomal microdissection will help to increase the marker density in regions of interest. Establishing the correct order of the ensuing markers would greatly benefit, however, from the availability of radiation hybrids in livestock species. The average size of the retained chromosome fragments could indeed be targeted to bridge the gap between the resolution obtainable with primary linkage maps and marker order deduced from STS content mapping of YACs, for instance.

The crux of the matter for animal geneticists, however, will be to establish high-resolution links with the human and mouse transcript maps, to take advantage of the spectacular progress of the human and mouse genome initiatives. As pointed out by Francis Collins (1995), a virtually complete human transcript map can be expected in the near future, which will shift the cloning method of choice from positional to positional candidate. For animal geneticists to benefit from this revolution, the development of efficient "trans-species shuttling" strategies is required. Coding sequences, typically characterized by homologies of ~70–80% between mammalian species, will undoubtedly be the preferred vehicle for such efforts.

The number of assigned coding sequences needs to be increased considerably to improve the resolution of comparative maps in livestock species. It is also clear that synteny maps will not be sufficient because the order of loci is often rearranged within segments of conserved synteny. Ordered maps have so far been constructed by in situ hybridization or by linkage mapping of polymorphic markers. These methods are laborious, however, and/or have limitations with regard to the resolution of order, and there is a strong need for improved mapping strategies. The establishment of radiation hybrid panels for domestic animals would be a useful resource because it would make it possible to order sequence tagged sites without the prior need to reveal polymorphism.

A more precise definition of the human chromosome region homologous to an animal locus of interest and defined by closely linked markers could be achieved by probing human YAC libraries with animal large-insert clones (e.g., BACs) containing the corresponding markers. Thus, 150–200 kb of insert DNA contained in a set of BAC clones might represent ~10 kb of coding sequences, which should be sufficient for obtaining above-background signals in most cases despite the technical hurdles associated with the use of large-insert clones, especially YACs. Identification of cross-hybridizing human YACs should immediately point toward the homologous human map position, because the vast majority of the human genome is now contained in YAC contigs (Chumakov et al. 1995). Given the depth of available human YAC libraries, each screening is expected to reveal several neighboring YAC clones. The prior mapping information available for the human YACs should greatly facilitate the interpretation of the hybridization patterns. Human coding sequences, for example, expressed sequence tags (ESTs), mapping to the identified human chromosome region would then be a source of candidate genes underlying the trait of interest, or could be used to screen the large insert libraries of the species of interest and construct physical maps of the targeted locus.

Cloning and characterization of QTL in livestock would be of paramount interest from both a scientific and practical breeding point of view. But positional cloning of QTL is a formidable undertaking for two major reasons. The genotype at a given QTL locus cannot be directly inferred from the phenotype due to the interference of other QTL and environmental effects. Therefore, with the exception of loci with very large effects, livestock QTL will be very difficult to map with the precision needed for positional cloning. The other major obstacle is that we are likely not looking for a defect in the gene product or in gene expression but rather a variant gene product or an altered gene expression. Thus, the polymorphism may be a regulatory as well as a structural mutation, and it will be more difficult to distinguish a linked polymorphism from the causative polymorphism than is the case for positional cloning of mutations causing a disorder.

Exploit Mapping Data in Breeding Programs by MAS

Continued support for livestock genomics will depend on the successful implementation of superior breeding programs that will incorporate

information on mapped or cloned production genes. The implementation of such breeding schemes, however, is far from trivial. The performances of MAS schemes will indeed be measured against the conventional "mass selection" approach that has proven so efficient and cost-effective over the years. Particularly relevant in the case of quantitative traits will be the question of whether a large enough proportion of the genetic variance will be explainable by mapped QTL. MAS, however, has the potential to overcome some of the limitations inherent in conventional selection methods that are based on the direct measurement of the phenotype of an animal and/or its relatives. While some phenotypes are expressed only in one sex and at a specific developmental stage, DNA-based diagnosis can be performed irrespective of sex and at any developmental stage and therefore often much earlier than the time of expression of the actual phenotype. Conventional breeding schemes may be limited in their scope by the difficulty and costs associated with the measurement of some phenotypes. With the growing sophistication of animal food products, the cost figures will increasingly be in favor of a DNA-based diagnosis rather than a direct measurement of the phenotype. The low costs of DNA-based diagnosis also mean that a larger pool of animals can eventually be screened for superior genotypes, allowing for an increase in the so-called selection differential and therefore genetic response.

