Microsatellite Markers Associated with Resistance to Marek's Disease in Commercial Layer Chickens

J. P. McElroy,* J. C. M. Dekkers,* J. E. Fulton,† N. P. O'Sullivan,† M. Soller,‡ E. Lipkin,‡ W. Zhang,* K. J. Koehler,* S. J. Lamont,* and H. H. Cheng§,[1]

*Department of Animal Science, 2255 Kildee Hall, Iowa State University, Ames, Iowa 50011; †Hy-Line International, PO Box 310, Dallas Center, IA 50063; ‡Department of Genetics, The Hebrew University of Jerusalem, Jerusalem 91904, Israel; and §USDA-ARS-ADOL, Avian Disease and Oncology Laboratory, 3606 East Mount Hope Road, East Lansing, Michigan 48823

ABSTRACT The objective of the current study was to identify QTL conferring resistance to Marek's disease (MD) in commercial layer chickens. To generate the resource population, 2 partially inbred lines that differed in MD-caused mortality were intermated to produce 5 backcross families. Vaccinated chicks were challenged with very virulent plus (vv+) MD virus strain 648A at 6 d and monitored for MD symptoms. A recent field isolate of the MD virus was used because the lines were resistant to commonly used older laboratory strains. Selective genotyping was employed using 81 microsatellites selected based on prior results with selective DNA pooling. Linear regression and Cox proportional hazard models were used to detect associations between marker genotypes and survival. Significance thresholds were validated by simulation. Seven and 6 markers were significant based on proportion of false positive and false discovery rate thresholds less than 0.2, respectively. Seventeen markers were associated with MD survival considering a comparison-wise error rate of 0.10, which is about twice the number expected by chance, indicating that at least some of the associations represent true effects. Thus, the present study shows that loci affecting MD resistance can be mapped in commercial layer lines. More comprehensive studies are under way to confirm and extend these results.

(*Key words*: chicken, Marek's disease, quantitative trait loci, survival, genetic resistance)

2005 Poultry Science 84:1678–1688

INTRODUCTION

Marek's disease (MD), a lymphoma caused by an avian herpesvirus, is a major disease affecting the poultry industry. It has been roughly estimated that, worldwide, Marek's disease costs the poultry industry $1 billion to $2 billion a year (Morrow and Fehler, 2004). The economic damage of MD is probably even greater because immunosuppression induced by the MD virus reduces resistance to other pathogens, which can lead to symptoms in young, market-weight broilers (Biggs et al., 1968; Abbassi et al., 1999), and lowers feed efficiency and other production traits (Groves, 1995; Islam et al., 2002). Vaccines have been produced that initially were effective in reducing MD incidence (Witter, 1985), but MD virus strains have evolved to the point that commercial vaccines are no longer fully protective.

An alternative method to reduce the incidence of MD is to genetically improve the chicken's innate resistance to this disease. Resistance or susceptibility to MD is a quantitative trait that is affected by multiple genes and the environment. Genetic improvement of quantitative traits can be achieved by selection of individuals with favorable phenotypic characteristics, by marker-assisted selection on genomic regions that harbor genes that confer the favorable phenotype, or both (Lande and Thompson, 1990). Marker-assisted selection is particularly useful for traits of low heritability and those that are sex-limited or difficult to measure. The MD resistance falls into all of these categories.

Use of marker-assisted selection requires knowledge of genes affecting a trait or of markers tightly linked to those genes (Dekkers and Hospital, 2002). A genome scan can be used to identify regions of the genome that harbor genes affecting a quantitative trait of interest, so-called QTL (Soller and Beckmann, 1983; Beckmann and Soller, 1983). Once a QTL region is identified, it can be more intensely studied to find the causative gene or a closely linked marker for use in selection programs.

Genomic regions associated with resistance to MD have been identified in several studies of noncommercial poultry populations. Vallejo et al. (1998) and Yonash et al. (1999) identified QTL on chromosomes 1, 2, 4, 7, and 8 that affect MD resistance; they used the same F_2 cross

©2005 Poultry Science Association, Inc.
Received for publication March 31, 2005.
Accepted for publication July 11, 2005.
[1]To whom correspondence should be addressed: hcheng@msu.edu.

