Genet. Sel. Evol. 36 (2004) 65–81

DOI: 10.1051/gse:2003051

Original article

Genetic analysis of a divergent selection for resistance to Rous sarcomas in chickens†

Marie-Hélène Pinard-van der Laan[a*], Denis Soubieux[b], Laurence Mérat[c], Danièle Bouret[d], Gillette Luneau[d], Ginette Dambrine[b], Pierrick Thoraval[b]

[a] UMR Génétique et diversité animales, Institut national de la recherche agronomique, 78352 Jouy-en-Josas Cedex, France
[b] Unité BioAgresseurs, Santé Environnement, Institut national de la recherche agronomique, 37380 Nouzilly, France
[c] Station de pathologie aviaire et parasitologie, Institut national de la recherche agronomique, 37380 Nouzilly, France
[d] Domaine du Magneraud, St-Pierre-d'Amilly, Institut national de la recherche agronomique, BP 52 17700 Surgères, France

(Received 9 December 2002; accepted 27 August 2003)

Abstract – Selection for disease resistance related traits is a tool of choice for evidencing and exploring genetic variability and studying underlying resistance mechanisms. In this framework, chickens originating from a base population, homozygote for the B^{19} major histocompatibility complex (MHC) were divergently selected for either progression or regression of tumors induced at 4 weeks of age by a SR-D strain of Rous sarcoma virus (RSV). The first generation of selection was based on a progeny test and subsequent selections were performed on full-sibs. Data of 18 generations including a total of 2010 birds measured were analyzed for the tumor profile index (TPI), a synthetic criterion of resistance derived from recording the volume of the tumors and mortality. Response to selection and heritability of TPI were estimated using a restricted maximum likelihood method with an animal model. Significant progress was shown in both directions: the lines differing significantly for TPI and mortality becoming null in the "regressor" line. Heritability of TPI was estimated as 0.49 ± 0.05 and 0.53 ± 0.06 within the progressor and regressor lines respectively, and 0.46 ± 0.03 when estimated over lines. Preliminary results showed within the progressor line a possible association between one *Rfp-Y* type and the growth of tumors.

chicken / selection / resistance / Rous sarcoma / *Rfp-Y*

† *This article is dedicated to the memory of Pierrick Thoraval (1960-2000).*
* Corresponding author: pinard@dga2.jouy.inra.fr

1. INTRODUCTION

For the analysis of genetic control of health traits in domestic animals, there is a growing interest for selection experiments as a powerful tool to explore the genetic variability of these traits and to create extreme phenotypes allowing the analysis of underlying mechanisms and the search for new genetic markers of disease resistance traits. Such tools are particularly developed in the chicken for the analysis of immunoresponsiveness [31] or resistance to specific diseases [3]. Resistance to viral diseases are examples of traits for which a genetic basis has been shown in many animal species [36]. For instance, selection for resistance to Marek's disease is one of the first successful selection experiments in chickens [9].

Resistance to another type of avian viral disease, the Rous Sarcoma virus (RSV), has been widely studied. Resistance to this disease is highly interesting as a model of resistance to tumor growth and its study has allowed new findings on related mechanisms and the genes involved. Indeed, early studies on RSV stipulated that only a very restricted number of genes and even one single gene would be involved in the control of tumor regression or progression. This was in some cases because of the easiness to select for the trait or because of the observation of the segregation of different phenotypes [21] or because of the particular genetic background of some inbred lines used intensively for the study of the fate of RSV tumors [10, 33, 39]. Naturally, most of the studies on RSV tumor control consider MHC as the natural candidate of choice as far as disease resistance is concerned, and showed an effect of the avian *B*-complex on either the progression or regression, as reviewed by Schierman and Collins [38]. Some major differences between genotypes in a given background have often been shown. For example, in an F2 cross of two highly inbred lines homozygous for B^2 and B^5, the most resistant genotype (B^2B^2) showed 5% of mortality and a mean Tumor Profile Index (TPI) of 2.94 and the most susceptible genotype (B^5B^5) showed a 93% mortality and a mean TPI of 4.93 whereas the heterozygote showed values closer to the resistant genotype than to the susceptible one [10]. Even if all the studies performed were not able to distinguish a possible direct effect from a closely linked effect, some clearly proved, using several recombinants in different backgrounds, that the genetic control is associated with the $B{-}F/B{-}L$ region rather than with the $B{-}G$ one [1, 2, 33]. Most reports studying the effect of MHC on the fate of RSV tumors were conducted from comparisons between congenic inbred lines or crosses between inbred lines, the possible amount of genetic variability expressed. Some of these studies, however, allowed at the same time to show evidence for non-MHC variation in the control of tumor fate when genetic background was found to play a major role [10–12]. Using backcrosses

from three partially congenic inbred lines, Cutting *et al.* [14] and Plachy [32] showed that resistance to RSV is the result of complementing action of MHC (or MHC-linked) genes and genes outside the MHC. The frequency of regressor chickens observed in the backcross mating and hybrids corresponded to the expected frequency of birds heterozygous for allelic genes at two independent loci. Indeed, the effect of non-MHC genes has been shown to be critical for regression of Rous sarcoma [7] using similar or identical MHC haplotypes in different genetic backgrounds and the relative influence of MHC and non-MHC genes was evaluated by Gebriel and Nordskog [16].

