POSSIBLE CONTRIBUTION OF MOLECULAR GENETICS TO THE RABBIT'S FUTURE

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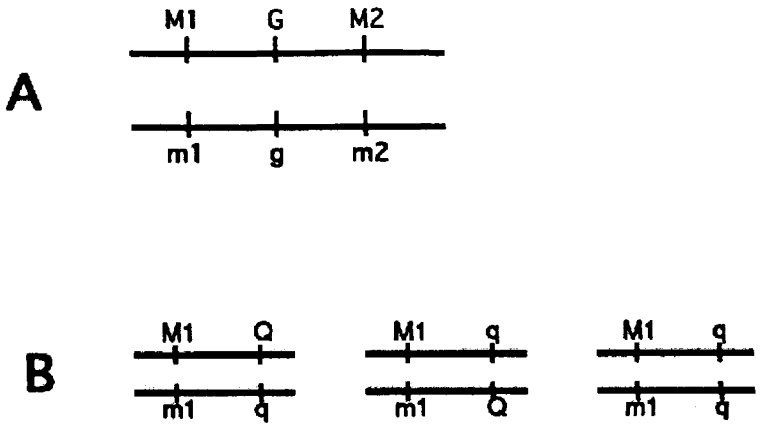
Abstract - Recent breakthroughs in DNA technology allow the rapid isolation of numerous polymorphic DNA markers. Once ordered into a genetic map, these markers can be efficiently used for the indirect detection of genes intervening in production traits, and the selection of the favourable genotypes. The present achievements of genome analysis in livestock species are presented, and the possible applications to the rabbit are discussed.

Remarkable progress has recently been made in the molecular analysis of the existing genetic variation within animal species. These studies are based on the development of simple and efficient methods of polymorphism detection at the DNA level. They were boosted by, and often took advantage of previous results in man and mouse. The main results have been: (i) for several already detected major genes, closely linked polymorphic marker loci have been identified, thus allowing marker-assisted selection (MAS), (ii) among these major genes intervening in production traits, a few have been characterized, and (iii) chromosomal regions containing previously undetected genes involved in zootechnical performances (Quantitative Trait Loci, or QTL) are now under study. The rabbit species, however, was left aside of this rapid development. The intent of this paper is to summarize the present achievements of genome analysis in livestock species, and to suggest how recent breakthroughs in genome analysis could be applied to the rabbit, at the expense of a reasonable effort.

1. OBJECTIVES

Mapping programmes in livestock species are intended to yield a skeleton of polymorphic markers, covering the whole genome. Provided a sufficient density of the map, which may be estimated at 1 marker every 30-40 centimorgans (cM) or 100-150 evenly spaced markers, this frame will allow to detect linkage between a major gene of interest and at least one of the mapped markers. Once linkage is detected, markers closely linked to this major gene will be used to deduce genotypes at the major gene locus from genotypes at marker loci (Figure 1A). It may be noticed that the power of this procedure is greatly improved when flanking tightly linked markers are available on both sides of the major gene locus. It will therefore be necessary to develop higher-resolution maps at the vicinity of major genes. Once available, linked marker loci can be used for the intra-breed selection of the favourable genotype (homozygous or heterozygous), or in introgression programmes, to accelerate the introduction of the introgressed gene in the recipient genome (MULSANT and ELSEN, 1996).

Figure 1 : Principle of marker-assisted selection (A) or QTL detection (B)



The second objective is the identification of important major genes, through a physiological or genetical approach. The former consists of elucidating the nature of the major gene through a thorough examination of the existing physiological data, and then using this information to clone the gene and identify causal mutations in its sequence. The latter approach, also known as positional cloning, aims at: (1) the narrowing of the interval between the gene and flanking markers to a size amenable to molecular biology analysis, i.e. in the range 0.5-2 cM, and (2) the isolation and assay of coding sequences in this region. The main advantage of this procedure is that it does not require previous extensive information on the biological effects of the gene of interest. In practice, both approaches will often be combined, an initial genetical analysis assigning the gene to a chromosomal region, and comparative mapping data being used to define the corresponding human or murine regions, which will then be screened for candidate genes.

