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On the need for combining complementary analyses to assess the effect of a candidate gene and the evolution of its polymorphism: the example of the Major Histocompatibility Complex in chicken

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Summary

The aim of this paper is to combine different but complementary approaches to check the neutrality of a given locus in a selected population. Analysis was undertaken through the polymorphism's evolution compared with that predicted under the effect of drift and through the analysis of the variance components of the measured traits, considering the effect of the locus as either a fixed or a random effect. This study deals with the case of the MHC locus, using both data from experimental lines of chicken selected for three different criteria of immune response, and frequencies of the genotyped haplotypes over time. Both the evolution of the polymorphism and the variance components approach have led to the conclusion that the MHC locus has an effect on the trait affecting antibody production against the Newcastle disease virus. Results have also highlighted the interest in using various methods in the case of low allelic frequencies. However, none of the common hypotheses, overdominance or frequency-dependent selection, was sufficient to explain the observed variation of the MHC polymorphism, which was displayed by the temporal variation of the allelic frequencies.

1. Introduction

The Major Histocompatibility Complex (MHC) plays an important role in the immune system of all vertebrates. In farm animals, knowledge of the MHC genes has been growing and, in several chicken studies, the MHC has been shown to be involved in immune response to pathogens and disease resistance traits (reviewed by Bacon, 1987; Bumstead *et al.*, 1991; Kaufman & Lamont, 1996). Yet, these data are still limited and selection experiments have mainly focused on the observed change in allelic frequencies between selected lines, but not its evolution over time.

The observed evolution over time of the polymorphism at a given locus results mainly from the joint effects of drift and selection, which are known to be two main factors affecting evolution of the genetic variability within a closed population. In order to check the neutrality of a given locus, results on its polymorphism's evolution should be compared with the expected results under the assumption of pure genetic drift. Another way is to analyse the variance components of a measured trait known to be selected at the phenotypic level and to estimate the effects of the different alleles on this trait.

The aim of this paper is to combine these different approaches for the case of the MHC locus, using data from experimental lines of chicken selected for different criteria of immune response.

2. Material

(i) *Selection design*

Four experimental lines of chickens have been developed since 1994 from an unselected base population of White Leghorn chickens (Pinard-van der Laan, 2002). Three of these lines were selected for high values according to three different criteria of immune response: antibody response 3 weeks after vaccination against the Newcastle disease virus (line 1, trait ND3),

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cell-mediated immune response at 9 weeks of age (line 2, trait PHA) and phagocytic activity at 12 weeks of age (line 3, trait CC). The three lines underwent mass selection with a restriction on the contribution of the different families (sizes of the different half-sib families were approximately balanced). The fourth line was a control line (line 4), in which the parents were chosen at random. Within each line and at each generation, 15 males and 30 females out of about 100 candidates of each sex were chosen as parents for the next generation. Mating was at random, except that full- and half-sib matings were avoided. This selection programme was conducted for nine discrete generations (generation 1 to generation 9, generation 0 being considered as the base population). Results of the selection response and the evolution of inbreeding were reported by Pinard-van der Laan (2002) and Loywyck *et al*. (2005). A total of 7550 animals have been measured for the three traits, whatever the status of their line (control or selected) and the nature of the selection criterion.

(ii) *MHC typing*

MHC is a complex of three regions comprised of several genes encoding for three classes of molecules: B-F and B-L gene products which are equivalent to class I and class II gene products of mammals, respectively, and B-G gene products which are equivalent to class IV gene products, specific to birds (Miller *et al*., 2004). From the begining of the selection, all birds were typed for the MHC antigens using alloantisera produced from the lines. Six B-G haplotypes were found: B^{15}, B^{19}, B^{21}, B^{34}, B^{121} and B^{124}. Each haplotype identified by serology corresponded to a unique B-G and B-F restriction fragment length polymorphism (RFLP) pattern, with the exception of the B^{21} and the B^{121} haplotypes which are serologically different and have different B-G RFLP patterns but the same B-F RFLP pattern. Because the different analyses did not reveal any significant difference between the two haplotypes, the results presented will consider five B-F haplotypes only: B^{15}, B^{19}, B^{34}, B^{124} and B^{21} (grouping the B-G^{21} and B-G^{121}). Twenty-one genotypes were observed within the four lines.

