QTL RESEARCH ON DURATION OF TONIC IMMOBILITY IN QUAIL

O. Roussot[1], F. Pitel[1], A. Vignal[1], J.M. Faure[2], A.D. Mills[2], D. Guémené[2], C. Leterrier[2], S. Mignon-Grasteau[2], P. Le Roy[3], M. Perez-Enciso[4] and C. Beaumont[2]

[1] INRA, Laboratoire de Génétique Cellulaire, 31326 Castanet-Tolosan, France
[2] INRA, Station de Recherches Avicoles, 37380 Nouzilly, France
[3] INRA, Station de Génétique Quantitative et Appliquée, 78352 Jouy-en-Josas, France
[1] INRA, Station d'Amélioration Génétique des Animaux, 31326 Castanet-Tolosan, France

INTRODUCTION

Although fear is an adaptive reaction, especially to allow the animal to respond appropriately to threatening stimuli, it may also have deleterious effects on animal welfare, health and productivity, in peculiar if intense or prolonged (Jones *et al*., 1994) or when fully expressed by animals in captivity. Fearfulness, i.e. propensity to respond fearfully to a wide range of potentially alarming stimuli (Jones *et al.,* 1994) is thus an important component of the animal's capacity of adaptation. But very little is known about its genetic control and relationships with other traits. This is particularly the case for emotionality, defined by Hall (1934) as related to the behavioural and peripheral changes hypothesized to accompany high sympathetic nervous system activity, which appears to be a complex trait depending on several components (Ramos and Mormède, 1998). Identifying genes involved in the control of fearfulness could help to better understand the mechanisms involved in the control of this trait and their relationships with other traits. It could also suggest new methods of reduction of stress.

A QTL (for Quantitative Trait Loci) research was initiated to identify the genome regions involved in the control of fearfulness. Focus was on tonic immobility reaction, an unlearned response characterized by a catatonic-like state of reduced responsiveness to external stimulation (Jones, 1986), which has been shown to be a behavioural tool to assess fearfulness (Jones, 1986). The QTL research was achieved on a second generation cross (F2) between two quail lines divergently selected for or against duration of tonic immobility for more than 25 generations (Mills and Faure, 1991). These lines differ for a large number of behavioural or physiological traits related to fearfulness (Faure and Mills, 1998). As no genetic map of quail is available yet, AFLP™ markers were developed (Vos *et al.,* 1995).

MATERIAL AND METHOD

Animals. *Low and high duration of tonic immobility lines.* Both lines were selected on the duration of tonic immobility (DTI). Tonic immobility was measured at 9 or 10 days of age. The quail was placed on its back in a U-shaped cradle. The number of inductions (NI, i.e. number of times the bird had to be restrained to obtain a tonic immobility lasting at least 10 seconds) and the duration of tonic immobility (DTI) were recorded. If the quail failed to quit the tonic immobility state after 5 minutes, the observation was censored and DTI set to 300. DTI was set at 0 if tonic immobility was not induced after 5 attempts (Mills and Faure, 1991).

F2 cross. A reciprocal cross was made between the high and low duration of tonic immobility lines and a second generation cross performed. A total of 1048 F2 quails issued from 9 sires and 18 dams were born in 10 hatches. They were measured for DTI and NI in the same

conditions as for selection. Body weight (BW) was also recorded at 2 weeks of age as well as corticosterone level (CORTICO) after a contention stress at 6 weeks of age (Satterlee and Johnson, 1988). Moreover latency before first movement (LAT), locomotion activity (LOC) and number of defecations (DEF) during the test were recorded in 3 out of the 10 hatches as indicators of open-field behaviour in a 3-minutes test.
Synthetic variable. A preliminary multidimensional analysis suggested to analyze the difference (DIFF) between DTI and NI, both centered and reduced. Indeed this trait summarizes all available information and is nearly normally distributed (Roussot *et al.,* 2001).