between 2 White Leghorn lines (Avian Disease and Oncology Laboratory lines 6 and 7; ADOL) that differed in MD resistance. Bumstead (1998) used a backcross of the same ADOL lines to map a QTL for MD resistance on chromosome 1. Many studies have shown that the MHC complex (B blood group) on chromosome 16 affects resistance to MD (Hanson et al., 1967; Bacon et al., 1981; Bacon, 1987; Schierman and Collins, 1987; Lamont, 1989; Bacon and Witter, 1994; Schat et al., 1994). The growth hormone gene (***GH1***) on chromosome 1 also has an allelic association with MD resistance (Kuhnlein et al., 1997; Liu et al., 2001b). Microarray analysis has shown that *GH1* expression is associated with differences in MD resistance (Liu et al., 2001a) and the GH1 protein has been shown to interact with the SORF2 protein, a protein only found in virulent MD virus strains (Liu et al., 2001b). Stem lymphocyte antigen 6 complex locus E (*LY6E*) on chromosome 2 has also been identified as an MD resistance gene through genetic, RNA, and protein analysis (Liu et al., 2003). Because all of these studies used experimental populations, it is important to confirm the association of these QTL regions with MD in commercial populations, which will enable selection upon the QTL in those populations. The objective of the current study, therefore, was to identify QTL associated with MD resistance (defined as survival time following challenge) in a cross between lines of commercial layer chickens.

MATERIALS AND METHODS

Experimental Population and Phenotyping

The population was a backcross between 2 partially inbred lines (as determined by foundation from narrow genetic bases) of commercial layer chickens. In a prior screening of the parental lines for 102 microsatellite markers, 60% were fixed in line 1 and 80% in line 2. The lines were also fixed for different serologically typed B blood group alleles: *B2* in line 1 and *B15* in line 2. Prior studies also have shown the parental lines to differ in susceptibility to experimental challenge with a very virulent MD virus; the percentage of MD mortality was 41.4 and 21.0 percentage points higher in line 1 than line 2 in 2 separate experiments (data not shown), defining line 2 as the more resistant of these 2 lines.

To produce the resource population (see Figure 1), 5 males from line 1 were pair mated to individual line 2 females to generate 5 full-sib F_1 families. Seven males from each F_1 family were each mated to 15 females from line 1 to generate 5 grandsire backcross groups, each consisting of 7 half-sib sire families.

A total of 656 backcross female chicks (85 to 160 per backcross group) were vaccinated with 500 plaque-forming units of bivalent HVT/SB-1 vaccine (Merial Select, Gainesville, GA) at 1 d of age and subcutaneously inoculated with 500 plaque-forming units of the very virulent plus (vv+) 648A MD virus strain (Witter, 1997) at 6 d of age. Age at death and presence or absence of tumors by visual examination was recorded from 30 to 140 d of age. Initiation of records at 30 d of age excludes typical early chick and brooding mortality. Birds surviving to the end of the study (140 d) were euthanized by CO_2 inhalation. Treatment of the birds met or exceeded accepted guidelines (ADSA, 1988). Birds were housed and phenotypic data were collected at Hy-Line International (Dallas Center, IA). Survival time, quantified based on number of days of survival after experimental challenge with a virulent MD virus, was the phenotype used for QTL mapping.

Markers and Genotyping

For DNA isolation, blood was collected from the jugular vein at 3 wk of age in syringes containing EDTA with 22-gauge needles. A Qiagen QIAamp DNA Blood Mini kit (Qiagen, Valencia, CA) was used for DNA isolation according to the manufacturer's instructions, except that 25 uL of whole blood plus 175 uL of PBS were used for the spin protocol, and the samples were incubated at 70°C.

The backcross progeny were selectively genotyped (Lander and Botstein, 1989; Darvasi and Soller, 1992) by genotyping the 20% (n = 133) of chicks with shortest survival times past 30 d and that had tumors and the 20% that were the longest survivors (n = 134) for 81 microsatellite markers. Individuals in the extremes of the phenotypic distribution contain the majority of information needed to identify markers linked to that trait (Lander and Botstein, 1989), which maximizes power with limited genotyping (Lebowitz et al., 1987). The presence of macroscopically visible tumors was used as a defining trait to place short-surviving birds into the category of MD-susceptible short survivors, thus minimizing the placement into this category of birds that died from causes that were not related to MD. All paternal grandparents and F_1 sires were also genotyped, but genotypes were not available for dams of the backcross chicks.