Mapping QTL segregating in crosses between divergent breeds allows animal breeders to envisage marker-assisted introgression of desired portions of an exotic genome into a commercially elite background. This approach is virtually impossible to envisage in animal production without the use of markers. Indeed, not only do linked markers allow monitoring of retention of the QTLs of interest, but—maybe more important given the prohibitively long generation interval of most domestic animals—markers spread across the rest of the genome also permit the accelerated recovery of the recurrent genome (Hospital et al. 1992). A nice illustration of this strategy is the production of a PSS/PSE-resistant Piétrain pig strain by introgression of the nondefective CRC allele (Hanset et al. 1995). The same strategy will be required in the future to move transgenes efficiently in appropriate commercial backgrounds. It remains to be seen how genetic background effects might interfere with the expression of the grafted genes.

Concerns will have to be addressed with regard to the risk of compromising long-term genetic response at the expense of an accelerated short-term response using MAS, due to the negative linkage disequilibrium that selection establishes between favorable major QTL alleles and the residual polygenes (Gibson 1994).

Whereas microsatellites have undoubtedly boosted the emergence of animal genomics, the costs associated with the present genotyping technology remain too high to warrant a widespread application in a commercial environment. There is a definite need either for a more cost-effective way to determine genotypes at microsatellite loci or for alternative marker systems that are more conveniently typed. Despite the considerable investments in the development and use of microsatellite markers, animal geneticists need to be prepared to accompany human and mouse geneticists in their ongoing technological revolutions.

Transgenics and the Second Phase of Genome Research in Livestock

Since the pioneering experiments of Palmiter and Brinster (Palmiter et al. 1982) that produced giant mice by injection of a rat growth-hormone structural gene under control of a metallothionein promotor into a fertilized mouse egg, the perspectives of transgenics as the ultimate tool for the genetic improvement of livestock have spurred the imagination of many scientists and laymen alike. While conventional breeding strategies are limited to the exploitation of the genetic variation preexisting within the species, if not the breed of interest, transgenics, on the contrary, opened possibilities for exploiting genetic variation across species boundaries and, even more, for exploiting "artefactual" genetic variants created in vitro.

If transgenesis has become an integral part of the arsenal used by plant breeders, the equivalent methods have proven much more difficult to implement in animal genetics. This reflects the convergence of a number of complicating factors, including technical and economic hurdles associated with the production of transgenic livestock, as well as concerns about public perception.

The recent production of sheep by nuclear transfer from a cultured cell line (Campbell et al. 1996) holds promise that a viable avenue toward the production of transgenic livestock (whether

exploiting homologous recombination or not) might become available in the future. It is often contended, however, that the implementation of transgenic techniques in livestock has been hampered considerably by the limited choice of suitable transgenes. The discipline of genomics will likely help to alleviate this (Georges 1996). The major boost will undoubtedly come from the massive investments that are presently being allocated to the analysis of the human and mouse genomes, and that are generating a myriad of candidate transgenes. In particular, one can expect a dramatic increase in the number of "knock-out" mice produced in the near future. Although most phenotypes associated with knock-out experiments reported so far are debilitating, to say the least (Brandon et al. 1995), it would be surprising if at least some of the obtained knock-out phenotypes did not point toward genes of potential interest to animal breeders and deserve further manipulation in livestock.

In addition, the production genes directly identified in livestock species using the genomic strategies described above will likely become prime candidate genes for further manipulation using transgenic techniques. Rather than being a purely sequential process—genomics followed by transgenics—one can anticipate a continuous interplay between both disciplines. Transgenic tools are indeed likely to become a key component for testing hypotheses with regard to function and regulation of underlying genes uncovered by genomic approaches.