Segregation analysis. The existence of a major gene was tested by segregation analysis (Le Roy, 1989) of DIFF. The model of analysis took into account the effects of the sex, hatch, sire and dam. The latter was supposed to be heterozygous. The existence of this gene was tested using the log likelihood ratio.

Development of AFLP™ markers. As no genetic map is available in quail, AFLP™ markers (Vos *et al.,* 1995) were developed using 24 primers sets and analyzed. As most AFLP™ markers are dominant, phenotype could be classified as recessive (absence of band, coded as 11) or dominant (presence of a band, coded as 20) ; in the latter case the genotype of individuals could in some informative families be deduced from the genotypes of their relatives and coded as 21 (heterozygous) or 22 (dominant homozygous).
Genotypings. Only extreme animals were genotyped (Lander and Botstein, 1989). They were chosen according to the results of the segregation analysis. Association between markers was tested using CRI-MAP version 2.4 (Green *et al.,* 1990).

Test of association between markers and trait. A first test was made using CRI-MAP and assuming the genotype of animals at the QTL as known based on their phenotypic value. The animals from the F0 lines as well as the extremes were coded as homozygous and the F1 as heterozygous ; the lod score between each marker and the coded phenotype was computed.
As an association between the phenotype and one of the markers was suspected, variance analysis was performed to test the association between DIFF and all the markers of the linkage group. Variance analysis was made on offspring of the informative sires (i.e. those heterozygous at the marker) ; animals with ambiguous genotypes (20) were discarded. The model of analysis took into account the effects of the hatch, of the sire, of the dam nested within sire and of the allele transmitted by the sire. The latter was nested within sire as the phase was unknown. In a second analysis, the effect of the allele transmitted by the dam was tested. The analysis was then restricted to the informative heterozygous dams. As before, offspring with ambiguous genotypes were discarded. The model of analysis took into account the effect of the dam, of the hatch and of the allele transmitted by the sire (nested within the dam).

RESULTS AND DISCUSSION

Means and standard deviations of the traits are summarized in table 1.

Table 1. Means and standard deviations of the traits : number of inductions (NI), duration of tonic immobility (DTI), difference between DTI and NI centered and reduced (DIFF), latency before first movement (LAT), locomotion activity (LOC), number of defecations (DEF), body weight (BW) at 2 weeks of age and corticosterone level (CORTIO) after a contention stress at 6 weeks of age

Trait	Number of observations	Mean	Standard-deviation
NI	1235	1.57	1.01
DTI	1235	70.7	65.88
DIFF	1235	-0.002	1.58
LAT	380	126.0	65.25
LOC	380	7.46	13.36
DEF	380	1.73	0.90
CORTICO	383	20.38	14.89
BW	1231	76.58	8.80

Segregation analysis. The mixed inheritance (major gene and polygene) was highly significantly more likely than the polygenic inheritance ($P < 0.001$). The effect of this gene (difference of average DIFF between homozygous genotypes) was estimated to be equal to 1.3 standard deviation of the trait. As it appeared to be nearly dominant it was not possible to distinguish between dominant homozygous and heterozygous offspring. Six sires whose probability to be heterozygous was higher than 0.90 were therefore chosen and 278 of their F2 offspring with extreme performance selected for genotyping. Thirty-two more F2 were also genotyped to complete one sire family (for the genetic map).

AFLP™ markers. A total of 432 markers are already available. Eight percent are co-dominant and 8 % nearly co-dominant while the others are dominant. The high number of AFLP™ markers that were developed should allow to compensate the smaller informativity of dominant markers in comparison with co-dominant microsatellites. Twenty percent of them are line specific (with different alleles in the high and low lines) and 20 % nearly specific (i.e. with very different allele frequencies in the two lines).