Markers used in the current study were chosen based on their associations with MD resistance in 2 preliminary selective DNA pooling analyses (unpublished data), following methods described in Darvasi and Soller (1994) and Lipkin et al. (1998). Fifty-six of the 81 genotyped markers were chosen based on a selective DNA pooling analysis of 117 markers in this population (data not shown), and the 25 additional markers were chosen from 120 tested markers based on a selective DNA pooling analysis of the reciprocal backcross population (data not shown). Markers that were included in the initial pooling analyses were chosen by position to get maximal genomic coverage. The 81 markers were distributed among 17 chromosomes; 16 were on chromosome 1, 14 on 2, 10 on 3, 9 on Z, 8 on 5, 7 on 4, 4 on 15, 2 each on 6, 8, and 18, and 1 each on 7, 9, 13, 17, 23, 27, and E22. The average marker interval for chromosomes with multiple markers was 37 cM.

Statistical Analyses

Line Origin Probabilities. The objective of the statistical analyses was to identify associations of marker alleles

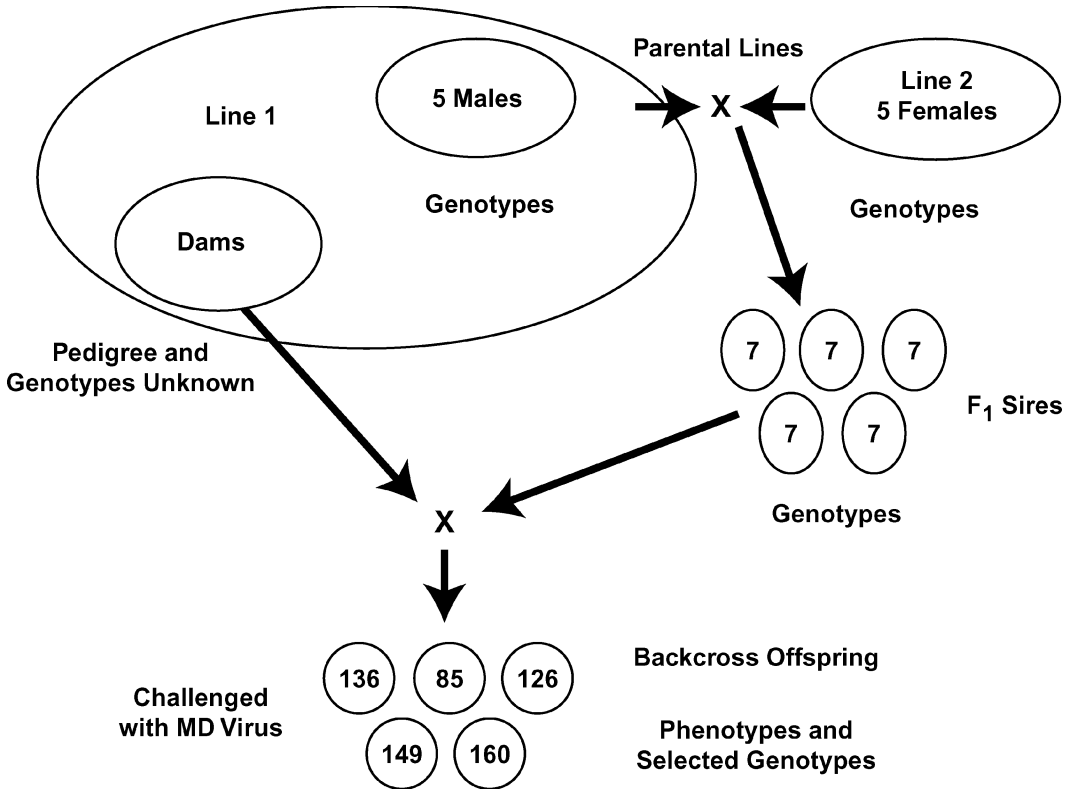

Figure 1. Population design used to generate each of the 5 grandparental backcross families. Numbers of individuals are indicated by the numbers in the circles. Five line 1 males were pair-mated to 5 line 2 females to generate 5 full-sib F_1 families. Seven sires from each of the 5 F_1 families were each mated to approximately 15 line 1 females to generate 5 groups (by grandsire) of backcross individuals. Length of survival was recorded for all backcross individuals. Genotypes for 81 microsatellite markers were known for all grandparents, F_1 sires, and selected backcross individuals. Genotypes were not observed for the dams of the backcross individuals. MD = Marek's disease.