In this context, the selection experiment analysed hereafter was set up with animals which were all serologically defined homozygous for BG^{19} [15]. The selection would therefore explore MHC polymorphism outside the *BG* region and all the non-MHC variation. The aim of the study was to analyze 18 generations of selection for either progression or regression of RSV induced tumors, to estimate genetic parameters of one resistance trait (TPI) and to present a preliminary result on the association between the fate of the tumor and *Rfp-Y* types, the second MHC gene polymorphic cluster in the chicken outside the *B*-complex [5].

2. MATERIALS AND METHODS

2.1. Selection lines

A divergent selection for resistance to Rous sarcoma virus was initiated in 1982 from a White Leghorn base population (generation G_0) for 18 generations. The chicken line was bred at the Domaine du Magneraud (Inra, France) in specified pathogen-free conditions. A serological survey of breeder stocks was performed to ascertain the absence of specified pathogens including Marek's disease virus, avian leucosis virus, Newcastle disease virus, Gumboro disease virus, reovirus, infectious bronchitis disease virus, adenovirus, pseudoadenovirus, salmonella pullorum and gallinarum, mycoplasma gallisepticum and synoviae. Challenges were performed in filtered-air negative-pressure rooms at the Station de pathologie aviaire et parasitologie at Nouzilly (Inra, France).

The first generation of selection was performed by a progeny test. Progeny was inoculated in the subcutaneous tissue of the wing web at 4 weeks of age with 1000 focus-forming units per bird of a Rous Sarcoma virus strain D identified as the Schmidt-Ruppin strain of subgroup D (provided by P. Vigier, Institut Curie, France). The volume of the tumors was calculated 10 days post inoculation (PI) from the three maximum dimensions of the tumor using a slide calliper. Then the volumes were recorded every three days for one month. The means of the maximum volume of the tumor scored at any time during this

period were calculated for each sire progeny. Sires producing the upper third and lower third of this mean distribution were assigned as "progressor" and "regressor", respectively. Dams were selected on the basis of their divergence to sires, *i.e.*, dams whose progeny showed a higher or lower mean of the maximum volume of tumors than the sire family were classified as progressor or regressor, respectively. At this step, 7 sires and 21 dams (hatched in 1982 and originating from 3 males and 8 females) and 7 sires and 21 dams (hatched in 1982 and originating from 3 males and 6 females) were selected and assigned as "progressor" and "regressor", respectively.

Subsequent selections, from G1 to G18, were based on full-sib family performances, carrying out the same protocol of the Schmidt-Ruppin strain virus challenge and according to the same selection criterion, *i.e.*, maximum volume of tumors. The numbers of animals tested are given in Table I. One generation was produced per year, except in 1989, 1993 and 1995 where two generations were hatched. In years 1986, 1987 and 1989, no selection was performed due to the occurrence of positive serology to the Marek's disease virus. The tested animals were produced in one hatch, except in 1982, 1984 and 1991 and in 1983, where two and three hatches were produced, respectively.

From G10 onwards, the animals were selected still on full-sibs but reproduced within separate sublines in each line. Four sublines were derived in the regressor line, called pe5, pe10, pe11 and pe58. Seven sublines were derived in the progressor line, called pd2, pd4, pd5, pd8, pd10, pd1317 and pd1321. These sublines were produced and tested in balanced size.

2.2. Recorded resistance traits: TPI, mortality, time of death

From G1 onwards, the animals were inoculated and tested as previously described. Only, the length of the experiment may vary. For all generations, tumor size was recorded every week from 7 to 63 days PI. In addition, the animals from G6, G16 and G18 were measured until 70 days PI and the animals from G1, G2 and G3 were measured until 99, 126 and 105 days PI, respectively. Mortality was recorded daily. From the observation of the volume of the tumor and mortality, two classical criteria were defined: score and tumor profile index. Scores were defined weekly as follows: 0 = no palpable tumor; 1 = tumor up to 1 cm^3; 2 = tumor between 1 and 5 cm^3; 3 = tumor between 5 and 25 cm^3; 4 = tumor between 25 and 50 cm^3; 5 = tumor between 50 and 100 cm^3; 6 = tumor over 100 cm^3; 7 = death during the experiment. The scores were used to assign a tumor profile index (TPI) as slightly modified from Collins *et al.* [10]: 5 = a terminal tumor at 35 days PI; 4 = terminal tumor at 49 days PI; 3 = terminal tumor at 63 days PI; 2 = tumor up to 1 cm^3; 1 = otherwise (tumor less than 1 cm^3 , no tumor or complete regression by the end of the experiment).

In this study, besides mortality and age at death, TPI was only analyzed since it is the most synthetic criterion describing the resistance to the Rous sarcoma virus. The detailed analysis of tumor growth of this selection experiment will be the subject of another study.