3. Methods

(i) *Variance component analysis and estimation of haplotypes effects*

In a first step, variance components of the three immune traits (ND3, PHA and CC) were estimated by the Restricted Maximum Likelihood (REML) method, using VCE software (Groeneveld, 1997). Because previous results obtained from the first six generations showed no genetic correlation between the three traits (Pinard-Van der Laan, 2002), single-trait analyses were performed. Performance and pedigree data from all generations, up to the base population, were used. On the basis of simulation results by Sorensen *et al*. (2003), for a given trait data from both the line selected for this trait and the control line were used.

For each trait, three animal models were compared: the first did not include the effect of the MHC genotype and the other two differed in the way this effect was taken into account:

$$Y_{ijl} = \mu + gener_i + sex_j + A_{ijl} + E_{ijl} \quad \text{(model 1)}$$

$$Y_{ijkl} = \mu + gener_i + sex_j + z_k + A_{ijkl} + E_{ijkl} \quad \text{(model 2)}$$

$$Y_{ijkl} = \mu + gener_i + sex_j + Z_k + A_{ijkl} + E_{ijkl} \quad \text{(model 3)}$$

In these equations, subscripts i, j, k and l refer to the generation number, the sex of the animal, its MHC genotype and the animal itself, respectively; Y is the performance; μ is the overall mean; *gener* and *sex* are the environmental fixed effects of the generation and the sex of animals, respectively; A is the additive genetic value, assumed to be of polygenic origin; and E is the random error. The effect of the MHC genotype is represented by z or Z, this effect being considered as fixed (model 2) or random (model 3).

In a second step, the breeding value (A) of each animal and the random effect (Z) of each MHC genotype were predicted by the BLUP method under model 3, using the genetic parameters estimated during the first step under the same model, and assuming no correlation between the two variables A and Z. PEST software (Groeneveld, 1990) was used to perform this analysis. Due to the presence of rare alleles, only model 3 was used here, because considering the genotype effect as random was the only way to avoid estimation problems for several genotypes with a very small number of available performance data.

In a third step, the effects of the different haplotypes were compared by the method of contrasts, under model 2. This method consists of comparing the mean of the effects of genotypes including one haplotype with the mean of the effects of genotypes including another haplotype, assuming that the genotype effect may be considered as two additive haplotype effects. As the use of the usual statistical test was complicated because the degrees of freedom due to error that are not defined in a mixed-effect model (model 3), only model 2 was used here. Moreover, grouping in the analysis the rare genotypes with less rare ones prevents the estimation problems evoked in the second step. The method was performed using data from all generations over the four lines. First, the effects of each haplotype on the three traits were estimated; second, the effects of homozygous

Table 1. *Estimation of the genetic parameters for each trait as a percentage of the total phenotypic variation (SE, in percentage), according to the three different models considered: with (fixed or random) or without an MHC effect*

Trait	Parameter	Model 1: without MHC effect	Model 2: MHC as a fixed effect	Model 3: MHC as a random effect
ND3 [line 1]	σ^2_A	33·4 (1·1)	32·1 (1·0)	31·4 (1·1)
	σ^2_{MHC}	–	–	2·3* (0·3)
	σ^2_E	66·6 (0·8)	67·9 (0·7)	66·2 (0·8)
PHA [line 2]	σ^2_A	11·9 (2·0)	11·9 (1·9)	11·9 (1·8)
	σ^2_{MHC}	–	–	0·1 NS (0·1)
	σ^2_E	88·1 (2·2)	88·1 (2·2)	88·0 (2·1)
CC [line 3]	σ^2_A	23·7 (13·0)	23·1 (13·1)	22·9 (13·0)
	σ^2_{MHC}	–	–	0·8 NS (2·3)
	σ^2_E	76·3 (11·4)	76·9 (12·3)	76·3 (12·2)

genotypes were compared with the effects of heterozygous genotypes in order to test the underlying hypothesis of additivity.

(ii) *Evolution of haplotype frequencies*

Within each line and at each generation, the haplotype frequencies were calculated. Thus, a possible effect of drift was tested. First, the inbreeding effective size (N_{eI}) was estimated from the observed rate of inbreeding (ΔF), according to the classical formula:

$$\hat{N}e_I = \frac{1}{2 \cdot \Delta F}$$

The rate of inbreeding was computed at each generation from the observed values of the average coefficient of inbreeding computed from pedigree data (Loywyck *et al.*, 2005). Next, the comparison between observed and expected change in haplotype frequencies was performed in two complementary ways.

