

## MICROSATELLITE DNA MARKERS FOR DUCK (*Anas platyrhynchos* and *Cairina moschata*)

M. E. Christel<sup>1\*</sup>, F. Katia<sup>2</sup>, G. Carine<sup>3</sup>, C. Gaëlle<sup>2</sup>, V. Florence<sup>2</sup>, and V. Alain<sup>2</sup>

<sup>1</sup> INRA, Station d'Amélioration Génétique des Animaux, B.P. 52627, 31326 Castanet-Tolosan.

<sup>2</sup> INRA, Laboratoire de Génétique Cellulaire, B.P. 52627, 31326 Castanet-Tolosan

<sup>3</sup> INRA, Unité de Recherche Génétique des Poissons, 78352 Jouy-en-Josas

\* Corresponding author e-mail : [marie@germinal.toulouse.inra.fr](mailto:marie@germinal.toulouse.inra.fr)

### ABSTRACT

A total of 158 microsatellite markers with dinucleotide to tetranucleotide patterns [(CA)<sub>n</sub>, (TG)<sub>n</sub>, (TAT)<sub>n</sub> and (GATA)<sub>n</sub>] were isolated from a genomic DNA library of common ducks (*Anas platyrhynchos*) and Muscovy ducks (*Cairina moschata*) containing 164.000 clones. Testing these markers on 16 DNA samples led to the selection of 127 functional markers, therefore available for genetic mapping in duck. Of them, 112 (88%) were polymorphic (with an average of 3 to 4 alleles), but some of them were specific to one or the other duck species. Through sequence comparison between these duck markers and the chicken assembly, an estimate of their positions on the chicken genome shows a good coverage of the chromosomes. This pool of microsatellite markers will be completed with published markers, in order to have genome coverage dense enough to carry out QTL detection.

**KEY WORDS:** Duck, Microsatellite markers, Genetic map.

### INTRODUCTION

Today, the improvement of waterfowl selection requires the development of molecular genetic tools. Among the markers available, the microsatellite markers are obvious markers for genetic mapping: indeed, they present many advantages (numerous, regularly distributed along the genome, codominants and highly polymorphic). However, their transposition from one avian species to another (chicken versus duck) is not easy, because of the divergence between species. We developed specific duck microsatellite markers from common and Muscovy ducks samples.

This paper presents a synthesis of the work carried out at Molecular Genetic Lab of INRA, thanks to the tools of the “genotyping platform” of Toulouse, from the DNA library construction, the markers development to the study of their informativity.

### EXPERIMENTAL PROCEDURES

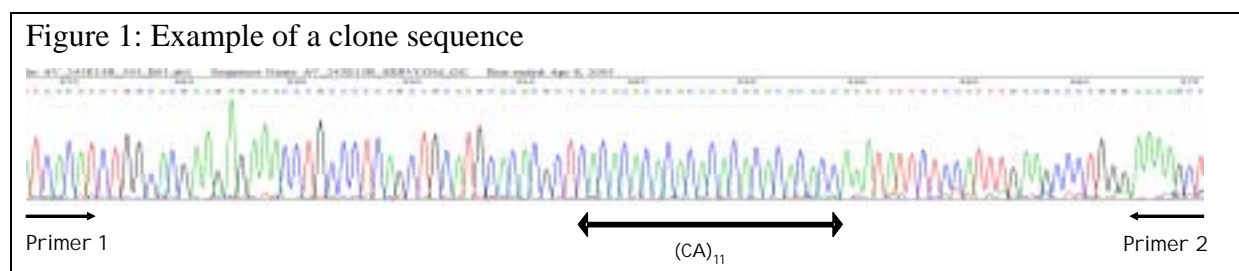
#### *DNA library construction*

The first step to obtain microsatellite markers required the construction of an ordered genomic DNA library. This library was obtained by digestion of a DNA mixture of common and Muscovy ducks; resulting fragments were integrated into bacterial clones, for a total of 164.000 clones ordered in 427 plates of 384 wells. Among these clones, 348 revealed a

positive signal of hybridization with probes of repeated patterns  $(CA)_n$ ,  $(TG)_n$ ,  $(TAT)_n$  and  $(GATA)_n$ , specific of the microsatellite markers (Genet et al., 2003).