Finally it is assumed that markers will be of help for the direct detection of genomic regions affecting a trait, also called Quantitative Trait Loci or QTL. The rationale of this approach is the following: if a genetic marker is linked to a QTL, performances will differ, in the progeny of an individual heterozygous at both the marker and the QTL, depending on the transmitted marker allele (Figure 1B). Systematic genome scanning of large populations should thus identify markers linked to a QTL, i.e. with apparent effect on an analyzed trait. However as genotypes at the QTL are not known *a priori*, transmission of an allele at a marker locus linked to a QTL will correspond to a positive, neutral or negative effect on progeny performances, and favourable marker alleles will be determined only intra-family. The efficiency of this procedure is therefore dependant on the size of the analysed families and the current programmes are being performed on 1000-1500 individuals. Under these conditions, the main benefit of this approach is that it should allow the detection of major genes previously unidentified, but also of genes with medium effects. One will notice, however, that the effect of a marker is dependant on both the magnitude of the QTL and the genetic linkage between marker and QTL.

2. CURRENT STATUS IN LIVESTOCK SPECIES

Mapping programmes

Low-resolution maps (10-30 cM intervals between markers) have been published for cattle, pig, chicken and sheep (for a review, see: BEATTIE, 1994) and will soon be available for goat. Higher density maps (about 1000 markers) are being developed for cattle and swine, and should be published in 1996. These maps now ensure an extensive coverage of cattle and swine genomes.

In most cases these maps have been developed by coordinate international consortia, the involved laboratories working on a few common informative families per species.

All these maps are mainly composed of microsatellites (also called Simple Sequence Length Polymorphisms or SSLP), due to: (1) their high degree of polymorphism and therefore informativeness, (2) their codominant nature, and (3) repetitive, possibly semi-automated typing methods. However these markers are generally species-specific, and methods have been developed to allow the comparison of the obtained maps with the much finer human or murine maps. These methods include the incorporation in the maps of coding sequences (known as Type I markers or anchorage loci, as suggested by O'BRIEN,1991), physical mapping of DNA sequences on chromosomes by in situ hybridisation on metaphases, and recently heterologous chromosomal painting: hybridisation on metaphases of one species of probes specific of an entire chromosome from another species, in order to define the inter-species correspondancies between large chromosomal regions (SCHERTHAN *et al.*, 1994).

Linkage of major genes

These newly developed maps have been used to obtain linkage evidence for several major genes, for instance the Weaver gene in cattle, the hornless (polled) genes of cattle and goat, muscle development genes in cattle (double-muscling) and sheep (callipyge), the rn meat quality gene in swine, the Booroola FecB prolificacy gene in sheep. In some cases, e.g. FecB, genetic distances between the gene and the closest markers are now small enough to allow marker-assisted selection in experimental introgression programmes. In this respect, the Booroola gene presents two interesting features. First, no effect of this gene has been demonstrated in carrier males. Marker-based selection is independent of gene expression and thus alleviates the need for time-consuming progeny-testing of male reproducers. Second, no candidate gene has been found in the corresponding human region, which further emphasizes the interest of positional cloning (which is currently under progress for the FecB gene, and for the above-mentioned genes).