References

Andersson, L., C.S. Haley, H. Ellegren, S.A. Knott, M. Johansson, K. Andersson, L. Andersson-Eklund, I. Edfors-Lilja, M. Fredholm, I. Hansson, J. Hkansson, and K. Lundström. 1994. Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science* **263:** 1771–1774.

Archibald, A.L. 1994. Fat pigs can blame their genes. *Curr. Biol.* **4:** 728–730.

Archibald, A.L., C.S. Haley, J.F. Brown, S. Couperwhite, H.A. McQueen, D. Nicholson, W. Coppieters, A. Van de Weghe, A.K. Wintero, M. Fredholm, N.J. Larsen, V.H. Nielsen, D. Milan, N. Woloszyn, A. Robic, M. Dalens, J. Riquet, J. Gellin, J.-C. Caritez, D. Hue, G. Burgaud, L. Ollivier, J.-P. Bidanel, M. Vaiman, C. Renard, H. Gelderman, R. Davoli, D. Ruyter, E.J.M. Vestege, M.A.M. Groenen, W. Davies, B. Hoyheim, A. Keiserud, L. Andersson, H. Ellegeren, M. Johansson, L. Marklund, R.J. Miller, D.V. Anderson-Dear, E. Signer, and A.J. Jeffreys. 1995. The PiGMaP consortium linkage map of the pig (*Sus scrofa*). *Mamm. Genome* **6:** 157–175.

Barendse, W., S.M. Armitage, L.M. Kossarek, A. Shalom, B.W. Kirkpatrick, A.M. Ryan, D. Clayton, L. Li, H.L. Neibergs, N. Zhang, W.M. Grosse, J. Weiss, P. Creighton, F. McCarthy, M. Ron, A.J. Teale, R. Fries, R.A. McGraw, S.S. Moore, M. Georges, M. Soller, J.E. Womack, and D.J.S. Hetzel. 1994. A genetic linkage map of the bovine genome. *Nature Genet.* **6:** 227–235.

Beattie, C. 1994. Livestock genome maps. *Trends Genet.* **10:** 334–338.

Bishop, M.D., S.M. Kappes, J.W. Keele, R.T. Stone, S.L.F. Sunden, G.A. Hawkins, S. Solinas Toldo, R. Fries, M.D. Grosz, J. Yoo, and C.W. Beattie. 1994. A genetic linkage map for cattle. *Genetics* **136:** 619–639.

Boehnke, M. 1994. Limits of resolution of linkage studies: Implications for the positional cloning of disease genes. *Am. J. Hum. Genet.* **55:** 379–390.

Bovenhuis, H,. J.A. Van Arendonk, and S. Korver. 1992. Associations between milk protein polymorphisms and milk production traits. *J. Dairy Sci.* **75:** 2549–2559.

Brandon, E.P., R.L. Idzerda, and G.S. McKnight. 1995. Targeting the mouse genome: A compendium of knockouts. *Curr. Biol.* **5:** 758–765, 873–881.

Broom M.F. and D.F. Hill. 1994. Construction of a large-insert yeast artificial chromosome library from sheep DNA. *Mamm. Genome* **5:** 817–819.

Bumstead, N. and J. Palyga. 1992. A preliminary linkage map of the chicken genome. *Genomics* **13:** 690–697.

Cai, L., J.F. Taylor, R.A. Wing, D.S. Gallagher, S.-S. Woo, and S.K. Davis. 1995. Construction and characterization of a bovine bacterial artificial chromosome library. *Genomics* **29:** 413–425.

Campbell, K.H.S., J. McWhir, W.A. Ritchie, and I. Wilmut. 1996. Sheep cloned by nuclear transfer from a cultured cell line. *Nature* **380:** 64–66.

Charlier, C., W. Coppieters, F. Farnir, L. Grobet, P. Leroy, C. Michaux, M. Mni, A. Schwers, P. Vanmanshoven, R. Hanset, and M. Georges. 1995. The *mh* gene causing double-muscling in cattle maps to bovine chromosome 2. *Mamm. Genome* **6:** 788–792.

