BREEDING AND GENETICS

Genetic Analysis of the Growth Curve of Rous Sarcoma Virus-Induced Tumors in Chickens

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ABSTRACT

White Leghorn chicks homozygous for B19 MHC haplotype were selected for 18 generations on tumor regression after inoculation in the wing web with an SR-D strain of Rous sarcoma virus (RSV) at 4 wk of age. Each chick was assigned a tumor profile index (TPI) based on age at death and size of the tumor. During 18 generations, 2,010 birds were divergently selected on TPI for either progression or regression of the tumor (P and R lines). A Brody growth curve was fitted for each bird. Brody function parameters included the asymptotic tumor volume (A), the factor for increased growth in progression phase (K1), the factor for decreased growth in regression phase (K2), age at maximum volume (Tmax), and maximum volume of the tumor (Vmax). Tumor growth curves were found to be different according to line, sex, and restriction fragment pattern Y complex Rfp-Y MHC haplotype (Yw*15, Yw*16, and Yw*17). Within the P line, birds from the Yw*16 haplotype reached Vmax at an earlier age than Yw*15 and Yw*17, but with a lower Vmax value. Within the R line, tumor growth curves of birds from Yw*16 and Yw*17 haplotypes were similar. Rank correlations between the different parameters and TPI were low (between −0.26 and 0.36). Heritability estimated by the sire component was high for Vmax (0.73). Heritabilities of Tmax and K2 were moderate (0.20 to 0.23 for Tmax and 0.18 to 0.21 for K2) allowing these traits to be used as selection criteria. Heritabilities of A and K1 were lower than 0.12. Modeling the growth curve should contribute to better distinction between progressors and regressors.

(Key words: Rous sarcoma, selection, growth curve, chicken, tumor)

2004 Poultry Science 83:1479–1488

INTRODUCTION

Studies focused on resistance or susceptibility to tumors in chickens frequently used Rous sarcoma virus (RSV), a member of the avian leukemia viruses, recently termed alpharetrovirus (Regenmortel et al., 2000). Rous sarcoma virus is a replication-competent virus bearing the v-src oncogene. Rous sarcoma virus-induced tumor growth is a complex multistep process beginning with virus infection and concomitant v-src transformation of target cells at the site of inoculation. Proliferation of transformed cells and continuous recruitment of transformed cells due to persistent spread of the virus leads to tumor formation. After tumor formation, different phenomena may be observed. Tumors may grow progressively more or less rapidly until death or be subject to varying degrees of regression. In the process of tumor development, selec-
ens possess 2 MHC gene clusters whose current nomenclature has been recently described (Miller et al., 2004): the classical B complex and the restriction fragment pattern-Y (Rfp-Y) complex, detected initially by polymorphic class I and class IIβ gene restriction fragment patterns presented by members of fully pedigreed families (Briles et al., 1993).

A selection experiment was performed within a histocompatible B19 line (Dambrine et al., 1986), effectively showing that homozygous serology-defined MHC antigens could not explain all the variability in resistance to RSV. Chickens of the B19 line shared identical genes in the MHC B complex while segregating for the Rfp-Y system (Thoraval et al., 1993). Three different sublines homozygous for the MHC Rfp-Y system were derived, defining respectively the Yw15, Yw16, and Yw17 haplotypes (Chaussé et al., 1990; Thoraval et al., 1993, 2003). The role of these MHC Rfp-Y haplotypes in the control of resistance to RSV in the B19 chickens has not been clearly established.

As in any genetic study, the accuracy of the phenotypic measure is of particular importance to precisely distinguish between progressors and regressors. In all these experiments, tumor development was measured by assigning a tumor profile index (TPI) based on age at death and tumor size (Carte et al., 1972; Collins et al., 1977). Another indicator, the score, was used to distinguish classes of tumor size (Collins et al., 1977). Pinard-Van der Laan et al. (2004) recently performed a genetic analysis of TPI on the former B19 selection experiment and showed high heritability of TPI (0.46) and a possible association between the growth of tumor and the Yw16 haplotype segregating in the B19 progressor line. However, such analysis did not take into account the kinetics of tumor growth and particularly the speed of progression or the degree of regression, which can be assessed over time by fitting a suitable mathematical model for growth.