2.3. Typing for MHC and *Rfp-Y*

Refined analysis and characterization of *Rfp-Y* types are described by Thoraval *et al.* [40]. Briefly, all animals of the progressor and regressor lines were serologically typed for the *B*-complex as homozygous BG^{19}. In addition, RFLP typing showed no polymorphism for class IV types but different patterns using class I and class II probes [8]. The relationship to polymorphism for the *Rfp-Y* system was further assessed, revealing three different *Rfp-Y* haplotypes: $Yw*^{15}$, $Yw*^{16}$ and $Yw*^{17}$. These assignments are tentative since sufficient careful comparisons remain to be done.

2.4. Statistical analysis

A comparison between lines when performed for a given generation were done, with a t-test for continuous traits, after checking for normality with the UNIVARIATE procedure. Frequency values were compared with a chi square test. The effects of *Rfp-Y* types on mortality were estimated using the CATMOD procedure. The effect of hatch, when applicable, was tested on TPI and mortality and was found non significant and therefore not included in further analyses. All these tests were performed using the SAS® library [34, 35].

The heritability of the selected TPI was obtained by using VCE software [20], applying the derivative-free restricted maximum likelihood method (DFREML) of Meyer [30], according to the following individual animal model (IAM):

$$TPI_{jkm} = \mu + G_j + S_k + U_{jkm} + e_{jkm} \quad (1)$$

where TPI_{jkm} = the TPI of the *m*th chick;

μ = a constant;

G_j = the fixed effect of the *j*th generation (0 to 18);

S_k = the fixed effect of the *k*th sex of the chick;

U_{jkm} = the random additive genetic effect on the TPI in the *m*th chick and e_{jkm} = a random error.

All relationships of the eighteen generations and data from all generations measured during this period were used (Tab. I). The fixed effect of the generation accounted for differences in environmental and experimental conditions between generations. Heritability for TPI was estimated across lines and within both selected lines. Individual inbreeding coefficients were estimated using the method of Meuwissen and Luo [29] using the PEDIG software [4].

Table I. Number of animals measured, data recorded and *Rfp-Y* type analysed, per line and generation.

		Line			
Year	G[1]	P[2]	R[3]	TPI[4]	*Rfp-Y* type[5]
82	0	262		X[6]	ND[7]
83	1	157	155	X	ND
84	2	158	146	X	ND
85	3	96	84	X	ND
86	4	.	.	ND	ND
87	5	.	.	ND	ND
88	6	19	38	X	ND
89a	7	.	.	ND	ND
89d	8	.	.	ND	ND
90	9	53	49	X	X
91	10	83	47	X	X
92	11	69	49	X	X
93a	12	32	34	X	X
93d	13	55	35	X	X
94	14	59	41	X	X
95a	15	45	27	X	X
95d	16	42	27	X	X
96	17	48	24	X	X
97	18	52	24	X	X

[1]Generation n; [2]numbers of animals recorded in the progressor (P) line; [3]numbers of animals recorded in the regressor (R) line; [4]tumor profile index (TPI) recorded (X[6]) or not done (ND[7]); [5]*Rfp-Y* type analysed (X[6]) or not done (ND[7]).

The average inbreeding level of each line was then calculated per generation. Estimated breeding values (EBV) for TPI were estimated with the PEST software [19] by applying model 1 and using the heritability value estimated by VCE. The selection response was evaluated by averaging these EBV per line and generation.

The effects of *Rfp-Y* type on TPI were separately estimated in the selected lines, using the following model:

$$TPI_{jklm} = \mu + G_j + S_k + \textit{Rfp-Y}_l + U_{jklm} + e_{jklm} \quad (2)$$

Table II. LSMean values (± SE) for the tumor profile index (TPI) and time of death (d), and mortality (%) in the progressor (P) and regressor (R) line, in the generations 1, 14 and 18.
N.B. Means are presented for the first and last generations (1 and 18, respectively) and at the maximum of response (14).

	Generation					
	1		14		18	
	Line					
Trait	P	R	P	R	P	R
TPI	2.84±0.08[a]	2.04±0.08[b]	3.45±0.13[a]	1.11±0.16[b]	1.91±0.14[a]	1.22±0.21[b]
Mortality (%)	66.24[a]	35.48[b]	74.58[a]	0.00[b]	25.00[a]	0.00[b]
Time of death (d)	49.52±1.77[a]	56.23±2.30[b]	34.15±2.40	.	55.82±4.41	.

[a,b] Values with different superscripts indicate differences ($P < 0.01$) between lines within generation.

where *Rfp-Y*$_l$ = the fixed effect of the *l*th *Rfp-Y* type and all the other terms are as defined in model (1). The solutions were obtained using the PEST program and the heritability values estimated previously in the lines. Differences between *Rfp-Y* types were tested as contrasts by a F-value generated by PEST.

3. RESULTS

3.1. Phenotypic selection response for TPI

Phenotypic responses to selection for TPI during 18 generations expressed as the mean TPI per line and generation is shown in Figure 1. A significant difference of 0.8 TPI was obtained already after the first generation of selection between the progressor and regressor line (Tab. II). The significance of the TPI difference between the lines remained unstable until generation 10. From generation 11 onwards, the lines differed significantly for TPI with a maximum difference of 2.34 in generation 14, the progressor line reaching its highest value during the selection at 3.45 TPI (Tab. II).