Identifying genes

The nature of the phenotypic variation at a few loci has been elucidated. For instance, chicken [dw] dwarf individuals were found to bear mutations in the growth hormone receptor gene, as is in some cases of human dwarfism. The alphaS1 Casein gene of goat was shown to be involved in large hereditary differences in the protein content of goat milk (review: GROSCLAUDE *et al.*, 1994). These genes were chosen for analysis on the basis of the sole physiological information. On the other hand, the gene responsible for halothane sensitivity of homozygous pigs was identified as the Calcium Release Channel, or Ryanodine Receptor (RYR1) gene on the basis of a combination of physiological and genetical data, in both man and swine. The Hal gene was localised in a pig chromosomal region whose homologous human counterparts were identified. The RYR1 candidate gene was then shown to be linked at 0 cM to a human hereditary disease, similar to the Hal gene. Screening of the pig RYR1 gene finally identified a single mutation, separating wild type from affected individuals (OTSU *et al.*, 1991). The swine Hal gene can be considered as a remarkable model system of an efficient use of comparative mapping for the identification of a major gene. Whereas selection on linked genetic markers enabled the elimination of the detrimental halothane-sensitive allele in same breeds (Landrace) but not all (Piétrain), the knowledge of the causal mutation has allowed the development of a simple mutation-specific PCR typing assay and the rapid and error-free assessment of swine genotypes at this locus. This examplifies one main benefit of gene identification, which enables direct genotyping in populations. On the other hand, marker-based typing procedures are dependant on allelic associations, and can be valid only within families in the absence of strong linkage disequilibrium.

QTL detection

QTL genome scans require both a complete genetic map and large families, on which performances will be measured for many zootechnical traits. Most experiments in livestock species are therefore currently under progress. Two studies have been published (reviewed by HALEY, 1995) : in dairy cattle, association was searched in 14 families between alleles at 159 microsatellite loci and performances of male reproducers (measured by evaluation of their daughters). It is of interest that, on the 4 chromosomal regions identified, only one had a significant effect in more than one family. In addition, although this analysis was carried out intra-breed, large effects were demonstrated. These results indicate that major QTLs are still segregating in selected populations. In pig, chromosome 4 was shown to contain QTL region(s) associated with growth rate and fat content. The studied population however was a cross between wild boar and Large White, and the revealed QTL can possibly differentiate wild boars from commercial animals and be fixed in current commercial breeds. Some simplified surveys have been carried out, aiming at testing whether one particular gene or region is playing a role in a zootechnical trait, either directly or through linkage to a QTL. For instance, in cattle, a genetic effect of alleles at the prolactin locus on lactation performances was demonstrated, and associations are being searched between prolificacy and allele transmission at the bovine equivalents of the Booroola FecB markers.

3. RABBIT

Current status

As the costs of DNA marker analysis can be considered as independent of the value of the animals, the studied species were initially selected on the basis of their economical or scientific importance. Therefore molecular genetics in livestock species was first restricted to species where it is classical to use few reproducers of high value at each generation. There was no coordinate mapping programme of the rabbit, similar to those initiated in other livestock species and, consequently, no genetic map of rabbit has been published since 1990 (FOX, 1990). On the other hand, the rabbit is an animal system of choice for physiological studies. The last releases of Genbank and Embl databases thus contain over 1000 rabbit DNA sequences, mostly cDNAs. Among them, 51 contain microsatellite sequences with a sufficient number of repetitions to be polymorphic.
Rabbit chromosomes can be accurately classified, and physical mapping by in situ hybridisation is therefore possible (e.g. GELLIN *et al.*, 1985; MARTIN-DELEON, 1989). Interspecific rabbit-rodent somatic cell hybrid panels have been produced, which have segregated rabbit chromosomes (ECHARD *et al.*, 1982). They should be of use for synteny studies (genes situated on the same carrier chromosome). Their utilisation for regional localisation (YERLE *et al.*, 1996) awaits the cytogenetic characterisation of the subset of rabbit chromosomes

and chromosomal fragments which has been retained in each hybrid clone. Irradiation hybrids, which allow the fine regional assignment of linked markers, are not yet available.