First, a 95% confidence interval of the frequency was determined for each haplotype separately in each generation and in each line. Populations with discrete generations, random choice of parents and random mating were simulated. Because generations did not overlap and due to the stability of the management rules which involved a balance between families, the pure drift situation was simulated using the concept of effective population size. For a given line and a given haplotype, a population was simulated with an effective size equal to the estimated value of N_{eI} and with an initial frequency equal to the observed initial value. In each generation, the bounds of the 95% confidence interval were empirically determined on the basis of the frequencies observed in 5000 independent replicates.

Second, the MHC locus was considered as a whole: an estimate of the standardized temporal variance in allelic frequency, f (Waples, 1989), was computed for each line over the nine generations; the f_c estimator of f, proposed by Nei & Tajima (1981), was used:

$$\hat{f}_c = \frac{1}{k}\sum_{i=1}^{k}\frac{[x_i - y_i]^2}{\frac{x_i + y_i}{2} - x_i \cdot y_i}$$

where k is the number of segregating alleles, x_i is the frequency of allele i at generation 0 and y_i the frequency of this allele at generation t. The estimate of the variance effective size (N_{eV}) of each selected line was directly deduced from the value of f_c, using the equation of Nei & Tajima (1981), as there is no sampling variance because the exact values for frequencies were available (all individuals at each generation had been genotyped):

$$\hat{N}e_V = \frac{t}{2\hat{f}_c}.$$

This value was compared with the value of the inbreeding effective size (N_{eI}). Moreover, as in Goldringer & Bataillon (2004), the observed value of f_c was compared with the distribution of f_c obtained from a series of simulations of populations with the same initial allelic frequencies and the same inbreeding effective size.

4. Results

(i) *Variance components analysis*

Table 1 shows the estimates of the genetic parameters for each trait, according to the three different models considered. When the MHC effect was not taken into account (model 1), the estimated value of the heritability was 0·33, 0·12 and 0·24 for traits ND3, PHA and CC, respectively. These values for traits ND3 and PHA are consistent with those reported

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Table 2. *Estimation of the effects of the MHC haplotypes on the three traits, using the method of contrasts and considering the MHC effect as fixed*

Haplotype	Trait PHA		
15	$0{\cdot}175^{ab}$	$0{\cdot}049^{a}$	$0{\cdot}008^{bc}$
19	0^{b}	$0{\cdot}008^{a}$	$0{\cdot}006^{bc}$
21	$0{\cdot}449^{a}$	0^{a}	$0{\cdot}010^{ab}$
34	$0{\cdot}441^{a}$	$0{\cdot}050^{a}$	$0{\cdot}033^{a}$
124	$0{\cdot}356^{a}$	$0{\cdot}047^{a}$	0^{c}

Values are given considering the lowest effect as the reference, e.g. values of the effects of haplotypes on trait ND3 are given as a comparison with the value of the effect of B^{19} haplotype.
^{abc} Two haplotypes sharing the same letter have effects that are not significantly different.

by Pinard-van der Laan (2002) – 0·35 and 0·1 3, respectively – but the estimate for trait CC was higher than that of Pinard-van der Laan (0·15). In comparison with model (1), taking into account the MHC effect led to changes in estimated values for trait ND3 only: for ND3, when the MHC effect was considered as fixed, the estimated value of the additive variance (σ^2_A) was reduced by 5·9 %; when the MHC effect was considered as random, the variance of this effect was found to be significantly different from zero and represented 6·9 % of the total genetic variance (including both the polygenic and the MHC components).

(ii) *Effect of the MHC haplotypes and comparison between heterozygotes and homozygotes genotypes*

Table 2 shows the estimates of the effects of the MHC haplotypes using the method of contrasts. Compared with B^{21}, B^{34} and B^{124}, the B^{19} haplotype had a significant negative effect on trait ND3 and, compared with B^{21} and B^{34}, the B^{124} haplotype had a significant negative effect on trait CC. No significative effect of any haplotype was observed for trait PHA.