Charlier, C., B. Denys, J.I. Belanche, W. Coppieters, L. Grobet, M. Mni, J. Womack, R. Hanset, and M. Georges. 1996a. Microsatellite mapping of a major determinant of *White Heifer Disease*: The bovine *roan* locus. *Mamm. Genome* **7:** 138–142.

Charlier, C., F. Farnir, P. Berzi, P. Vanmanshoven, B. Brouwers, H. Vromans, and M. Georges. 1996b. Identity-by-descent mapping of recessive traits in livestock: Application to map the bovine *syndactyly* locus to chromosome 15. *Genome Res.* **6:** 580–589.

Cheng, H.H., I. Levin, R.L. Vallejo, H. Kathib, J.B. Dodson, L.B. Crittenden, and J. Hillel. 1996. Development of a genetic map of the chicken with markers of high utility. *Poult. Sci.* (in press).

Chumakov, I.M., P. Rigault, I. LeGall, C. Bellanne-Chantelot, A. Billault, S. Guillou, P. Soularue, G. Guasconi, E. Poullier, I. Gros, et al. 1995. A YAC contig map of the human genome. *Nature* **377:** 175–297.

Cockett, N.E., S.P. Jackson, T.D. Shay, D. Nielsen, R.D. Green, and M. Georges. 1994. Chromosomal localization of the callipyge gene in sheep (*Ovis aries*) using bovine DNA markers. *Proc. Natl. Acad. Sci.* **91:** 3019–3023.

Cockett, N.E., S.P. Jackson, T.D. Shay, F. Farnir, S. Berghmans, G. Snowder, D. Nielsen, and M. Georges. 1996. Polar overdominance at the ovine callipyge locus. *Science* **273:** 236–238.

Collins, F.S. 1995. Positional cloning moves from perditional to traditional. *Nature Genet.* **9:** 347–350.

Crawford, A.M., K.G. Dodds, A.J. Ede, C.A. Pierson, G.W. Montgomery, H.G. Garmonsway, A.E. Beattie, K. Davies, J.F. Maddox, S.W. Kappes, R.T. Stone, T.C. Nguyen, J.M. Penty, E.A. Lord, J.E. Broom, J. Buitkamp, W. Schwaiger, J.T. Epplen, P. Matthew, M.E. Matthews, D.J. Hulme, K.J. Beh, R.A. McGraw, and C. Beattie. 1995. An autosomal genetic linkage map of the sheep genome. *Genetics* **140:** 703–724.

Crooijmans, R.P.M.A., P.A.M. Van Oers, J.A. Strijk, J.J. Van der Poel, and M.A.M. Groenen. 1996. Preliminary linkage map of the chicken (*Gallus domesticus*) genome based on microsatellite markers: 77 new markers mapped. *Poult. Sci.* (in press).

Darwin, C. 1859. The origin of species by means of natural selection or the preservation of favoured races in the struggle for life. Mentor edition (1958), NAL Penguin, New York, NY.

Duriez, B., M.-L. Sobrier, P. Duquesnoy, M. Tixier-Boichard, E. Decuypere, G. Coquerelle, M. Zeman, M. Goossens, and S. Amselem. 1993. A naturally occuring growth hormone receptor mutation: In vivo and in vitro evidence for the functional importance of the WS motif common to all members of the cytokine receptor superfamily. *Mol. Endocrinol.* **7:** 806–814.

Edfors-Lilja, I., U. Gustafsson, Y. Duval-Iflah, H. Ellegren, M. Johansson, R.K. Juneja, L. Marklund, and L. Andersson. 1995. The porcine intestinal receptor for Escherichia coli K88ab, K88ac: Regional localization on chromosome 13 and influence on IgG response to the K88 antigen. *Anim. Genet.* **26:** 237–242.

Eggen, A and R. Fries. 1995. An integrated cytogenetic map of the bovine genome. *Anim. Genet.* **26:** 215–236.

Ellegren, H., C.J. Davies, and L. Andersson. 1993. Strong association between polymorphisms in an intronic microsatellite and in the coding sequence of the BoLA-DRB3 gene; implications for microsatellite stability and PCR-based DRB3 typing. *Anim. Genet.* **24:** 269–275.