Understanding tumor growth may have profound implications in controlling disease. A survey of the literature showed that there is a dearth of reports on the kinetics of RSV-induced tumor growth in chickens. However, several reports are available on the mathematical assumptions on the growth of tumor cells in human (Michelson et al., 1987; Solyanik et al., 1995; Wiarda and Travis, 1997) and physiological growth curves in chicken (Barbato, 1991; Mignon-Graesteau et al., 1999). The aim of the current study was therefore to fit a model for tumor growth (both regressive and progressive phases) and to study the heritable nature of the variables derived from the mathematical model for the growth of RSV-induced tumors in chicken. This was performed in the same selection experiment and with the same data as in Pinard-Van der Laan et al. (2004). The present study may contribute to more accurate distinctions between progressors and regressors, and help determine the role of MHC Rfp-Y haplotypes, and thus help to identify genes involved in the control of regression.

**MATERIALS AND METHODS**

**Creation of B19 Lines**

After definition of the B complex haplotypes by hemagglutination tests, B19 chickens were obtained from the INRA-B19 White Leghorn flock originating from the B19 line isolated at Birmingham (French, 1975). The INRA-B19 flock has been bred at the Domaine Expérimental du Magneraud (Institut National de la Recherche Agronomique, France) in specific pathogen-free conditions since 1977. Serological survey of breeder stocks was performed to ascertain the absence of specific pathogens including Marek’s disease virus. Lack of transmission of avian leukosis viruses was checked at each generation. Three sublines homozygous for the respective Yw15, Yw16, and Yw17 MHC Rfp-Y haplotypes of the MHC were derived from B19 chicken line on the basis of the polymorphism of the MHC RFLP patterns identified by Southern blotting with F-10, a chicken MHC class I probe derived from BF cDNA (Chaussé et al., 1990). Briefly, an initial screening was performed on 65 chickens from the eighth generation. It resulted in the presence of 6 different RFLP patterns: 5 homozygous combinations called Yw15, Yw16, and Yw17 (2, 17, and 7 typed chickens among 65, respectively), and 3 heterozygous combinations called Yw15/Yw16, Yw15/Yw17, and Yw16/Yw17 (12, 2, and 25 typed chickens among 65, respectively). Males and females with identical homozygous patterns were then mated along with 2 additional pedigrees of chickens showing heterozygous patterns. The 3 homozygous sublines B19 Yw15, B19 Yw16, and B19 Yw17 were derived from the next generation by mating only males and females showing the same homozygous patterns (Chaussé et al., 1990; Thoraval et al., 1993, 2003). Each subline was obtained from 4 to 5 founding families, each composed of 1 sire and 3 dams.

**Methods of Selection**

At 4 wk of age, each progeny chick was injected subcutaneously in the wing web with a dose of 1,000 focus forming units of RSV SR-D virus (subgroup D Schmidt Ruppin strain of RSV, provided by P. Vigier, Institut Curie, Orsay, France). Tumors were assessed 10 d postinoculation (DPI) by gross observation and at 7- to 14-d intervals. Maximum age measured varied between 63 and 126 d with generation, and the mean number of measurements per bird was 9.12. Measurements were taken at the 3 largest positions (length, height, and width) using calipers. Estimates of tumor volume were calculated as half the product of the 3 dimensions (Cutting et al., 1981).

Depending on the volume of the tumor and whether the bird had died, each bird was assigned a tumor profile index (TPI), slightly modified from Collins et al. (1977). Five levels were defined as: survived with tumor volume less than 1 cm3 (level 1), survived with tumor volume greater than 1 cm3 (level 2), died between 50 and 63 DPI (level 3), died between 36 and 49 DPI (level 4), and died before 35 DPI (level 5). Mortality and age at death, when appropriate, were recorded for each bird.
Based on the TPI, a selection experiment was initiated in 1982 to derive divergent lines, hereafter called P for progressor line and R for regressor line. Progressor sires were selected from the upper third of tumor volume family averages, and regressor sires belonged to the families from the lower third of tumor volumes. Dams were selected based on the divergence of tumor volume of their own family and the corresponding sire family. Matings were arranged within lines and the offspring of the progressor and regressor lines were challenged with RSV, as described above. In the following generations, selection was not based on offspring performances, but on full sibs performances. In 4 years (1982, 1983, 1984, and 1991), chicks from more than one hatch were used for the experiment. From the tenth generation onward, selection was performed within separate sublines. Five separate sublines were created, corresponding to the Yw*15, Yw*16, and Yw*17 haplotypes in the P line, and to Yw*16 and Yw*17 in the R line.