3.2. Phenotypic selection response for mortality and time of death

Mortality in the progressor and regressor lines showed very similar evolution as presented for the TPI in Figure 1 (data not shown). A significant difference in mortality of 30.76% was observed between the lines at generation 1 (Tab. II). The difference remained significant ($P < 0.01$) during the whole selection except in generation 9. The difference was maximum in generation 14 with 74.58% and 0% mortality for the progressor and regressor lines, respectively and tended to decrease afterwards. From this generation

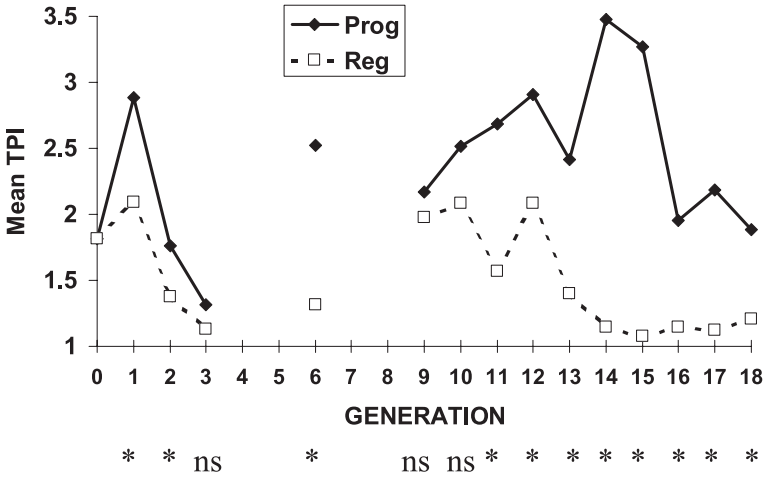

Figure 1. Phenotypic response for the tumor profile index (TPI) in the regressor (Reg) and progressor (Prog) lines during 18 generations. "*" indicates differences ($P < 0.01$) in mean TPI between the lines for a given generation. "ns" indicates no significant difference.

14 onwards, mortality was null in the regressor line. Average time at death was compared when relevant between progressor and regressor lines (Tab. II). After the first and third generations, the birds from the progressor line died significantly ($P < 0.01$) earlier than did those from the regressor line. Afterwards, there was no clear difference for the time of death between the lines nor for its direction nor significance.

3.3. Inbreeding of the lines

The evolution of the average inbreeding level was similar for the progressor and regressor lines. Inbreeding increased in a linear way of about +3.51% per generation and reached after 18 generations high levels of 66.54% and 61.06% in the progressor and regressor lines, respectively.

3.4. Heritability of the TPI

The heritability of the Tumor Profile Index, estimated using all data and pedigree information on all lines over 18 generations, was 0.46 ± 0.03. When estimated in selected lines separately, the analyses gave similar values in the progressor line (0.49 ± 0.05) and in the regressor line (0.53 ± 0.06).

3.5. Genetic selection response for TPI

3.5.1. In progressor and regressor selected lines

The evolution per line and generation of the mean of the breeding values for the TPI estimated using all data and pedigree information is shown in Figure 2. The difference between the progressor and regressor lines remained significant although the importance of the divergence between the lines varied widely during the course of the selection. Three phases may be seen with the lines diverging from each other before becoming closer in terms of genetic values: generations 0-3, 3-8 and 8-18. The second phase (3-8) corresponds to a period where only one generation of selection could be actually performed (generation 6). As observed for phenotypic values, genetic divergence was maximum at generation 14 (divergence of 1.75 estimated TPI) but diminished at the end of the period analyzed here (divergence of 0.96 estimated TPI).

3.5.2. Within sublines of the progressor and regressor selected lines

Since from generation 10 onwards the animals were selected and bred within separate sublines, the estimated breeding values for the TPI were averaged per subline as well. In the regressor line, there were no large changes in the ranking of the sublines during the last eight generations (data not shown). At generation 18, the pe10 regressor subline showed a significantly higher genetic value for the TPI than the other sublines (pe58, pe11 and pe5) (Tab. III). In the progressor line, various trends were observed depending on the sublines as shown in Figure 3. Finally, in generation 18, there were two significantly distinct groups within the progressor line with a higher progressor group (pd2, pd1321, pd8 and pd1317) *versus* a lower progressor group (pd10, pd4 and pd5) (Tab. III).

3.6. Generation effect on the TPI

Generation effects, estimated from model 1, showed large variations across generations, with "favorable" generations like generations 1 (+0.6 TPI), 12 (+0.5 TPI) or 14 (+0.3 TPI) and "unfavorable" ones like the last three generations (−0.6 TPI).

3.7. Effects of sex on TPI, mortality and time of death

Sex effect was estimated on the TPI and time at death on the whole selection. For both criteria, females appeared more sensitive, showing a higher TPI (+0.159 TPI) and dying earlier (−4.38 days) ($P < 0.01$).