with survival in the backcross offspring, based on line origin of the marker allele that was inherited from the F_1 sire. Line origin could be determined for 38 markers that were fixed for alternative alleles in the parental lines and for 23 markers for which distinct alleles were segregating in the parental lines. For 20 of the 81 markers, however, parental lines segregated at least one common allele. For such markers, an offspring was not fully informative when identically heterozygous to the F_1 sire. On average across these markers, 55% of the offspring were not fully informative. Although genotypes of the backcross dams were unknown, allelic frequencies in the line were known from previous genotyping of individuals from line 1. These frequencies were used to infer the probability that a dam transmitted a given allele to the noninformative offspring and, equivalently, the probability that the sire transmitted the alternate allele. The probability that a backcross offspring with marker genotype A_1A_2 inherited a line 1 allele from its F_1 sire ($p(L_1)$) was then computed as

$$p(L_1) = p(A_1 = L1 | A_1 = F1)\, p(A_1 = F1) + p(A_2 = L1 | A_2 = F1)\, p(A_2 = F1)$$

where $p(A_j = L1 | A_j = F1)$ is the probability that allele A_j originated from line 1 given that it came from the F_1 sire (= 1, 0.5, or 0 following Mendelian inheritance) and can be computed based on allele frequencies among dams, $f(A_j)$, as

$$p(A_j = F1) = 1 - f(A_j) \,/\, [f(A_1) + f(A_2)].$$

Note that because $p(L_1) = 1 - p(L_2)$, all information on line origin is captured by $p(L_1)$.

Statistical Models. Only individuals that were genotyped were included in the analyses. The phenotypic data were right-skewed and censored (some individuals survived to the end of the study and therefore did not have a date of death), and only phenotypic extremes were genotyped (selective genotyping). For this reason the Cox proportional hazards (CPH) model (Cox, 1972) was used for analysis, in addition to regular least squares regression (Legendre, 1805). Aside from the proportional hazards assumption, estimation for the CPH model is rank based (distribution free) and accommodates survival data with censoring (Smith, 2002); therefore, it may be more appropriate than least squares regression for analysis of these data. Effect estimates from the CPH model can be interpreted as natural logarithms of ratios of hazards. The least squares regression model was also considered because it is computationally easier to use than the CPH model, and the effect estimates from linear regression have a more convenient interpretation with respect to mean survival time.

In preliminary analyses under the CPH model, effects of grandsire, blood group genotype, their interaction, and the interactions of these effects with $p(L_1)$ did not occur

more often than expected by chance, considering a comparison-wise error rate (CWER) of 0.05 (fewer than 5% of the tests had a CWER = 0.05 for each of the effects). Therefore, these effects were not included in the final models for analysis.

The final model used for linear regression analysis was

$$S_i = \beta p(L_1)_i + \varepsilon_i$$

where S_i is the survival time of animal i, in days; β is the increase in the mean survival time associated with inheriting the line 1 versus the line 2 marker allele; $p(L_1)_i$ is as defined previously for animal i; and ε_i is the residual for animal i.

In accordance with Smith (2002), the model used for CPH analysis was

$$S(t;\eta_i) = [S_0(t)]^{\exp(\eta_i)}$$

where $S(t;\eta_i)$ is the probability that animal i survived at least until time t, and $S_0(t)$ is the baseline survivor function:

$$S_0(t) = e^{\int h_0(t)dt}$$

where $h_0(t)$ is the baseline hazard function and

$$\eta_i = \beta p(L_1)_i.$$

The corresponding hazard function is

$$h(t) = \exp(\eta_i)h_0(t) = \exp(\beta p(L_1)_i)h_0(t),$$

where β is the allelic effect on the natural log of the ratio of hazards for inheriting the line 1 versus line 2 marker, and $p(L_1)_i$ is as defined previously. This formulation of the model allows the use of standard CPH statistical software for estimation of β, and the baseline hazard is not needed to estimate β (Smith, 2002). This approach provides an approximation to a partial likelihood estimator for β, as discussed in the appendix of this paper. Simulation of data under the null hypothesis of no QTL effect was used to ensure that the standard probability values that were obtained from each model were appropriate. To account for multiple testing, the false discovery rate (FDR; Benjamini and Hochberg, 1995; Weller et al., 1998) and the probability of false positives (PFP; Fernando et al., 2004; Heifetz, 2004) statistics were used as an indication of the strength of associations of markers with survival.

The linear regression and CPH models were compared based on their abilities to identify markers associated with MD survival and for their agreement in comparison-wise probability values and estimates of marker effects. The β coefficient in the linear regression model is interpreted in units of days, whereas β in the CPH model is interpreted in terms of the conditional odds of dying during a short period of time after any particular time point given that an animal survives up to that time point. Because of their different scales, a correlation was used to compare the effect estimates and probability values of the 2 models.