**Fitting the Growth Curve**

To study the kinetics of tumor development in each bird, a tumor growth curve model (Brody, 1945) was fitted using nonlinear regression with PROC NLIN (SAS Institute, 1999) as follows:

**For the Growth Phase.** Before the maximal volume is reached, the tumor growth is modeled as follows:

\[ V_t = V_{\text{max}} \times \exp^{K_1(t - T_{\text{max}})} \]

where \( V_t \) is the observed volume of the tumor at time \( t \), \( V_{\text{max}} \) the observed maximum volume of the tumor, \( T_{\text{max}} \) the observed time at maximum tumor volume, and \( K_1 \) the estimated factor for increased growth in the progression phase.

**For the Decreasing Phase.** After maximal volume has been reached, tumor growth curve is modeled as follows:

\[ V_t = A - (A - V_{\text{max}}) \times \exp^{-K_2(t - T_{\text{max}})} \]

where \( A \) is the estimated asymptotic volume and \( K_2 \) the estimated factor for decreased growth in the regression phase.

All estimated parameters were constrained to be positive; \( K_2 \) was constrained to be lower than 1,000. For birds that did not develop a tumor, \( V_{\text{max}} \) was set at zero, and \( T_{\text{max}} \) the observed time at maximum tumor volume, \( K_1 \), and \( K_2 \) were set at 'missing'. For birds that developed a tumor that did not regress (i.e., \( V_{\text{max}} \) equal to the last measured volume), \( K_2 \) and \( A \) were set at 'missing'.

**Statistical Analysis**

Data collected over 18 generations were used for analysis. The fixed effect of generation accounted for differences in environmental and experimental conditions between generations. The effects of hatch, sex, line, and MHC haplotype (within line) on mortality were analyzed using logits and the PROC CATMOD procedure (SAS Institute, 1999). As growth curve parameters were discrete or their distributions were not normal, or both, a nonparametric ANOVA (Kruskall-Wallis test) was used to test the line or type effect, with the NPAR1WAY procedure (SAS Institute, 1999). Medians were considered instead of means, because they were more representative of the population. Rank correlations between these variables, and TPI or age at death were estimated using the PROC CORR (SAS Institute, 1999) procedure. As no significant hatch effect was found for any of the traits studied, it was ignored for further analysis.

**Estimates of Heritability**

As distributions of growth curve parameters were far from normality, several classes were defined for each trait. Three classes of equal frequency ranging from 1 to 3 were defined for \( T_{\text{max}} \), \( K_1 \), \( K_2 \), and \( A \). Definition of classes is given in Table 1. A fourth class was created for \( V_{\text{max}} \), corresponding to birds that did not develop a tumor \( (V_{\text{max}} = 0) \). A program dedicated to the analysis of discrete data was used to estimate sire and dam heritabilities of traits and corresponding breeding values of animals (Chapuis et al., 2000). This program was based on the threshold model of Gianola and Foulley (1983). The model used was the following:

\[ Y_{ijkl} = \mu + G_i + S_j + D_k + e_{ijkl} \]

where \( Y_{ijkl} \) is the growth parameter for \( i \)th animal, \( \mu \) the general mean, \( G_i \) the effect of the \( i \)th generation, \( S_j \) the effect of the \( j \)th sire, \( D_k \) the effect of the \( k \)th dam, and \( e_{ijkl} \) the residual pertaining to the \( i \)th animal.

**RESULTS**

**Tumor Growth Curve**

Among the 2,010 recorded birds, tumor growth curves could be fitted to 1,848 birds over all generations. Elementary statistics on estimated parameters are summarized in Table 1. Effects of line, MHC Rf-Y haplotypes, and sex are described in Table 2. Of the birds recorded, 15% did not develop a tumor, the frequency being slightly higher in males than in females \( (P < 0.001) \). As expected, the R line had a higher percentage of nonresponders than the P line \( (P < 0.001) \). There were also differences in percentages of nonresponders in the various MHC Rf-Y haplotypes within the P line, i.e., 7.3% for Yw*15, 13.3% for Yw*16, and 4.3% for Yw*17 \( (P < 0.001) \). Similarly, within the R line, the percentage of nonresponders was greater for Yw*16 than for Yw*17 (17.6 and 14.7%, respectively, \( P = 0.10 \)) but the difference was not significant.