In addition, significantly ($P<0{\cdot}005$) lower values for trait CC were associated with heterozygote genotypes than with the homozygote genotypes. Moreover, there was a significantly ($P<0{\cdot}003$) lower value for trait ND3 associated with the heterozygote genotype B^{15}–B^{19} than with the corresponding homozygote genotypes B^{15}–B^{15} and B^{19}–B^{19}, and a significantly ($P<0{\cdot}008$) lower value for trait CC associated with the heterozygote genotype B^{19}–B^{124} than with the corresponding homozygote genotypes B^{19}–B^{19} and B^{124}–B^{124}.

(iii) *Evolution of haplotype frequencies*

Fig. 1 presents the evolution of the observed frequencies of the haplotypes within each selected line and within the control line. The B^{19} haplotype was lost within line 1 at generation 6, and the B^{34} haplotype was lost within line 2 at generation 7 and within line 3 at generation 5. Only the evolution of the frequency of the B^{19} haplotype within line 1 exceeded the 95 % confidence interval under the asumption of drift. For all the other haplotypes in all four lines, no deviation was observed from the confidence interval (for the sake of clarity in the figures, this interval was not shown in these cases).

Table 3. *Estimated effective sizes of the population (for each line over the nine generations) based on the temporal variation approach N_{eV}, and on the pedigree approach, N_{eI}*

Line	f_c at MHC locus (P value)	N_{eI}	N_{eV} [95 % CI]
1	0·0884 (0·599)	34	51 [6–142]
2	0·0694 (0·663)	36	65 [8–181]
3	0·1103 (0·393)	38	41 [5–114]
4	0·0573 (0·713)	40	79 [10–219]

As shown in Table 3, the estimated effective size of the population based on the temporal variation approach (for each line over the nine generations), N_{eV}, was always higher than the estimate of the effective size based on the pedigree approach, N_{eI}. This indicated that the MHC locus was globally evolving at a lower rate than expected based on the pedigree data. The probability of obtaining in the simulated distribution an f_c, value equal to or greater than the observed f_c was high for the three selected lines (P value = 0·599, 0·663 and 0·393 for lines 1, 2 and 3, respectively) as well as for the control line (P value = 0·713), indicating that the four observed f_c values were not extreme, compared with the simulated f_c distribution.

5. Discussion

The estimate of the MHC effect on the three selected immune traits (ND3, PHA and CC) varied according to the method used. What lessons may be drawn from this study?

(i) *The interest in combining complementary analysis*

The interest in considering the MHC effect as random was twofold. First, it reduced the effect of rare genotypes, since in particular the initial frequency of the B^{34} haplotype was low, and second, it allowed consideration of the effect of the whole locus. On the contrary, assuming MHC as a fixed effect allowed us to have an approach focused on haplotypes instead of genotypes, and more particularly with the emphasis laid on each haplotype. Comparing the two