Simulation Analysis. To determine whether standard probability values were appropriate for the analyses that were conducted, survival data with properties similar to the observed data were simulated with a backcross model under the null hypothesis of no QTL, following procedures described by Vincent Ducrocq (Station de Genetique Quantitative et Appliquee, Institut National de la Recherche Agronomique, France, personal communication). To simulate new samples of survival times that reflect the features of the observed data, a survival function (S(t)) was estimated from the observed data using the nonparametric Kaplan-Meier estimator (Kaplan and Meier, 1958) of a survivor function:

$$\hat{S}(t) = \prod_{j=1}^{k} \frac{n_j - d_j}{n_j}$$

for $t_{(k)} \le t < t_{(k+1)}$, where j is the rank of a particular day (t) among all chronologically ordered days in which death occurred, k is the rank of the day at which the survival function is being evaluated, n_j is the number of animals at risk on day j, and d_j is the number of birds dying on day j. Note that n_j excludes any birds that died or were censored before day j, but includes any bird censored at day j. $\hat{S}(t) = 1$ for $0 \le t < t_{(1)}$. In this application birds were only censored at 140 d. A simulated sample of death times was obtained by drawing a sample from a uniform [0,1] distribution and inverting the Kaplan-Meier estimate of the survivor function to obtain death times. Birds with death times exceeding 140 d were censored at 140 d. Each bird was also independently assigned 1 of 2 marker alleles based on a random draw from a binomial distribution with a 50% chance of inheriting either allele, simulating a marker linked to a QTL with no effect. To simulate selective genotyping data, the simulated birds were ranked based on survival time, and the 20% shortest and longest survivors were analyzed using the linear regression and CPH models. Birds surviving to 140 d were considered censored for the CPH model and as dying at 140 d for the linear regression model. This process was repeated for 1,000 replicates of 700 birds, and the proportions of replicates with a probability value less than CWER levels of 0.1, 0.05, 0.01, and 0.001 were compared with the expected false positive rate for those levels. A 2-tailed binomial test (Miller and Miller, 1999) was used to identify deviations from expectations.

RESULTS

Distribution of Survival Times

Survival times in the backcross population over the recording time (30 to 140 d) ranged from 33 to 140 d (Figure 2). Survival times followed a right-skewed distribution with a mean of 65.5 d, a median of 59.0 d, and a standard deviation of 23.9 d. Twenty-eight individuals

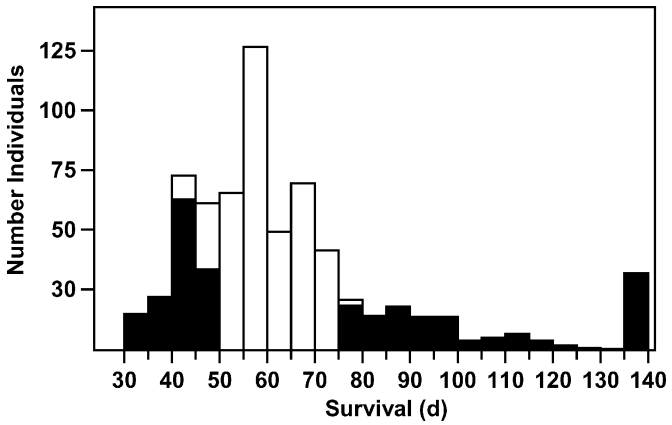

Figure 2. Distribution of length of survival in the backcross population. Dark shading indicates the selectively genotyped individuals.

(4.3%) survived to the end of the study and were considered censored in the analyses.

False Positive Rates

Table 1 shows the percentage (of 1,000 simulated replicates) of tests that had a comparison-wise probability value less than 0.1, 0.05, 0.01, or 0.001 from analyzing the selective genotyping survival data simulated under the null hypothesis (no QTL effect) with the linear regression and CPH models. None of the false positive rates were significantly different ($P = 0.05$) from the expected rates (the four CWER thresholds) based on a 2-tailed binomial test. This finding indicates that the comparison-wise probability values obtained from the actual data correspond to tests with valid type I error levels for both methods of analysis.

Marker Analysis

As mentioned above, in preliminary analyses, proportions of significant effects of grandsire and blood group did not deviate from the expected by chance using a CWER of 0.05 under the CPH model and, therefore, they were not included in the final models for analysis. Our results, summarized in Table 2 and Figure 3, show 7 and 6 markers that exceeded a 0.2 threshold using PFP and FDR, respectively. Results for an additional 10 markers, which were significant relative to a CWER of 0.10 but did not reach a PFP of FDR threshold of 0.2, are also summarized in Table 2 and Figure 3. Although the evidence of an association of these markers with survival is not as strong, these results are included here so that they can be compared with results from other experiments that are or will be reported in the literature and so that trends can be observed in identified genomic regions reported in the present study. The corresponding locations from the consensus genetic linkage map of the chicken are also indicated in Table 2 and Figure 3.