Regression was observed in 53.0% of birds that developed a tumor, the percentage being slightly higher in males than in females \( (P = 0.04) \). The expected difference was found between the P line and the R line, with regression rates of 41.9 and 70.7%, respectively \( (P < 0.001) \).
Within the P line, regression rates did not differ (\( P = 0.57 \)). Within the R line, \( Yw^{177} \) had the highest regression rate (78.7 vs. 67.7\% for \( Yw^{176} \), \( P = 0.06 \)).

The median value for \( T_{\text{max}} \) was 49 d, and the mode was 63 d (11.6\% of birds). Birds of the P line reached \( T_{\text{max}} \) (25.6 vs. 13.6 cm\(^3\)) even when considering only birds with a tumor up to 53 cm\(^3\). Lines P and R differed in similar proportion, the mean \( V_{\text{max}} \) being equal to 31.6 cm\(^3\) in the P line and only 1.2 cm\(^3\) in the R line (45.8 and 3.3 cm\(^3\), respectively, if only birds without a tumor were considered).

Finally, MHC \( Yw^*17 \) haplotypes also differed for this parameter, with respective values of 49.6 cm\(^3\) for \( Yw^*15 \), 17.6 cm\(^3\) for \( Yw^*16 \), and 40.0 cm\(^3\) for \( Yw^*17 \) within the P line, 0.4 cm\(^3\) for \( Yw^*16 \) and 0.8 cm\(^3\) for \( Yw^*17 \) within the R line.

The MHC \( Rfp-Y \) haplotype effect was not significant for \( K_1 \) (\( P = 0.26 \)), although type \( Yw^{177} \) of line had higher \( K_1 \) values than the other MHC \( Rfp-Y \) haplotypes (Table 2). The line effect for \( K_2 \) was also in the expected direction, median being lower in the P (0.12 d\(^{-1}\)) than in the R line (0.23 d\(^{-1}\), \( P < 0.001 \)). The MHC \( Rfp-Y \) haplotype effect also approached significance for \( K_2 \), reflecting the opposition between the P and R lines (\( P = 0.10 \)). Males tended to develop tumors more quickly (\( K_1 = 0.109 \) d\(^{-1}\) for males vs. 0.105 d\(^{-1}\) for females, \( P = 0.21 \)) and to fight them more effectively than females (\( K_2 = 0.189 \) d\(^{-1}\) for males vs. 0.154 d\(^{-1}\) for females, \( P = 0.22 \)) but differences were not significant.

Finally, no effect was found for \( A \). Growth curves, as established from median values for lines, sexes, and MHC \( Rfp-Y \) haplotypes, can be seen in Figures 1 to 3.

**TABLE 2.** Effect of line, sex, and MHC \( Rfp-Y \) haplotypes on proportion of birds with no tumor development, regression, and median of tumor growth parameters\(^1\)