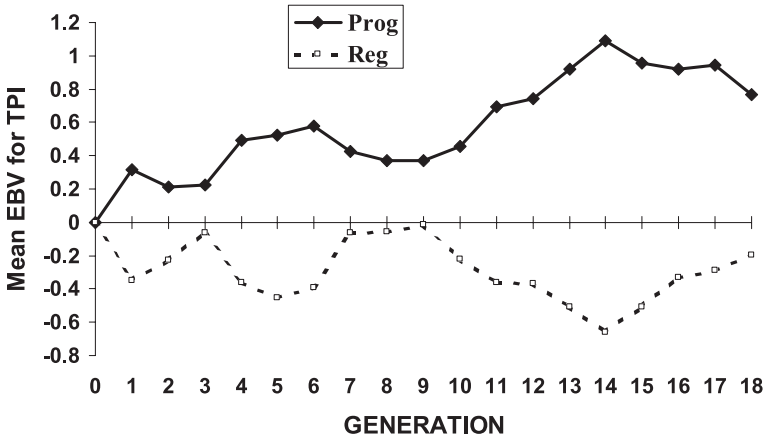

Figure 2. Genetic response for the tumor profile index (TPI) expressed as the mean estimated breeding values (EBV) in the regressor (Reg) and progressor (Prog) lines during 18 generations.

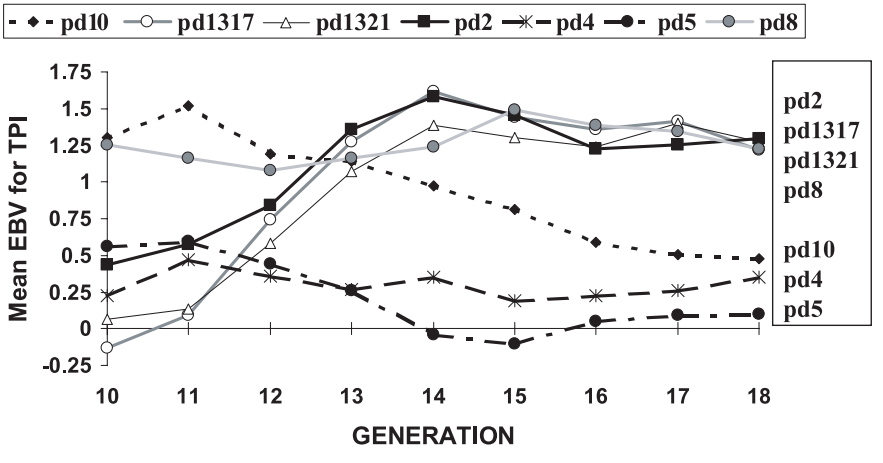

Figure 3. The mean estimated breeding values (EBV) for the tumor profile index (TPI) per subline in the progressor line from generations 10 to 18.

3.8. Effects of *Rfp-Y* types on TPI and time of death

The effects of *Rfp-Y* types were estimated on the TPI in both lines and on the time of death in the progressor line from generations 9 to 18. The results are shown per line in Table IV. The different sublines of progressor and regressor differed in *Rfp-Y* types but the use of the IAM could take into account these differences to estimate the *Rfp-Y* type. The effect of $Yw*^{15}$could not be estimated in the regressor line because it was absent there. In the regressor line,

Table III. Least Square Means of estimated breeding values for tumor profile index (TPI) in the different sublines of the regressor (R) and progressor (P) lines in generation 18.

Line			
R		P	
Subline	LSMean ± SE	Subline	LSMean ± SE
pe10	0.115 ± 0.055^{a}	pd2	1.298 ± 0.101^{a}
pe58	-0.232 ± 0.055^{b}	pd1321	1.277 ± 0.109^{a}
pe11	-0.327 ± 0.055^{b}	pd8	1.226 ± 0.109^{a}
pe5	-0.339 ± 0.055^{b}	pd1317	1.216 ± 0.109^{a}
		pd10	0.476 ± 0.089^{b}
		pd4	0.349 ± 0.089^{b}
		pd5	0.103 ± 0.089^{b}

[a,b] Estimates with different superscripts indicate differences ($P < 0.01$) between sublines within line.

Table IV. Estimates of *Rfp-Y* type effect on the tumor profile index (TPI) in the regressor (R) and progressor (P) lines and on the time of death in the progressor (P) line in the generations 9 to 18.

	Trait		
	TPI		Time of death (d)
	Line		
Rfp-Y type	R	P	P
$Yw*^{16}$	0.000^{a}	0.000^{a}	0.00^{a}
$Yw*^{17}$	0.047^{a}	-0.930^{b}	5.55^{b}
$Yw*^{15}$	.	-1.119^{b}	8.11^{b}

[a,b,c] Estimates with different superscripts indicate differences ($P < 0.01$) between *Rfp-Y* types within line.

there was no significant effect of the *Rfp-Y* type on TPI. In the progressor line, the $Yw*^{16}$ type was associated with a higher sensitivity, the animals showing a higher TPI and dying earlier than the $Yw*^{17}$ and $Yw*^{15}$ carriers.