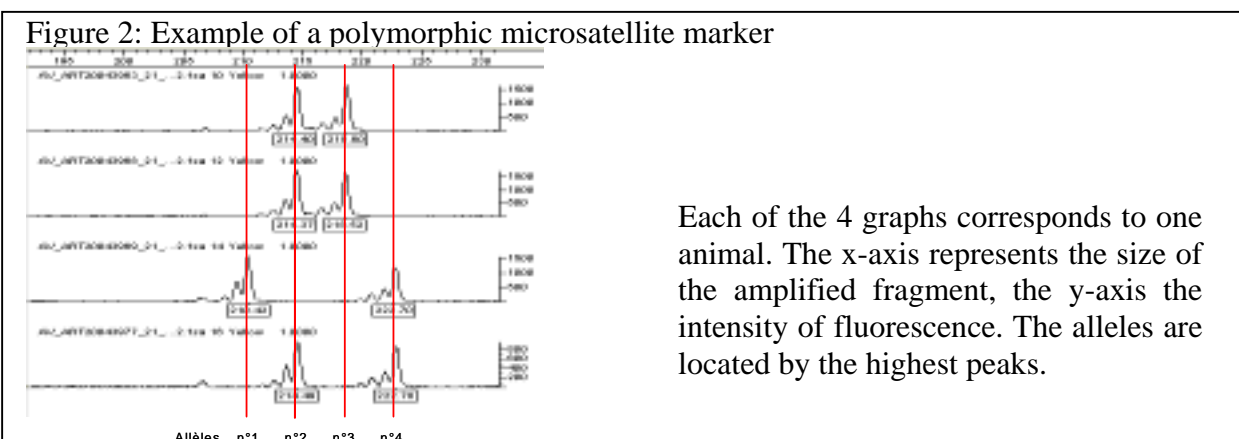
### Sequencing of clones

To confirm the presence of a microsatellite sequence in each fragment, these 348 clones were sequenced. 158 primer pairs flanking the microsatellite area were designed (figure 1): each primer pair was specific of a genome area and of a single marker (Pitel and Riquet, 1999).



### Markers development

All primer sets were tested for amplification and polymorphism on a panel that consisted of 16 DNA samples of unrelated ducks raised at the INRA experimental farm: 8 Muscovy ducks and 8 common ducks, of which 4 came from 2 INRA strains (I444 and I37 strains) and 4 were crosses between these 2 strains and potential sires of a QTL program under progress. First, PCR conditions for these markers were defined (Tagu *et al*, 1999) on the 16 duck DNA samples, in order to obtain markers optimized for automated fluorescent genotyping. Thus, 4 PCR conditions (various temperatures and magnesium concentrations) were retained, allowing to obtain an amplification for 136 developed markers out of the 158 (figure 2).



Among these 136 markers, 127 markers displayed patterns interpretable on automatic sequencer, and only 9 markers were removed after analysis.

## RESULTS AND DISCUSSION

### Polymorphism and informativity of markers

Knowing that the microsatellite markers are interesting only if they present different alleles, i.e. different sizes, we studied the polymorphism of our markers. Thus Figure 2 illustrates a polymorphic marker with 4 alleles of different sizes. Finally, of the 127 markers

selected, 112 were polymorphic with an average of 3 to 4 different alleles per marker. The other 15 markers were monomorphic.

We can note specific markers of the duck species: thus, 22 markers do not show any amplification with Muscovy duck DNA and 13 other markers with common duck DNA (table 1). This characteristic may be due to mutations or lack of homologous sequence on the genome of a species in the primer area. It illustrates the genetic distance between Muscovy duck (*Cairina moschata*) and common duck (*Anas platyrhynchos*).