<table>
<thead>
<tr>
<th>Effect(^2)</th>
<th>No tumor (%)</th>
<th>Regression (%)</th>
<th>( K_1 ) (d(^{-1}))</th>
<th>( K_2 ) (d(^{-1}))</th>
<th>( T_{\text{max}} ) (d)</th>
<th>( V_{\text{max}} ) (cm(^3))</th>
<th>( A ) (cm(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Line effect</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>11.9</td>
<td>41.9</td>
<td>0.11</td>
<td>0.12</td>
<td>49</td>
<td>31.6</td>
<td>0.0</td>
</tr>
<tr>
<td>R</td>
<td>21.2</td>
<td>70.7</td>
<td>0.10</td>
<td>0.23</td>
<td>42</td>
<td>1.2</td>
<td>0.0</td>
</tr>
<tr>
<td>( P )</td>
<td>(&lt;0.001)</td>
<td>(&lt;0.001)</td>
<td>0.91</td>
<td>(&lt;0.001)</td>
<td>0.02</td>
<td>(&lt;0.001)</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>Sex effect</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>19.8</td>
<td>56.0</td>
<td>0.11</td>
<td>0.19</td>
<td>49</td>
<td>4.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Females</td>
<td>11.7</td>
<td>51.2</td>
<td>0.10</td>
<td>0.15</td>
<td>42</td>
<td>13.8</td>
<td>0.0</td>
</tr>
<tr>
<td>( P )</td>
<td>(&lt;0.001)</td>
<td>0.04</td>
<td>0.21</td>
<td>0.22</td>
<td>(&lt;0.001)</td>
<td>(&lt;0.001)</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>MHC ( Rfp-Y ) haplotype effect</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( Yw^{175} ) — P line</td>
<td>7.3</td>
<td>38.1</td>
<td>0.13</td>
<td>0.18</td>
<td>42</td>
<td>49.6</td>
<td>0.0</td>
</tr>
<tr>
<td>( Yw^{176} ) — P line</td>
<td>13.3</td>
<td>32.5</td>
<td>0.13</td>
<td>0.37</td>
<td>35</td>
<td>17.6</td>
<td>0.0</td>
</tr>
<tr>
<td>( Yw^{177} ) — P line</td>
<td>4.3</td>
<td>33.3</td>
<td>0.14</td>
<td>0.21</td>
<td>42</td>
<td>40.0</td>
<td>0.0</td>
</tr>
<tr>
<td>( Yw^{176} ) — R line</td>
<td>17.6</td>
<td>67.7</td>
<td>0.13</td>
<td>1.70</td>
<td>35</td>
<td>4.0</td>
<td>0.0</td>
</tr>
<tr>
<td>( Yw^{177} ) — R line</td>
<td>14.7</td>
<td>78.7</td>
<td>0.17</td>
<td>1.70</td>
<td>28</td>
<td>0.8</td>
<td>0.0</td>
</tr>
<tr>
<td>( P )</td>
<td>0.01</td>
<td>(&lt;0.001)</td>
<td>0.26</td>
<td>0.12</td>
<td>0.10</td>
<td>(&lt;0.001)</td>
<td>0.83</td>
</tr>
</tbody>
</table>

\(^1\)\( K_1 \) = factor of increased growth of the tumor in the progression phase; \( K_2 \) = factor of decreased growth of the tumor in the regression phase; \( T_{\text{max}} \) = age at maximum tumor size; \( V_{\text{max}} \) = maximum tumor volume; and \( A \) = asymptotic tumor volume.

\(^2\)P = progressor; R = regressor; \( Yw^{15} \), \( Yw^{16} \), and \( Yw^{17} \) = sublines homozygous for the \( Yw^{15} \), \( Yw^{16} \), and \( Yw^{17} \) MHC \( Rfp-Y \) haplotypes, respectively.
Rank Correlations Between Growth Curve Parameters and TPI

The rank correlations between the parameters estimated from the Brody curves, TPI, and age at death are presented in Table 3. Absolute values of correlations between the different Brody curve parameters were low, ranging from 0.01 to 0.40. However these were significantly different from zero, except for the correlations between $K_2$ and $K_1$ or between $K_2$ and $V_{\text{max}}$. Correlations between $K_1$ and $T_{\text{max}}$ or between $K_1$ and $V_{\text{max}}$ were negative and moderate. Correlations between TPI and the other traits were low, ranging from $-0.26$ with $T_{\text{max}}$ to $0.36$ with $V_{\text{max}}$. Finally, age at death was more highly correlated with TPI, $T_{\text{max}}$, and $V_{\text{max}}$. These weak correlations between TPI and Brody curve parameters were consistent with the fact that birds could have very different tumor growth curves with the same TPI value, as shown in Figure 4 for 4 representative birds with the same TPI value. In the first figure, the chicken develops a tumor, but there is no regression ($V_{\text{max}} = 565 \text{ cm}^3$, $A =$ missing). For the second bird, a regression occurs after 63 DPI, and the final asymptotic volume of the tumor is zero ($V_{\text{max}} = 346 \text{ cm}^3$, $A = 0 \text{ cm}^3$). In the third one, a regression also occurs, but the tumor does not completely disappear, i.e., the asymptotic volume is not equal to zero, and the maximum tumor volume is much lower than for the first 2 birds ($V_{\text{max}} = 73 \text{ cm}^3$, $A = 50 \text{ cm}^3$). Finally, the last bird shows progression and regression, but with very small tumor volumes, compared with the other 3 ($V_{\text{max}} = 1 \text{ cm}^3$, $A = 0 \text{ cm}^3$).

Genetic Parameters and Evolution

Heritabilities of Brody curve parameters are presented in Table 4. They are very low for $K_1$ and $A$, consistent with the nonsignificant evolution of these traits with generations in both P and R lines. Heritability was moderate for $K_2$ and $T_{\text{max}}$, with similar values for sire and dam heritabilities. A high discrepancy for $V_{\text{max}}$ was observed between the very low dam heritability and the very high sire heritability.