4. DISCUSSION

Divergent selection for progression and regression to RSV showed here a rapid and successful response since after one generation, the two selected lines diverged significantly for TPI, mortality and age at death. A fast response to selection for regression of tumors to a Bryan strain of RSV was first reported

by Gyles and Brown [21], who used individual performances during the first three generations and a mixture of individual and full-sib family performances later. It agreed with a previous assumption that the number of genes controlling resistance to RSV would be limited [15]. The number of birds showing complete regression increased from 14% in the base population to 59% after six generations, which represented 30% more than in the genetic control line. In the data presented here, the effect of the selection in the following generations may be questioned even more. Even if the phenotypic differences between the progressor and the regressor lines were mostly significant for TPI or mortality, the end values in the progressor lines were less severe than in the first, 12th or 14th generations. Time of death did not show either any obvious correlated response to selection but it should be analyzed more accurately, using dedicated models. Selection in the regressor line was successful but likely limited downwards in the last generations by this obligatory biological threshold of no mortality nor tumor, without a finer selection criterion. The assumption that in the last generation, the regressor birds would no longer be able to be infected due to a loss of receptors for ALV-D cannot be excluded either.

The genetic analysis of the selection using estimated breeding values from an animal model provides a more accurate estimate of response to selection since it takes into account all the numerous relationships between individuals. Indeed the genetic trends obtained were smoother than the phenotypic ones, showing more clearly the different phases of selection but also showing obviously smaller differences between the lines than did the phenotypic means.

The values of heritability for TPI were rather high for a disease resistance trait (0.46 overall lines) but in agreement with successful selections. The estimates of heritabilities were equivalent in both lines in agreement with a rather symmetric response to selection. Using other types of animal material (inbred lines deriving from either noninbred progressor or regressor lines) and estimation methods (sire component from least squares analysis), Gyles *et al.* [23] found significant additive genetic variation in the regression process but not in the progression of tumors. Urban *et al.* [41] using a nested analysis of variance estimated a comparable value of heritability for TPI (0.41) in an outbred line. In addition, these authors indicate the likely presence of dominance or maternal effects.

In the present study, females appeared more susceptible than males suggesting interactions between the hormonal system and resistance mechanisms. The effects of sex are widely varying with the disease trait or the genetic background. In an F2 cross of B^2B^2 and B^5B^5 lines, Collins *et al.* [10] did not show any effect of sex on the fate of the RSV tumor. But Collins *et al.* [13] by analysis of the metastasis later found that females display fewer disseminated lesions than males. Gyles *et al.* [22] compared sexes within progressor and

regressor groups and found no difference in the progressor group but in the regressive one, females showed higher scores, larger tumors and took more days to regress.

The *Rfp-Y* system was recently found to be an independent system from the *B*-complex [5], the different subtypes identified previously using class I and class II probes [8] being in fact *Rfp-Y* types. The studies on the association between the *Rfp-Y* system and resistance to diseases are scarce (*e.g.* resistance to Marek's disease [42, 43]). LePage *et al.* [26] analysed the fate of RSV (Bryan high-titer strain of subgroup A) tumors for three haplotypes combined into five different *Rfp-Y* genotypes obtained in a B^2B^5 background. A significant effect of *Rfp-Y* on TPI and mortality was found, with large differences in mortality between the most resistant and the most susceptible genotype (14.3% and 72.2%, respectively) and for TPI (1.4 and 3.4, respectively). We found a significant but more moderate effect of the *Rfp-Y* type on the TPI (difference of 1 TPI) in the progressor line. The absence of an effect of *Rfp-Y* in the regressor line could be due to an interaction with the genetic background or simply that *Rfp-Y* genes did not play a major role in the coselection of regressor genes. Indeed, the $Yw*^{15}$ type disappeared in the regressor line although it was associated with a low TPI in the progressor line. Moreover, despite using an animal model, the results should be cautiously interpreted due to the specific family structure (sublines bred separately in this typed phase) and high inbreeding. Several segregating crosses to break linkage disequilibrium accumulated over generations would be needed to accurately estimate all possible *Rfp-Y* genotypes against a more random background. Even more interesting would be to combine these different *Rfp-Y* genotypes with different *B* genotypes since complementing effects have been suggested by LePage *et al.* [26]. Also, as found for the MHC, interactions between the effect of the *Rfp-Y* system on the fate of RSV tumors and other factors like age at inoculation [24], virus strain [27] or dose of virus [37] should be investigated. Senseney *et al.* [37] in a cross segregating for two haplotypes (B^{Q} and B^{17}) found no effect of MHC on the regression of tumors at a high dose of virus but an effect at a lower dose of the same virus and in the same genetic stock with an allelic complementation between the two alleles, the heterozygote state showing an advantage towards tumor regression. The superiority of other heterozygote combination were found elsewhere [6, 27, 39]. The effect of the resistance genes may clearly depend on the degree of pathogenicity of the virus.

This study opens ways to search for other genes controlling the fate of RSV tumors. Other genetic systems have been reported as being associated with the fate of tumors. Non-MHC alloantigens (Ea-L) affected tumor size, TPI and mortality depending on the MHC background [28] or not [25]. Non-MHC

T-lymphocyte and B-cells alloantigens were found to have an effect on regression, resulting from specific interactions between alleles and genetic background [17, 18]. Also the endogenous viral genes have been found to be associated with progression or regression [32]. The current lines are segregating for some of the ALVE genes and the role of ALVE1 is now being investigated.