Ellegren, H., B.P. Chowdhary, M. Johansson, L. Marklund, M. Fredholm, I. Gustavsson, and L. Andersson. 1994. A primary linkage map of the porcine genome reveals a low rate of genetic recombination in the pig. *Genetics* **137:** 1089–1100.

Falconer, D.S. and T.F.C. Mackay. 1996. Introduction to quantitative genetics, 3rd edition. Longman Scientific and Technical, Essex, UK.

Fuji, J., K. Otsu, F. Zorzato, S. Deleon, V.K. Khanna, J.E. Weiler, P.J. O'Brien, and D.H. MacLennan. 1991. Identification of a mutation in the porcine ryanodine receptor associated with malignant hyperthermia. *Science* **253:** 448–451.

Geldermann, H., U. Pieper, and B. Roth. 1985. Effects of marked chromosome sections on milk performance in cattle. *Theor. Appl. Genet.* **70:** 138–146.

Georges, M. 1996. Recent progress in mammilian genomics and its implication for the selection of candidate transgenes in livestock species. In *Transgenic animals: Generation and use* (ed. L.M. Houdebine). Harwood Academic Publishers, New York, NY.

Georges, M., R. Drinkwater, A. Lefort, F. Libert, T. King, A. Mishra, D. Nielsen, L.S. Sargeant, A. Sorenson, M. R. Steele, X. Zhao, J.E. Womack, and J. Hetzel. 1993a. Microsatellite mapping of a gene affecting horn development in *Bos taurus*. *Nature Genet.* **4:** 206–210.

Georges, M., M. Lathrop, A.B. Dietz, A. Lefort, F. Libert, A. Mishra, D. Nielsen, L.S. Sargeant, M.R. Steele, X. Zhao, H. Leipold, and J.E. Womack. 1993b. Microsatellite mapping of the gene causing *weaver* disease in cattle will allow the study of an associated QTL. *Proc. Natl. Acad. Sci.* **90:** 1058–1062.

Georges, M., D. Nielsen, M. Mackinnon, A. Mishra, R. Okimoto, A.T. Pasquino, L.S. Sargeant, A. Sorensen, M.R. Steele, X. Zhao, J.E. Womack, and I. Hoeschele. 1995. Mapping quantitative trait loci controlling milk production by exploiting progeny testing. *Genetics* **139:** 907–920.

Gibson, J.P. 1994. Short-term gain at the expense of long-term response with selection of identified loci. In *Proceedings of the 5th World Congress on Genetics Applied to Livestock Production*, August 7-12, 1994, University of Guelph, Ontario, Canada. (ed. C. Smith, J.S. Gavora, B. Benkel, J. Chesnais, W. Fairfull, J.P. Gibson, B.W. Kennedy, E.B. Burnside), **21:** 201–204.

Guérin, G., Y. Duval-Iflah, M. Bonneau, M. Bertaud, P. Guillaume, and L. Ollivier. 1993. Evidence for linkage between K88ab, K88ac intestinal receptor to *Escherichia coli* and transferrin loci in pigs. *Anim. Genet.* **24:** 393–396.

Haley, C.S., S.A. Knott, and J.M. Elsen. 1994. Mapping quantitative trait loci in crosses between outbred lines using least squares. *Genetics* **136:** 1195–1207.

Hanset, R., C. Dasnois, S. Scalais, C. Michaux, and L. Grobet. 1995. Effets de l'introgression dans le génome Piétrain de l'allèle normal au locus de sensibilité à l'halothane. *Genet. Sel. Evol.* **27:** 77–88.

Hastbacka, J., A. de la Chapelle, I. Kaitila, P. Sistonen, A. Weaver, and E. Lander. 1992. Linkage disequilibrium mapping in isolated founder populations: Diastrophic dysplasia in Finland. *Nature Genet.* **2:** 204–211.

Hoeschele, I. and T.R. Meinert. 1990. Association of genetic defects with yield and type traits: The *weaver* locus effect on yield. *J. Dairy Sc.* **73:** 2503–2515.