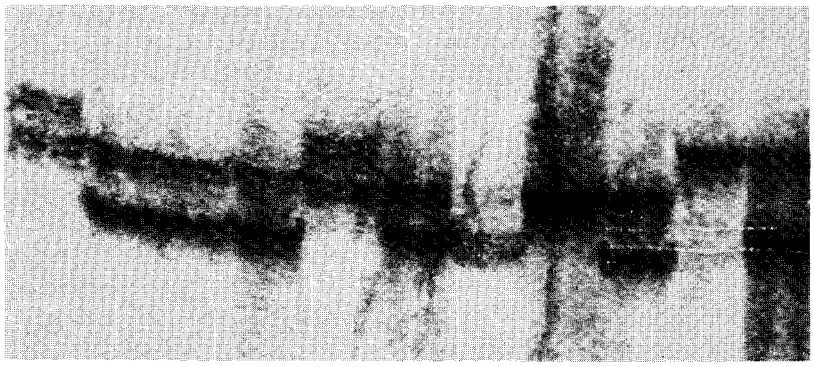
A mapping programme

The rabbit has evident advantages for mapping purposes. Its small size, high prolificacy and short generation interval enable the rapid constitution of the necessary families: initial programmes in other species consisted of a dozen three-generation families, with between 100 and 200 F2 individuals. In order to be informative in both the map families as in the populations to be analysed later, markers must be as polymorphic as possible. This is achieved through the use of microsatellite markers, and for mapping purposes, by crossing largely different breeds.

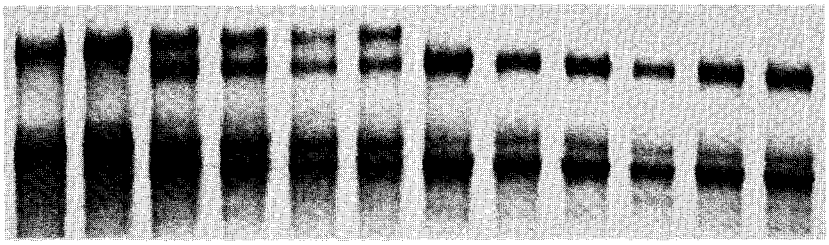
Due to the present scarcity of rabbit microsatellite sequences, it will be necessary to isolate additional markers, mostly microsatellites. It may be considered to add some gene sequences to the set of markers. Typing methods have been developed recently (as reviewed by GROMPE, 1993), which allow the efficient separation of DNA sequences differing by single nucleotide changes. In spite of their frequent biallelic character, which roughly halves their genetic informativeness, the inclusion of gene sequences would have the important benefit to enable comparative mapping between the rabbit map and the detailed human or murine maps, as mentioned previously. In addition, a large number of rabbit gene sequences are already available in data banks. Gene DNA sequences being more conserved between species than microsatellite sequences, it is often possible to amplify rabbit gene fragments from consensus primers between different species. Examples of polymorphism in rabbit at both a microsatellite locus and in a gene sequence are depicted in Figure 2, exemplifying the higher informativeness of microsatellite sequences.

Figure 2 : Examples of polymorphism in a microsatellite (A) and a gene sequence (B)
A. A microsatellite in the rabbit αS1-casein gene (length polymorphism)
B. Fragment of the rabbit FGF5 coding sequence (conformation polymorphism)

A



B



Gene identification

Rabbit populations exhibit considerable phenotypic variations in growth, reproduction or fur quality, as reviewed by ROCHAMBEAU (1988). Studies in mouse or man now provide candidate genes, whose variation could explain the observed phenotypic variations. For example, it has recently been shown that the inactivation of the gene coding for the FGF5 growth factor was responsible for the angora phenotype of the mouse, in both natural and artificial "knock-out" mutants. We are currently testing the hypothesis that the angora mutation (l allele *vs* L for WT) of rabbits is also due to a defect in the FGF5 gene: heterozygous (Ll) animals were

crossed with homozygous (ll) angora individuals and we are looking for a complete cosegregation of FGF5 and angora alleles in the progeny. If the expected absence of recombinants is indeed observed, genes from wild-type or angora individuals will be screened, in order to identify the causal mutation. As the rabbit FGF5 gene has not been cloned, coding sequences have to be amplified with consensus primers between human and mouse (Figure 2B).