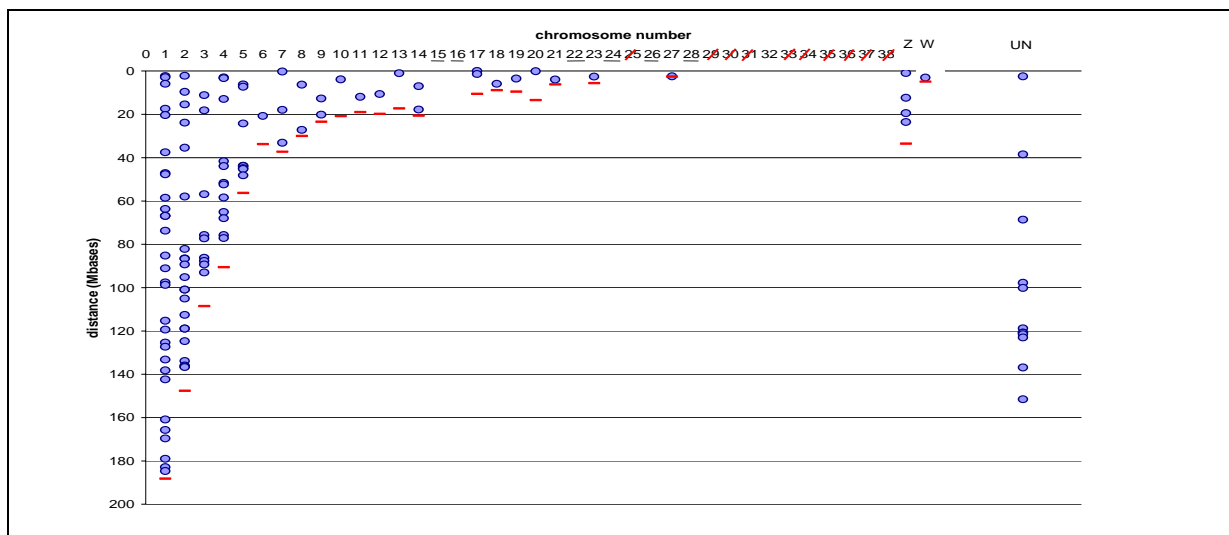
Table 1: Description of species' specificity

112 polymorphic markers		
22 markers with amplification in common ducks only	77 markers with amplification in both species	13 markers with amplification in Muscovy ducks only

The informativity of a marker was defined by its ability to distinguish unambiguously two groups of progeny according to the marker allele received from their parents. It supposes that the relative is heterozygous at marker loci, i.e. presents 2 alleles of different sizes. In our study, for the 4 F1 common ducks, we obtained 51 markers for which at least 1 of the males was heterozygous, therefore potentially informative for a QTL design.

**Markers position on chromosome**

The first chicken genome sequence assembly was released in March 2004 (International Chicken Genome Sequencing Consortium, 2004). This sequence of 907 Mb covers approximately 86% of the genome: on the 38 autosomes, 10 microchromosomes do not have an assigned sequence, but those sequences are gathered into a chromosome called “unknown” ([http://www.ensembl.org/Gallus\\_gallus/index.html](http://www.ensembl.org/Gallus_gallus/index.html)).



*chr*: lack of sequence on these chromosomes (10 chromosomes)  
*chr*: chromosomes with no microsatellite markers (7 chromosomes)

Figure 3: Position of our duck microsatellite markers on chicken genome

Through a BLAST analysis between our markers and the complete sequence of the chicken genome ([http://www.ensembl.org/Gallus\\_gallus/blastview.html](http://www.ensembl.org/Gallus_gallus/blastview.html)), we localised

chicken loci orthologous to 120 of our duck microsatellite markers on the chicken chromosomes (figure 3).

The validity of this approach of sequences comparison was shown by Kayang and al. (2006) between the chicken and quail species. The chromosomes with known sequences present a good coverage by these markers. However, 7 microchromosomes remain without any marker. It is likely that among the 38 markers without localisation on the chicken sequence (too low sequence similarity), some would be localised on duck microchromosomes. Moreover, the lower concentration of microsatellite markers on the microchromosomes compared to the macrochromosomes is well-known in birds (Primmer *et al.*, 1997).

## CONCLUSION

Thanks to the development of duck microsatellite markers in the Molecular Genetic Lab of INRA, we have 127 markers available for genetic mapping in this species. On average, for a given experimental animal design, approximately 50% of them are informative. It will be necessary to complement this panel of markers, by new markers located in the areas with a low coverage. In order to do that, 326 microsatellite markers (developed by Chinese, Japanese and German teams) are available in the international data bases or recently published (Huang *et al.*, 2005). This work will give us an essential tool to detect Quantitative Trait Locus in waterfowl production. An animal design is in progress at INRA experimental farm, aiming at identifying genomic regions affecting the zootechnical traits of forced fed duck. For such an analysis, it will be necessary to have approximately 150 informative genetic markers.

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