Hospital, F., C. Chevalet, and P. Mulsant. 1992. Using markers in gene introgression breeding programs. *Genetics* **132:** 1199–1210.

Huang, N., L.A. Cogburn, S.K. Agarwal, H.L. Marks, and J. Burnside. 1993. Overexpression of a truncated growth hormone receptor in the sex-linked dwarf chicken: Evidence for a splice mutation. *Mol. Endocrinol.* **7:** 1391–1398.

Johansson, M., H. Ellegren, L. Marklund, U. Gustavsson, E. Ringmar-Cederberg, K. Andersson, I. Edfors-Lilja, and L. Andersson. 1992. The gene for dominant white color in the pig is closely linked to ALB and PDGFRA on chromosome 8. *Genomics* **14:** 965–969.

Johansson, M., L. Marklund, K. Sandberg, and L. Andersson. 1994. Cosegregation between the chestnut coat colour in horses and polymorphisms at the melanocyte stimulating hormone (MSH) receptor locus. *Anim. Genet.* **25 (Suppl. 2):** 35.

Johansson, M., H. Ellegren, and L. Andersson. 1995. Comparative mapping reveals extensive linkage conservation—but with gene order rearrangements—between the pig and human genomes. *Genomics* **25:** 682–690.

Johansson Moller, M., R. Chaudhary, E. Hellmén, B. Höyheim, B. Chowdhary, and L. Andersson. 1996. Pigs with the dominant white coat color phenotype carry a duplication of the KIT gene encoding the mast/stem cell growth factor receptor. *Mamm. Genome.* (in press).

Klungland, H., D.I. Vage, L. Gomez-raya, S. Adalsteinsson, and S. Lien. 1995. The role of melanocyte-stimulating hormone (MSH) receptor in bovine coat color determination. *Mamm. Genome* **6:** 636–639.

Langford, C.F., H. Telenius, J.R. Miller, P.D. Thomson, and E.M. Tucker. 1993. Preparation of chromosome-specific paints and complete assignment of chromosomes in the pig flow karyotype. *Anim. Genet.* **24:** 261–267.

Leeb, T., G. Rettenberger, H. Hameister, G. Brem, and B. Brenig. 1995. Construction of a porcine YAC library and mapping of the cardiac-muscle ryanodine receptor gene to chromosome 14q22-q23. *Mamm. Genome* **6:** 37–41.

Levin, I., L. Santangelo, H. Cheng, L.B. Crittenden, and J.B. Dodgson. 1994. An autosomal genetic linkage map of the chicken. *J. Hered.* **85:** 79–85.

Libert, F., A. Lefort, R. Okimoto, and M. Georges. 1993. Construction of a bovine genomic library of large yeast artificial chromosome clones. *Genomics* **18:** 270–276.

Ma, R.Z., J.E. Beever, Y. Da, C.A. Green, I. Russ, C. Park, D.W. Heyen, R.E. Everts, S.R. Fisher, K.M. Overton, A.J. Teale, S.J. Kemp, H.C. Hines, G. Guérin, and H.A. Lewin. 1996. A male linkage map of the cattle (*Bos taurus*) genome. *J. Hered.* (in press).

MacLennan, D.H. and M.S. Phillips. 1993. Malignant hyperthermia. *Science* **256:** 789–794.

Mariani, P., K. Lundstrom, U. Gustafsson, A.-C. Enalt, R.K. Juneja, and L. Andersson. 1995. A major locus (Rn) affecting muscle glycogen content is located on pig chromosom 15. *Mamm. Genome* **7:** 52–54.

Mariani, P., M. Johansson, B. Høyheim, L. Marklund, W. Davies, H. Ellegren, and L. Andersson. 1996. The extension coat color locus and the loci for blood group O and tyrosine aminotransferase are on pig chromosome 6. *J. Hered.* (in press).

Marklund, L., W. Davies, H. Ellegren, M. Fredholm, B. Hoyheim, M. Johansson Moller, K. Juneja, P. Mariani, W. Coppieters, and L. Andersson. 1996. A comprehensive pig linkage map based on wild pig–Large White intercross. *Anim. Genet.* **27:** (in press).