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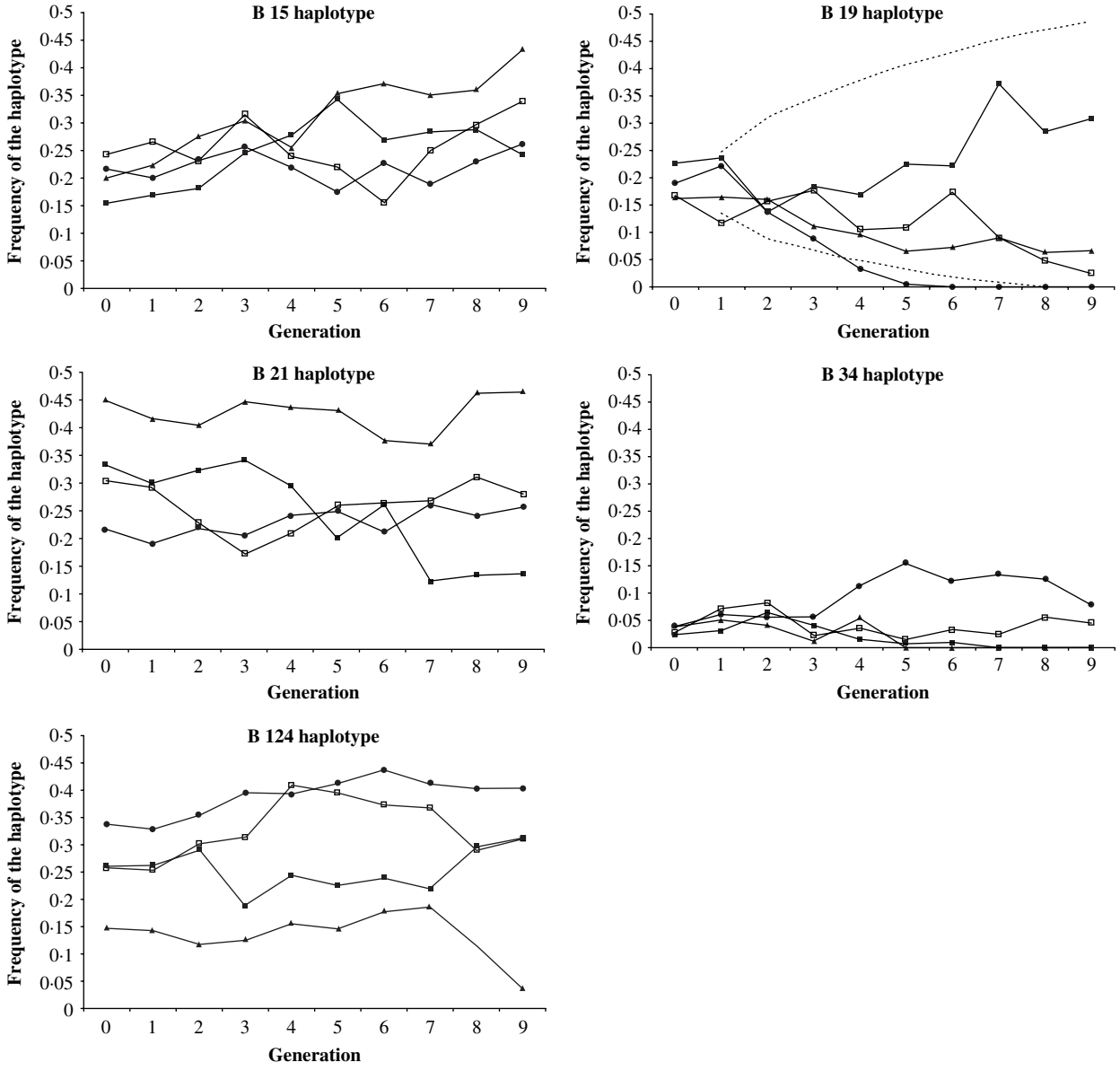

Fig. 1. Evolution of the observed frequencies of the haplotypes B^{15}, B^{19}, B^{21}, B^{34} and B^{124} within each selected line (unbroken lines: line 1, circles; line 2, squares; line 3, triangles) and within the control line (line 4, empty squares). Bounds of the 95% confidence interval are drawn (dotted lines) for the B^{19} haplotype within line 1.

hypotheses (fixed effect vs random effect) let us know whether the locus-scale variations detected when MHC is considered as a fixed effect may be large enough to be detected at the genome scale. Indeed, the negative effect of the B^{19} haplotype on trait ND3 was confirmed whereas the negative effect of the B^{124} haplotype on trait CC was not.

Likewise, the temporal method relies on the assumption that all variance in allele frequencies is due only to drift (Waples, 1989) and considers the locus as a whole. Effect of selection on the B^{19} haplotype within line 1 (trait ND3), revealed by the evolution of the haplotype frequency, was not strong enough to be detected using the temporal variation method. However, one limitation of the temporal method was shown by Pollack (1983): selection of constant intensity has a minor effect on f if t/N_e is small, which is the case in our study. In addition, in a multiallelic case, it might not detect even large variation in one specific allele frequency, provided the rest of the variation is alloted between the other different alleles. Therefore, the approach of the evolution of haplotype frequencies, haplotype by haplotype, is more efficient for detecting any impact of selection when the selection rules are maintained over generations and when a specific haplotype is selected.