Figure 5 shows the increase in breeding value for $V_{\text{max}}$ in P line, due to its increase in haplotypes $Yw^{15}$ and $Yw^{17}$, whereas no genetic trend was observed in birds of haplotype $Yw^{30}$ for this parameter. The decrease in $V_{\text{max}}$ in R line was similar in both sublines. Genetic evolution of $T_{\text{max}}$ (Figure 6) was due to a decrease in R line and an increase in P line but erratic evolution was observed in
TABLE 3. Rank correlations between tumor profile index (TPI), age at death, and Brody curve parameters for all birds with a tumor (n = 1571)

<table>
<thead>
<tr>
<th>Variables</th>
<th>K2</th>
<th>Tmax</th>
<th>Vmax</th>
<th>A</th>
<th>TPI</th>
<th>Age at death</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>-0.04</td>
<td>-0.29</td>
<td>-0.21</td>
<td>-0.11</td>
<td>-0.11</td>
<td>-0.37</td>
</tr>
<tr>
<td>K2</td>
<td>0.09</td>
<td>0.01</td>
<td>0.17</td>
<td>0.36</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Tmax</td>
<td>0.29</td>
<td>0.40</td>
<td>0.23</td>
<td>0.40</td>
<td>0.13</td>
<td>0.32</td>
</tr>
<tr>
<td>Vmax</td>
<td>0.04</td>
<td>0.03</td>
<td>0.96</td>
<td>0.73</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.13</td>
<td>0.32</td>
<td>0.32</td>
<td>0.32</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>TPI</td>
<td>-0.29</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

1K1 = factor of increased growth of the tumor in the progression phase; K2 = factor of decreased growth of the tumor in the regression phase; Tmax = age at maximum tumor size; Vmax = maximum tumor volume; and A = asymptotic tumor volume.

2Values in italics are not significantly different from zero (P ≥ 0.05).

FIGURE 4. Variability of tumor growth curves for birds with the same tumor profile index (TPI) value (TPI = 2). Each figure represents a different bird (A, B, C, and D). Observed values are represented by dotted points and estimated values by solid lines.

DISCUSSION

Biological Significance of the Estimated Parameters

Fitting the growth curve summarizes all the information taken during the 63-d period of observation (i.e., up to 18 measurements) of 5 parameters. The classical growth models such as logistic or Gompertz equations have been used extensively to describe tumor growth processes (Michelson et al., 1987; Marusic et al., 1994; Yakovlev et al., 1999). A common feature among these models is that growth only increases and tends to an asymptote that is not zero (Werker and Jaggard, 1997). Although appropriate in many cases, it represents a serious limitation in the present case, because it does not allow regression of the tumor. Quadratic and exponential models have been used to describe biphasic curves for antibody production (Siegel et al., 1984; Weigend et al., 1997). In the current study, we needed to fit with the same function either monophasic or biphasic curves, which were observed in 54.5 and 45.5% of birds, respectively. It is the reason why, of all curves used to fit growth curves (Mignon-Grasteau

TABLE 4. Heritabilities of growth curve parameters, as estimated from the sire component (h²s) and from the dam component (h²d)

<table>
<thead>
<tr>
<th>Variable</th>
<th>h²s</th>
<th>h²d</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>K2</td>
<td>0.23</td>
<td>0.18</td>
</tr>
<tr>
<td>A</td>
<td>0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>Tmax</td>
<td>0.23</td>
<td>0.20</td>
</tr>
<tr>
<td>Vmax</td>
<td>0.73</td>
<td>0.06</td>
</tr>
</tbody>
</table>

1K1 = factor of increased growth of the tumor in the progression phase; K2 = factor of decreased growth of the tumor in the regression phase; Tmax = age at maximum tumor size; Vmax = maximum tumor volume; and A = asymptotic tumor volume.

2SE not available.
FIGURE 5. Evolution of genetic values for maximum volume of tumor (V_max) with line (A) and haplotype (B). The progressor line and the regressor line are indicated by the solid and dotted line, respectively. Haplotypes Yw*15, Yw*16, and Yw*17 are represented by diamonds, squares, and triangles, respectively.

and Beaumont, 2000), the Brody curve was chosen. Moreover, as for the Gompertz curve, it is possible to give a physiological interpretation of estimated parameters of the growth curve: K_1 determines the rate of increment of the tumor in the first phase (tumoral growth) and K_2 the rate of decrease of the tumor in the second phase. A is the asymptotic volume, i.e., the theoretical volume at an infinite age.