Ménissier, F. 1982. Present state of knowledge about the genetic determination of muscular hypertrophy or the double muscled trait in cattle. In *Current topics in veterinary medicine and animal science*, vol. 16: *Muscle hypertrophy of genetic origin and its use to improve beef production* (ed. J.W.B. King and F. Ménissier), pp. 387–428. Martinus Nijhoff, The Hague, The Netherlands.

Milan, D., P. Le Roy, N. Woloszyn, J.C. Caritez, J.M. Elsen, P. Sellier, and J. Gellin. 1995. The RN locus for meat quality maps to pig chromosme 15. *Genet. Sel. Evol.* **27:** 195–199.

Milan, D., N. Woloszyn, M. Yerle, P. Leroy, M. Bonnet, J. Riquet, Y. Lahbibmansais, J.C. Caritez, A. Robic, P. Sellier, J.M. Elsen, and J. Gellin. 1996. Accurate mapping of the acid meat RN gene on genetic and physical maps of pig chromosome 15. *Mamm. Genome* **7:** 47–51.

Montgomery, G.W., A.M. Crawford, J.M. Penty, K.G. Dodds, A.J. Ede, H.M. Henry, C.A. Pierson, E.A. Lord, S.M. Galloway, A.E. Schmack, et al. 1994. The ovine Booroola fecundity gene (FecB) is linked to markers from a region of human chromosome 4q. *Nature Genet.* **4:** 410–414.

Moore, S.S., L. Sargeant, T.J. King, J.S. Mattick, M. Georges, and D.M.S. Hetzel. 1991. The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. *Genomics* **10:** 654–660.

Nadeau, J.H. and B.A. Taylor 1984. Length of

chromosomal segments conserved since the divergence of man and mouse. *Proc. Natl. Acad. Sci.* **81:** 814–818.

Neimann-Sorensen, A. and A. Robertson. 1961. The association between blood groups and several production characteristics in three Danish Cattle breeds. *Acta Agric. Scand.* **11:** 163–196.

O'Brien, S.J. 1991. Mammalian genome mapping: Lessons and prospects. *Curr. Opin. Genet. Devel.* **1:** 105–111.

O'Brien, S.J., J.E. Womack, L.A. Lyons, K.J. Moore, N.A. Jenkins, and N.G. Copeland. 1993. Anchored reference loci for comparative genome mapping in mammals. *Nature Genet.* **3:** 103–112.

Palmiter, R.D., R.L. Brinster, R.E. Hammer, M.E. Trumbauer, M.G. Rosenfeld, N.C. Birnberg, and R.M. Evans. 1982. Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature* **300:** 611–615.

Pearson, R.E., W.E. Vinson, and T.R. Meinert. 1990. The potential for increasing productivity through selection for increased milk and component yields. *Proceedings of the 4th World Congress in Genetics Applied to Livestock Production,* July 23–27, 1990, Edinburgh, UK (ed. W.G. Hill, R. Thomson, and J.A. Woolliams), **14:** 104–113.

Peters, J. and A.G. Searle. 1996. Linkage and synteny homologies in mouse and man. In *Genetic variants and strains of the laboratory mouse* (ed. M.F. Lyon, S. Rastan, and S.D.M. Brown), pp. 1256–1311. Oxford University Press, Oxford, UK.

Ponce de Leon, F.A., S. Ambadt, G.A. Hawkins, S.M. Kappes, M.D. Bishop, J.M. Robl, and C.W. Beattie. 1996. Cattle sheep and goat X-chromosome segment homologies assessed by chromosome painting: Development of a bovine linkage group. *Proc. Natl. Acad. Sci.* (in press).

Rettenberger, G., C. Klett, U. Zecher, J. Kunz, W. Vogel, and H. Hameister 1995. Visualisation of the conservation of synteny between humans and pigs by heterologous chromosomal painting. *Genomics* **26:** 372–378.

Rohrer, G.A., L.J. Alexander, J.W. Keele, T.P. Smith, and C.W. Beattie. 1994. A microsatellite linkage map of the porcine genome. *Genetics* **136:** 231–245.