Finally, evolution of the polymorphism at the MHC locus has shown that MHC had an effect on trait ND3 and the significant effect was confirmed by the variance components approach. This trait deals

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with the antibody production against an antigen: Newcastle disease virus. Such responses against a variety of pathogens were already found to be correlated with MHC, as for instance the response against *Salmonella* bacteria (Guillot *et al.*, 1995) or against Marek's disease virus (Bacon, 1987). However, the "background genome also has a substantial effect" (Lamont, 1998) and most studies rely only on the observation of differences in allelic frequencies between lines to deduce an association between the trait and the MHC locus. In some cases, complementary analyses such as the analysis of the variance components would allow separation of the effect of the MHC gene from the non-MHC gene effects. In this study, only the evolution of the polymorphism would lead us to conclude the effect of the B^{34} haplotype on PHA and CC, since this haplotype disappeared in lines 2 and 3, and the complementary methods have not shown it. Concerning the B^{124} haplotype, complementary analyses have not given a clear picture of the effect of this haplotype on trait CC but have laid the emphasis on contradictory forces in the evolution of the B^{124} haplotype frequency. Indeed, the variance components approach has revealed a significative negative effect of the B^{124} haplotype on trait CC, which should lead to a decrease in the haplotype frequency, as happened within line 1 for the B^{19} haplotype; however, the significantly higher effect of homozygotes over heterozygotes on trait CC may slow down the decrease in frequency and explain why the effect of the B^{124} haplotype was not detected at the locus scale.

(ii) *Estimation of effective population size*

The effective size (N_e) of the population is a key parameter for discerning allele frequency changes due to drift versus those due to selection and hitch-hiking. N_e is usually estimated from the rate of inbreeding (N_{eI}) or from the variance in allele frequencies over time (N_{eV}). Both methods were used in this study. The values of these two parameters N_{eI} and N_{eV} were expected to be equal since the two methods are based on the analysis of a neutral gene. However, as pointed out by Crow & Kimura (1970), N_{eI} is usually smaller than N_{eV} when a small number of parents generate a large number of offspring because 'the inbreeding effective number is more naturally related to the number of parents, while the variance effective number is related to that of the offspring'. Here, the two realized effective sizes were found to have different values in the experimental lines analysed, N_{eV} being in one case twice N_{eI}, but the above explanation may not be sufficient to explain such a large difference. Additionally, confidence intervals of N_{eV} in the four lines are rather large since there are only five haplotypes at a single locus. The method of calculation of the bounds of the 95% interval, provided by Nei & Tajima (1981) and used by Waples (1989), is 'only asymptotically correct' for a large number of independent alleles.

It seems that allele frequency variations at the MHC locus are weaker than those of the whole genome (see pedigree analysis). Combining the two approaches allows us to detect whether another force is needed in addition to drift to explain the evolution of the haplotype frequencies.

(iii) *Contrasting results on the selection response and the MHC locus effect*

Response to selection was significant but variable within the three selected lines (Loywyck *et al.*, 2005): the antibody response (ND3) was the trait with the highest and the most regular increase in its mean under selection and the highest estimated heritability (0·35), whereas the increase in the means of traits PHA and CC was low and fluctuating and their estimated heritability lower (0·13 and 0·15, respectively). Then, assessing the effect of the MHC locus may be more difficult: if the response to selection of the trait is low, the evolution of the polymorphism at the candidate gene is reduced.

Assuming MHC as a fixed effect raised the question about the underlying model, which supposes additivity, i.e. heterozygote genotypes showing intermediate values between the two corresponding homozygote genotypes. Here, the significantly lower values for the heterozygote genotypes compared with their homozygote counterparts led us to reject the underlying additive model.

The MHC locus is known to be extremely polymorphic and its variation is thought to be maintained by balancing selection either through heterozygous advantage or negative frequency selection (Hugues, 1998; Bodmer, 1972). However, both hypotheses are controversial (Slade & McCallum, 1992) and Takahata & Nei (1990) concluded that frequency-dependent selection (specifically, rare allele frequency) and overdominance could not be distinguished mathematically. The results of this study do not support the hypothesis of negative frequency-dependent selection, which assumes that it is advantageous to carry rare alleles to which pathogens are not adapted (Bodmer, 1972): here, the rare haplotype B^{34} disappeared in lines 2 and 3 although this haplotype had the highest effect on the two traits PHA and CC, respectively. As in a recent study on birds that looked for overdominance (De Boer, 2004), no evidence in favour of the hypothesis of a heterozygous advantage has been reported in the present study, since the significant difference between the effect of heterozygote genotypes compared with the effect of homozygote genotypes was in favour of homozygosity.