In addition, transgenesis in rabbits has been carried out repeatedly since the first experiments of HAMMER *et al.* (1985) and BREM *et al.* (1985). The current procedure of micro-injection adds one external gene to the resident endogenous ones and does not allow to control neither the integration site nor the copy number of the transferred gene sequences. It is therefore of poor value in present selection schemes. However, it enables to perform "gain of function" experiments, and for instance, it should be of use to test the direct involvement of the FGF5 gene in the angora phenotype, by transferring a functional FGF5 gene into angora embryos.

Such obvious candidate genes will not always be available. For other already identified major genes, a first mapping experiment is likely to be necessary to define more precisely the chromosomal region where they are located, and search for candidate genes within this region. Considering the importance of such an experiment (about 100-150 backcross individuals typed at 100-150 loci), protocols aiming at the rapid isolation of a first marker linked to the studied gene would be an interesting alternative. Such protocols allowing the direct isolation of tightly linked polymorphic DNA fragments, have been recently described (LISITSYN *et al.*, 1994). They need a large full-sib family from a parent heterozygous at the tested locus to be constituted (20-30 progeny individuals). Until now the efficiency of this method has been proven in only a few cases of mouse genetic defects. Its practical value could be tested in rabbits, where the required large full-sib families can easily be constituted.

QTLs

Complete genome scans comprise about 1000-1500 individuals in large families, typed at a minimal number of 100-150 loci. The tested markers will have to be polymorphic in the analysed populations. This implies the previous development of a medium-resolution map, with the isolation and localisation of a higher number of genetic markers. Besides, the cost of a QTL scan can be estimated at more than 300,000 euros, and will have to be weighted against the costs of classical selection schemes. Furthermore, the first QTL experiments in dairy cattle showed that, as could have been expected, the involved chromosomal regions vary between reproducers. It is therefore likely that the first rabbit QTL programmes will be restricted to zootechnical traits which are currently difficult to measure (for instance, disease resistance) and will be of the above-mentioned simplified type, a few candidate chromosomal regions being tested, in order to maintain the number of DNA typings to an acceptable level.

CONCLUSION

The objectives of molecular genetics are to localise loci of genes of interest on the relevant chromosomes, to control allele transmission at such loci through the use of linked markers and to identify their nature. A prerequisite is the development of a low-resolution map. Previous results in other species have clearly shown that the efficiency of such a mapping programme is increased by large inter-laboratories collaboration. INRA is beginning such a mapping programme between two commercial breeds, and we plan to reproduce this successful coordinated scheme, with a large circulation of DNAs, markers and results between partners. Based on the present information, it can be estimated that a first low-resolution map of rabbit could be obtained in less than two years. This map could rapidly be of use to elucidate the molecular nature of a few already proven major genes (e.g. the angora and rex genes), through both the assessment of available physiological data in rabbit and other species and the comparison of rabbit and man (or mouse) genetic maps.

The efficiency of rabbit mapping programmes to detect genes intervening in traits with multigenic control is more uncertain. In Western Europe, meat rabbit producers are fighting against poultry, pig and beef producers to maintain market shares. They need productivity gains, and genetic improvement is one way to obtain them. QTL scans are expected to accelerate genetic progress, by defining chromosomal regions containing genes controlling a zootechnical performance. However their extensive use in rabbits is limited by the high size and cost of the required experimental designs, and will therefore depend on further technical breakthroughs.

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Contribution potentielle de la génétique moléculaire à l'avenir du lapin - Les méthodes actuelles de biologie moléculaire permettent l'isolement de nombreux marqueurs moléculaires polymorphes et bien répartis sur les chromosomes des animaux. Ces marqueurs peuvent être ordonnés en cartes génétiques, et sont alors utilisés pour détecter des gènes intervenant dans des caractères de production, et sélectionner indirectement les animaux présentant des génotypes favorables. Les données actuelles dans les principales espèces domestiques sont présentées, et l'application de ces méthodologies au lapin est discutée.