To distinguish between complete regression and the absence of a tumor, the asymptotic value was set at zero in the former and missing in the latter. When no decrease in volume could be observed, K_2 was set at missing. Considering all 3 estimates thus allows categorization of birds into nonresponders (V_max = 0 and K_1, K_2, T_max, and A = missing), complete regressors (A = 0), complete progressors (V_max > 0 and K_2 = missing), or intermediate. Estimates are important for selection and for distinction between progressors and regressors, e.g., to identify the genes underlying this trait. In contrast, TPI distinguished between responders (TPI > 1) and nonresponders (TPI = 1), but did not make any distinction between regressors and progressors. For example, of the 855 birds with a TPI equal to 2, 302 did not regress, and 553 regressed. Even with higher TPI, there was a proportion of birds that fought the tumor (14.8% with TPI = 3, 5.9% with TPI = 4, and 2.0% with TPI = 5). Birds could thus be very different tumor growth curves with the same TPI value. These could have been better distinguished according to their growth curve parameters, and it could have been possible to modify the shape of the tumor growth curve by selecting based on these parameters instead of selecting based on TPI.

The estimated parameters varied widely, which is favorable for selection. However, they had a distribution far from normal. The estimated values were therefore categorized into different classes and analyzed as categorical data which were less informative than continuously distributed variables. The non-normal distribution is also the reason why nonparametric tests were used to test the different effects, and an average growth curve was drawn from median values, which in this case were more representative of the population than the mean (Meddis, 1984).

However, the precision of these estimations (whether categorical or not) is dependent on the interval between 2 successive observations (i.e., 1 wk), whereas a shorter interval would have increased the accuracy of this estimation. More importantly, regression appeared late in some birds and sometimes a small number of measurements were used to fit the second part of the growth curve. Measuring birds later would also contribute to a better description of the regression phase.

Indeed, as can be seen from changes of all parameters with generation (with the exception of K_2, for which data from most birds were missing), selection modified the whole curve. These parameters might allow more accurate selection on the shape of the growth curve, for example, for better study of the ability to regress by selecting animals with a biphasic tumor growth curve with higher or lower T_max and K_2 which, according to estimated heritabilities, should be efficient. It might also make it possible to refine comparisons, as could be seen from the curves drawn from median parameters for P and R lines or for males and females. Even when no regression occurred, the tumor developed later in the regressor line, suggesting that genes responsible for tumor development were also selected. Moreover, regression occurred earlier in the R line, thus suggesting that mechanisms involved in regression were activated more quickly in this line. This could help to identify them in the future.

Differences Between Lines

Selection experiments on RSV first focused on mortality (Gyles and Brown, 1971), and then on TPI (Carte et al., 1972; Collins et al., 1977), which combined information on mortality and tumor volume. Fitting tumor growth would put more emphasis on regression, which is im-
important with reference to cancer. Indeed, it could be seen that selecting based on TPI resulted in more prominent differences in the maximum tumor volume (V_max) and in the age at maximum tumor volume (T_max), than in K_1 and K_2. This result was consistent with the estimated correlations between TPI and either V_max, T_max, or A, which are higher than correlations between TPI and either K_1 or K_2. However, even small differences in the latter 2 parameters may result in significant changes in tumor volume.