Such divergent lines represent a powerful tool to look jointly for genes controlling the fate of RSV tumors and underlying mechanisms. Tumor fate was roughly analyzed by the TPI, the most synthetic criterion used so far. A finer analysis will consider the different aspects of the growth of tumors. Also, either the progression or regression of tumors involves complex and intricate immune mechanisms. There are indications that some families in the current lines may either show antiviral responses or antitumoral response. It will be of high interest to discover whether the *Rfp-Y* system or the endogenous viral genes might control these different pathways.

REFERENCES

[1] Aeed P.A., Briles W.E., Zsigray R.M., Collins W.M., Influence of different B-complex recombinants on the outcome of Rous sarcomas in chickens, Anim. Genet. 24 (1993) 177–181.

[2] Auclair B.W., Collins W.M., Zsigray R.M., Briles W.E., B-complex recombinants and sarcoma regression: role of B-L/B-F region genes, Poult. Sci. 74 (1995) 434–440.

[3] Bacon L.D., Hunt H.D., Cheng H.H., A review of the development of chicken lines to resolve genes determining resistance to diseases, Poult. Sci. 79 (2000) 1082–1093.

[4] Boichard D., PEDIG: a fortran package for pedigree analysis suited for large populations, in: Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, August 19-23, 2002, INRA, Castanet Tolosan, CD-ROM communication n° 28–13.

[5] Briles W.E., Goto R.M., Auffray C., Miller M.M., A polymorphic system related to but genetically independent of the chicken major histocompatibility complex, Immunogenetics 37 (1993) 408–414.

[6] Brown D.W., Collins W.M., Ward P.H., Briles W.E., Complementation of major histocompatibility haplotypes in regression of Rous sarcoma virus-induced tumors in noninbred chickens, Poult. Sci. 61 (1982) 409–413.

[7] Brown D.W., Collins W.M., Zsigray R.M., Briles W.E., A non-MHC genetic influence on response to Rous sarcoma virus-induced tumors in chickens, Avian Dis. 28 (1984) 884–899.

[8] Chaussé A.M., Thoraval P., Coudert F., Auffray C., Dambrine G., Analysis of B complex polymorphism in Rous sarcoma progressor and regressor chickens with B-G, B-F, and B-L beta probes, Avian Dis. 34 (1990) 934–940.

[9] Cole R.K., Studies on genetic resistance to Marek's disease, Avian Dis. 12 (1968) 9–28.
[10] Collins W.M., Briles R.W., Zsigray R.M., Dunlop W.R., Corbett A.C., Clark K.K., Marks J.L., McGrail T.P., The B locus (MHC) in the chicken: association with the fate of RSV-induced tumors, Immunogenetics 5 (1977) 333–343.
[11] Collins W.M., Heinzelmann E.W., Corbett A.C., Zsigray R.M., Dunlop W.R., Rous sarcoma regression in seven highly inbred lines of White Leghorns, Poult. Sci. 59 (1980) 1172–1177.
[12] Collins W.M., Brown D.W., Ward P.H., Dunlop W.R., Briles W.E., MHC and non-MHC genetic influences on Rous sarcoma metastasis in chickens, Immunogenetics 22 (1985) 315–321.
[13] Collins W.M., Dunlop W.R., Zsigray R.M., Briles R.W., Fite R.W., Metastasis of Rous sarcoma tumors in chickens is influenced by the major histocompatibility (B) complex and sex, Poult. Sci. 65 (1986) 1642–1648.
[14] Cutting J.A., Watanabe D.H., Strebel F.R., McBride R.A., Complementing MHC- and non-MHC-linked genes and resistance to avian sarcoma virus-induced tumours in inbred lines of chickens, J. Immunogenet. 8 (1981) 215–223.
[15] Dambrine G., Millet N., Boyer J.P., Coudert F., Cauchy L., Immunogénétique et résistance au développement des sarcomes aviaires, Sci. Tech. Anim. Lab. 11 (1986) 15–24.
[16] Gebriel G.M., Nordskog A.W., Genetic linkage of subgroup C Rous sarcoma virus-induced tumour expression in chickens to the IR-GAT locus of the B complex, J. Immunogenet. 10 (1983) 231–235.
[17] Gilmour D.G., Collins W.M., Fredericksen T.L., Auclair B., Clark K.K., Urban W.E. Jr., Influence of non-MHC T lymphocyte alloantigens on regression of Rous sarcomas in the chicken, Immunogenetics 17 (1983) 43–54.
[18] Gilmour D.G., Collins W.M., Fredericksen T.L., Urban W.E. Jr, Ward P.F., DiFronzo N.L., Genetic interaction between non-MHC T- and B-cell alloantigens in response to Rous sarcomas in chickens, Immunogenetics 23 (1986) 1–6.
[19] Groeneveld E., PEST User's Manual, University of Illinois, Urbana, 1990.
[20] Groeneveld E., VCE User's Manual, Neustadt, Germany, 1995.
[21] Gyles N.R., Brown C.J., Selection in chickens for retrogression of tumors caused by Rous sarcoma virus, Poult. Sci. 50 (1971) 901–905.
[22] Gyles N.R., Miley J.L., Brown C.J., Size, score and speed of development of tumors in chickens in response to subcutaneous inoculations with Rous sarcoma virus, Poult. Sci. 46 (1967) 789–795.
[23] Gyles N.R., Johnson L.W., Brown C.J., Whitfill C.E., Heritabilities of regression and progression of Rous sarcomas in the chicken, Poult. Sci. 61 (1982) 835–841.
[24] Heinzelmann E.W., Clark K.K., Collins W.M., Briles W.E., Host age and major histocompatibility genotype influence on Rous sarcoma regression in chickens, Poult. Sci. 60 (1981) 2171–2175.
[25] LePage K.T., Briles W.E., Kopti F., Taylor R.L. Jr, Nonmajor histocompatibility complex alloantigen effects on the fate of Rous sarcomas, Poult. Sci. 79 (2000) 343–348.