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6. Conclusion

This study highlights the interest in using various sources of information and methods in analysing a complex phenomenon such as testing whether a candidate gene is neutral or not. Different but complementary points of view have to be handled by either considering the locus as a whole or by analysing each allele separately, so that results may be contrasted or nuanced.

References

Bacon, L. D. (1987). Influence of the MHC on disease and productivity. *Poultry Science* **66**, 802–811.

Bodmer, W. F. (1972). Evolutionary significance of the HLA system. *Nature* **237**, 139–145.

Bumstead, N., Millard, B., Barrow, P. & Cook, J. K. A. (1991). Genetic basis of disease resistance in chickens. *Breeding for Disease Resistance in Farm Animals* (ed. J. B. Owen & R. F. E. Axford), pp. 10–23. Melksham, UK: CAB International.

Crow, J. F. & Kimura, M. (1970). *An Introduction to Population Genetics Theory*. New York: Harper & Row.

De Boer, R. J., Borghans, J. A. M., van Boven, M., Kesmir, C. & Weissing, F. J. (2004). Heterozygote advantage fails to explain the high degree of polymorphism of the MHC. *Immunogenetics* **55**, 725–731.

Goldringer, I. & Bataillon, T. (2004). On the distribution of temporal variations in allele frequency: consequences for the estimation of effective population size and the detection of loci undergoing selection. *Genetics* **168**, 563–568.

Goudet, J. (1995). FSTAT (version 1.2): a computer program to calculate *F*-statistics. *Journal of Heredity* **86**, 485–486.

Groeneveld, E. (1990). *PEST User's Manual*. Urbana, Illinois: University of Illinois.

Groeneveld, E. (1997). *VCE4 User's Guide and Reference Manual*. Neustadt, Germany.

Guillot, J. F., Beaumont, C., Bellatif, F., Mouline, C., Lantier, F., Colin, P. & Protais, J. (1995). Comparison of resistance of various poultry lines to infection by *Salmonella enteritidis*. *Veterinary Research* **26**, 81–86.

Hughes, A. L. & Yeager, M. (1998). Natural selection and the evolutionary of major histocompatibility complex loci. *Frontiers in Bioscience* **3**, 509–516.

Kaufman, J. F. & Lamont, S. J. (1996). The chicken major histocompatibility complex. In *The Major Histocompatibility Complex in Domestic Animal Species* (ed. L. B. Schook & S. J. Lamont), pp. 35–64. Boca Raton, Florida: CRC Press.

Lamont, S. J. (1998). Impact of genetics on disease resistance. *Poultry Science* **77**, 1111–1118.

Loywyck, V., Bijma, P., Pinard-van der Laan, M. H. & Verrier, E. (2005). A comparison of two methods for prediction response and rates of inbreeding in selected populations with the results obtained in two selection experiments. *Genetics Selection, and Evolution* **37**, 273–289.

Miller, M. M., Bacon, L. D., Hala, K., Hunt, H. D., Ewald, S. J., Kaufman, J., Zoorob, R. & Briles, W. E. (2004). Nomenclature for the chicken major histocompatibility (B and Y) complex. *Immunogenetics* **56**, 261–279.

Nei, M. & Tajima, F. (1981). Genetic drift and estimation of effective population size. *Genetics* **98**, 625–640.

Pinard-van der Laan, M. H. (2002). Immune modulation: the genetic approach. *Veterinary Immunology and Immunopathology* **87**, 199–205.

Pollak, E. (1983). A new method for estimating the effective population size from allele frequency changes. *Genetics* **104**, 531–548.

Slade, R. W. & McCallum, H. I. (1992). Overdominant vs. frequency-dependent selection at MHC loci. *Genetics* **132**, 861–862.

Sorensen, D., Guldbrandtsen, B. & Jensen, J. (2003). On the need for a control line in selection experiments: A likelihood analysis. *Genetics Selection and Evolution* **35**, 3–20.

Takahata, N. & Nei, M. (1990). Allelic genealogy under overdominant and frequency-dependent selection and polymorphism of major histocompatibility complex loci. *Genetics* **124**, 967–978.

Waples, R. S. (1989). A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* **121**, 379–391.

Weir, B. S. & Cockerham, C. C. (1984). Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.

Wright, S. (1969). Evolution and the genetics of population. *The Theory of Gene Frequencies*, vol. 2. Chicago: University of Chicago Press.