As for TPI (Pinard-Van der Laan et al., 2004), the MHC Rfp-Y haplotype also has a role in growth kinetics. Indeed, the MHC Rfp-Y haplotype effect was significant on V_max, K_1, K_2, and T_max. However, TPI was not sufficiently precise to enable understanding of mechanisms involved in the effect of MHC Rfp-Y haplotype effect on tumor growth. In fact, Pinard-Van der Laan et al. (2004) observed a higher TPI and a lower age at death in Yw^{16}, and concluded that this haplotype had a greater sensitivity. These results are consistent with the observation in the current study that the lowest T_max was in Yw^{16}, with birds of this haplotype developing tumors sooner than those with Yw^{15} and Yw^{17} haplotypes. However, a greater sensitivity of Yw^{16} haplotypes cannot be concluded, as the death rate in the P line was higher in Yw^{17} (54.6%) than in Yw^{16} (44.4%). Moreover, in the P line, the percentage of birds that did not develop a tumor was higher in Yw^{16} (13%) than in Yw^{15} (7%) or Yw^{17} (4%). It was concluded that the 3 haplotypes did not differ in their sensitivity, but by the mechanism involved in the response to RSV. The lowest maximum volume reached by the tumors in Yw^{16} could indicate an antitumoral response (i.e., may be effective even if only the v-src oncogene is inoculated), whereas the later development of the tumor in Yw^{17} would more likely correspond to an antiviral response. Fate of tumors induced by inoculation of RSV or by v-src DNA constructs.
in Yw*16 and Yw*17 chickens support this hypothesis (unpublished results). Both antitumoral and antiviral responses could rely on the demonstration of the expression of at least one class I gene of the MHC Rfp-Y system, which would have a specialized antigen recognition function compared to the classical B complex (Afanassieff et al., 2001). Indeed, MHC Rfp-Y haplotypes Yw*16 and Yw*17 have been shown to play a moderate role in skin allograft rejection (Thoraval et al., 2003), as has been described for other MHC Rfp-Y haplotypes (Pharr et al., 1996). It appears justified to assign to MHC Rfp-Y genes a similar role in allograft or tumor rejection.

**Differences Between Sexes**

From the higher percentage of mortality, greater V_{max}, greater A, and faster growth of tumor at later ages, it was concluded that females have a higher susceptibility to RSV infection and tumor development than males (Pinard-Van der Laan et al., 2004), which also resulted in faster evolution of a tumor. The mean age at maximum volume of the tumor (T_{max}) was earlier in females than in males. The exact physiological mechanism is not understood. While demonstrating a main association between the B locus and the fate of RSV-induced tumors in chickens, Collins et al. (1977) reported that sex had a slight, non-significant effect on TPI. In another study, Collins et al. (1986) reported higher metastatic growth of tumors in males than in females in various internal organs in response to RSV inoculation. These differences and the current results on B^{19} chickens may be due to the genetic background of the chickens, i.e., to an interaction between genotype and sex. Similarly, differences between sexes in susceptibility to cancer were observed in rodents and with other cancers (Lipkin, 1970; Hart et al., 1981), which suggests the existence of such differences.

**Rank Correlations Between Parameters**

The higher rank correlations observed between TPI and A and V_{max}, as well as T_{max}, explained why selection on TPI resulted in significant differences in these parameters, whereas no significant differences were observed for K_{2}, which was less correlated with TPI and less heritable. These results also emphasized that TPI cannot summarize all the information, as the highest correlation was only 0.36. Considering the entire growth may make it possible to distinguish progressors (with higher T_{max} and V_{max}) from regressors (with a higher K_{2}).

**Estimated Heritabilities**

Heritability estimates for tumor growth curve parameters were especially high for V_{max} when estimated from the sire component of variance. The difference between sire and dam estimates probably partially resulted from maternal effects, but also from nonoptimal differentiation of both sire and dam effects, as observed in such all-or-none traits (Beaumont et al., 1999). The TPI is dependent on age at death and maximum volume of the tumor, both of which are correlated with T_{max}. This trait has a lower heritability, but one compatible with selection. Heritability of K_{2} was close to 0.20, although no significant genetic difference was observed between lines, probably because selection on TPI mainly resulted in the absence of a tumor more than its regression. Selecting on a combination of T_{max} and V_{max} would probably result in a very similar response. Including K_{2} in the selection criteria, however, would make it possible to select for the regression phase, which is not taken into account in selection based on TPI.

The other 2 parameters had lower estimates of heritability, which could be deduced from the definition of A. Indeed, because A was missing if no regression appears (75% of birds) and nearly equal to zero in 62% of the remaining birds (i.e., A < 4 × 10^{-4}, this variable was not very informative when divided into classes for genetic analysis.

In conclusion, the current selection experiment showed that, in addition to the distinction between progressors and regressors, birds exhibited different tumor growth curves after inoculation with RSV. Similarly, males and females had different susceptibility as well as different tumor growth curves. Finally, our study confirmed the role of MHC in the type of reaction to the virus. The later development of tumor in Yw*17 was in agreement with an antiviral response, whereas the smallest maximum volume reached in Yw*16 was consistent with an antitu-

**REFERENCES**