Genotypings and test of association. A total of about 156.000 genotypings have been achieved. A first set of 334 markers have been studied first.
Linkage analysis. Preliminary two point linkage analysis revealed 37 linkage groups with a maximum of 28 markers in a group. This is a first step towards the development of a genetic map in the quail.
Test of association. In the largest group, a lod score of 2.73 was observed between one line-specific marker and tonic immobility. The recombination fraction was 0.23. Within this group, the effect of the allele within sire was significant ($P < 0.05$) for one marker on 182 informative offspring issued from 4 sires. The effect of the allele transmitted by the dam was significant ($P < 0.05$) for two other markers, on 205 and 142 animals issued from 8 and 5 dams respectively. Discarding ambiguous genotypes and uninformative animals resulted in a large loss of information as AFLP™ are dominant. This may explain why no other association was observed for the other markers (but association was close to significance ($P < 0.10$) for 3 other markers).

All these results strongly suggest that a QTL could lie within this linkage group. QTL have already been identified for various behavioural traits, (see Ramos and Mormede, 1998 for a review), among which fear-like behaviours (as in Gershenfeld and Paul, 1997) but, to our knowledge at least, it would be the first QTL controlling a behavioural trait identified in birds. However a multi-point analysis is needed to definitely confirm this result. This will be done using a method based on identity by descent (IBD) probability (as in Perez-Enciso *et al.,* 2000) taking advantage of the band intensity to estimate the homozygous or heterozygous status of the animals (Perez-Enciso and Roussot, 2002) and thus increasing the markers informativity. The results will be presented at the conference.
The genome scan will be achieved to map QTL in other genomic regions. If confirmed, the effect of the QTL in the other recorded traits will be investigated, which will allow to better understand the genetic relationships between fearfulness and the various measures of emotionality. In the longer term, the identification of the genes underlying this QTL could benefit from genes already identified in other species, particularly in laboratory species.

CONCLUSION

In a species where no genetic map was available, it was possible to rapidly develop AFLP™ markers, perform a QTL research and highly suspect the existence of a QTL underlying duration of tonic immobility in quail.

REFERENCES

Faure, J.M. and Mills, A.D. (1998) In "Genetics and the Behavior of Domestic Animals", p. 235-264, Academic Press.

Gershenfeld, H.K. and Paul, S.M. (1997) *Genomics* **56** : 1-8.

Green, P., Falls, K. and Crooks, S. (1990) "Documentation for CRI-MAP version 2.4", Washington University School of Medicine, St. Louis, USA.

Hall, C.S. (1934) *J. Comp. Psychol*. **18** : 385-403.

Jones, R.B. (1986) *World. Poult. Sci*. **42** : 82-96.

Jones, R.B., Mills, A.D., Faure, J.M. and Williams, J.B. (1994) *Physiol. Behav*. **56** : 529-534.

Lander, E.S. and Botstein, D. (1989) *Genetics* **121** : 185-199.

Le Roy, P. (1989) Thèse de l'Université Paris XI, Orsay, France.

Mills, A.D. and Faure, J.M. (1991) *J. Comp. Psychol.* **105** : 25-38.

Mills, A.D., Crawford, L., Domjan, M. and Faure, J.M. (1997) *Neurosc. Biobehav. Rev.* **21** : 261-281.

Perez-Enciso, M., Varona, L. and Rothschild, M. (2000) *Genet. Sel. Evol*. **32** : 467-482.

Perez-Enciso, M. and Roussot, O. (2002) Submitted.

Ramos, A. and Mormède, P. (1998) *Neurosc. Biobehav. Rev*. **22** : 53-57.

Roussot, O., Pitel, F., Vignal, A., Faure, J.M., Mills, A., Guémené, D., Leterrier, C., Mignon-Grasteau, S., Le Roy, P. and Beaumont, C. (2001) *J. Rech. Avicoles :* 369-372.

Satterlee, D.G. and Johnson, W.A. (1998) *Poult. Sci.* **77 :** 25:32.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. (1995) *Nucleic Acids Res.* **23** : 4407-4414.