[26] LePage K.T., Miller M.M., Briles W.E., Taylor R.L. Jr., Rfp-Y genotype affects the fate of Rous sarcomas in B2B5 chickens, Immunogenetics 51 (2000) 751–754.

[27] McBride R.A., Cutting J.A., Schierman L.W., Strebel F.R., Watanabe D.H., MHC gene control of growth of avian sarcoma virus-induced tumours in chickens: a study on the role of virus strains, J. Immunogenet. 8 (1981) 207–214.

[28] Medarova Z., Briles W.E., Taylor R.L. Jr., Alloantigen system L affects the outcome of Rous sarcomas, Exp. Biol. Med. 227 (2002) 158–163.

[29] Meuwissen T.H.E., Luo Z., Computing inbreeding coefficient in large populations, Genet. Sel. Evol. 24 (1992) 305–313.

[30] Meyer K., Restricted maximum likelihood to estimate variance components for animal models with several random effects using a derivative-free algorithm, Genet. Sel. Evol. 21 (1989) 317–340.

[31] Pinard-van der Laan M.-H., Siegel P.B., Lamont S.J., Lessons from selection experiments on immune response in the chicken, Poult. Avian Biol. Rev. 9 (1998) 125–141.

[32] Plachy J., Korec E., Hlozanek I., Zdenkova E., Effect of the expression of an endogenous viral gene on the growth of tumours induced by Rous sarcoma virus in chickens, Folia Biol. (Praha) 31 (1985) 235–240.

[33] Plachy J., Benda V., Location of the gene responsible for Rous sarcoma regression in the B-F region of the B complex (MHC) of the chicken, Folia Biol. (Praha) 27 (1981) 363–368.

[34] SAS® Institute, SAS®/STAT User's Guide, Version 6, Fourth edn., SAS® Institute Inc., Cary, NC, USA, 1989.

[35] SAS® Institute, Procedure Guide, Version 6, Third edn., SAS Institute Inc., Cary, NC, USA, 1990.

[36] Schat K.A., Davies C.J., Viral diseases, in: Axford R.F.E., Bishop S.C., Nicholas F.W., Owen J.B. (Eds.), Breeding for disease resistance in farm animals, 2nd edn., CAB International, 2000, pp. 271–300.

[37] Senseney H.L., Briles W.E., Abplanalp H., Taylor R.L. Jr, Allelic complementation between MHC haplotypes B(Q) and B17 increases regression of Rous sarcomas, Poult. Sci. 79 (2000) 1736–1740.

[38] Schierman L.W., Collins W.M., Influence of the major histocompatibility complex on tumor regression and immunity in chickens, Poult. Sci. 66 (1987) 812–818.

[39] Schierman L.W., Watanabe D.H., McBride R.A., Genetic control of Rous sarcoma regression in chickens: linkage with the major histocompatibility complex, Immunogenetics 5 (1977) 325–332.

[40] Thoraval P., Afanassieff M., Bouret D., Luneau G., Esnault E., Goto R.M., Chaussé A.-M., Zoorob R., Soubieux D., Miller M.M., Dambrine G., Role of nonclassical class I genes of the chicken major histocompatibility complex Rfp-Y locus in transplantation immunity, Immunogenetics, in press.

[41] Urban W.E. Jr, Zervas N.P., Collins W.M., Buck R.W., Aeed P.A., Briles W.E., Estimates of heritability of response to Rous sarcomas of chickens, Poult. Sci. 64 (1985) 1405–1410.
[42] Vallejo R.L., Pharr G.T., Liu H.C., Cheng H.H., Witter R.L., Bacon L.D., Non-association between Rfp-Y major histocompatibility complex-like genes and susceptibility to Marek's disease virus-induced tumors in 6(3)x7(2) intercross chickens, Anim. Genet. 28 (1997) 331–337.
[43] Wakenell P.S., Miller M.M., Goto R.M., Gauderman W.J., Briles W.E., Association between the Rfp-Y haplotype and the incidence of Marek's disease in chickens, Immunogenetics 44 (1996) 242–245.