Rohrer, G.A., L.J. Alexander, Z. Hu, J.W. Keele, T.P. Smith, and C.W. Beattie. 1996. A comprehensive map of the porcine genome. *Genome Res.* **6:** 371–391.

Rothshild, M., C. Jacobson, D. Vaske, C. Tuggle, L. Wang, T. Short, G. Eckardt, S. Sasaki, A. Vincent, D. McLaren, O. Southwood, H. van der Steen, A. Milehm, and G. Plastow. 1996. The estrogen receptor locus is associated with a major gene influencing litter size in pigs. *Proc. Natl. Acad. Sci.* **93:** 201–205.

Rudolph, J.A., S.J. Spier, G. Byrns, C.V. Rojas, D. Bernoco, and E.P. Hoffman. 1992. Periodic paralysis in quarter horses: A sodium channel mutation disseminated by selective breeding. *Nature Genet.* **2:** 144–147.

Schmitz, A., P. Chardon, I. Gainche, B. Chaput, M.N. Guilly, G. Frelat, and M. Vaiman. 1992. Pig standard bivariate flow karyotype and peak assignment for chromosomes X, Y, 3, 7. *Genomics* **14:** 357–362.

Schmitz, A., A. Oustry, B. Chaput, I. Bahri-Darwich, M. Yerle, D. Millan, G. Frelat, and E.P. Cribiu. 1995. The bovine bivariate flow karyotype and peak identification by chromosome painting with PCR-generated probes. *Mamm. Genome* **6:** 415–420.

Schmutz, S.M., T.G. Berryere, J.S. Moker, T.D. Thue, and D.C. Winkelman. 1994. Gene mapping from a bovine 1:29 DNA library prepared with chromosome microdissection. *Mamm. Genome* **5:** 138–141.

Smith, T.P., L.J. Alexander, T.S. Sonstegard, J. Yoo, C.W. Beattie, and M.F. Broom. 1996. Construction and characterization of a large insert bovine YAC library with five-fold genomic coverage. *Mamm. Genome* **7:** 155–156.

Solinas-Toldo, S., C. Lengauer, and R. Fries. 1995. Comparative genome map of man and cattle. *Genomics* **27:** 489–496.

Spelman, R.J., W. Coppieters, J.A.M. van Arendonk, and H. Bovenhuis. 1996. Quantitatve Trait Loci analysis for five milk production traits on chromosome six in the dutch Holstein-Friesian population. *Genetics* (in press).

Steele, M. and M. Georges. 1991. Generation of bovine multisite haplotypes using random cosmids. *Genomics* **10:** 889–904.

Sun, H.S. and B.W. Kirkpatrick. 1996. Exploiting dinucleotide microsatellites conserved among mammalian species. *Mamm. Genome* **7:** 128–132.

Vaiman, D., O. Koutita, A. Oustry, J.M. Elsen, E. Manfredi, M. Fellous, and E.P. Cribiu. 1996. Genetic mapping of the autosomal region involved in XX sex-reversal and horn development in goats. *Mamm. Genome* **7:** 133–137.

Voegeli, P., R. Fries, G. Leemann, H.U. Bertschinger, and G. Stranzinger. 1994. Genes for the AO inhibition (S-locus) are closely linked to the genes specifying receptors of F107 fimbriated Escherichia coli strains causing oedema disease and post-weaning diarrhoea in the pig. *Anim. Genet.* **25 (Suppl. 2):** 66.

Womack, J.E. and Y.D. Moll. 1986. Gene map of the cow: Conservation of linkage with mouse and man. *J. Hered.* **77:** 2–7.

Yerle, M., A. Schmitz, D. Milan, B. Chaput, L. Monteagudo, M. Vaiman, G. Frelat, and J. Gellin. 1993. Accurate characterization of porcine bivariate flow karyotype by PCR and fluorescence in situ hybridization. *Genomics* **16:** 97–103.

Yu, T.P., C.K. Tuggle, C.B. Schmitz, and M.F. Rotschild. 1995. Association of PIT1 polymorphisms with growth and carcass traits in pigs. *J. Anim. Sci.* **73:** 1282–1288.