Table 1. False positive rates for the linear regression and Cox's proportional hazards models from simulation under the null hypothesis of no QTL effect, for different comparison-wise significance levels[1]

	Comparison-wise significance level[2]			
Model	0.1	0.05	0.01	0.001
Linear regression	0.111	0.053	0.01	0
Cox proportional hazard	0.108	0.066	0.007	0

[1]Results are based on 1,000 replicates.

[2]With a 2-tailed binomial test, none of the values were different (comparison-wise $P \leq 0.05$) from the expected values based on significance level.

Correlations of Effect Estimates and Probability Values

The correlation between effect estimates from the linear regression and CPH analyses of all markers was −0.96, indicating that CPH estimates can be accurately predicted from linear regression estimates. The negative relationship arises from the difference in the interpretation of the parameters in the 2 models; a shorter expected survival time from the regression model corresponds to a larger hazard ratio in the CPH model. The correlation between the probability values from the 2 models was 0.83, suggesting good correspondence in the degree of significance.

DISCUSSION

Comparison of Analyses

Analysis of the simulated selective genotyping survival data showed that standard determinations of CWER resulted in valid false positive rates and, therefore, in valid probability values for the CPH and linear regression models. This result was expected for the CPH model because, assuming the proportional hazards assumption was met, the data did not violate assumptions of the model. This result was not necessarily expected for linear regression because the assumption of normally distributed data was violated (Larsen and Marx, 1990). The CWER for the linear regression analysis appeared robust to violation of this assumption, likely because the numbers of individuals were large enough for the central limit theorem to become a factor (Miller and Miller, 1999). The estimated coefficients, therefore, had a large sample normal distribution, and so the significance tests also had an approximate normal distribution. This finding does not, however, mean that the effect estimates obtained from either of these models are valid. Linear regression overestimates QTL effects when selective genotyping is used, even if phenotype is normally distributed in the complete data set (Lander and Botstein, 1989). Darvasi and Soller (1992) and Ronin et al. (1998) proposed methods to correct this bias for normally distributed traits, but these methods are not appropriate for survival data because of skewness and censoring.

From analysis of the actual data, linear regression appears to be as, or more, powerful than the CPH model

Table 2. Markers associated (comparison-wise $P \leq 0.10$) with Marek's disease survival

			Linear regression		Cox's proportional hazards	
Marker	Chromosome	Position[1]	*P*-value	Effect (d)[2]	*P*-value	Effect[2]
ADL0309	2	90	0.014[3,4]	–10.71	0.051	0.26
ADL0176	2	115	0.011[3,4]	–12.01	0.006[3,4]	0.40
ADL0300	2	225	0.074	–7.65	0.191	0.17
MCW0034	2	230	0.086	–7.79	0.232	0.17
MAXL	4[5]	220	0.031	9.28	0.124	–0.20
LEI0116	5	5	0.018[3]	10.32	0.067	–0.25
ADL0253	5	45	0.007[3,4]	20.01	0.008[3,4]	–0.64
ADL0023	5	100	0.081	7.53	0.135	–0.20
HUJ0005	6	40	0.094	7.24	0.161	–0.19
ADL0278	8	95	0.161	–6.00	0.083	0.23
MCW0231	15	30	0.029	–16.13	0.057	0.42
ADL0022	Z	0	0.001[3,4]	–14.63	0.001[3,4]	0.46
MCW0331	Z	20	0.000[3,4]	–15.27	0.001[3,4]	0.45
MCW0055	Z	30	0.001[3,4]	–14.54	0.001[3,4]	0.44
MCW0258	Z	35	0.092	–7.22	0.066	0.24
ADL0273	Z	75	0.076	–7.61	0.049	0.26
LEI0121	Z	130	0.103	–7.00	0.077	0.23

[1]Locations from the consensus genetic linkage map of the chicken (Groenen et al., 2000; Schmid et al., 2000).

[2]Negative effects from the linear regression model (in days) and positive effects from the Cox proportional hazards (in terms of the effect on the natural log of the hazard ratio) model indicate that the favorable allele was derived from the more resistant line.

[3]Significant based on the proportion of false positive rate ≤ 0.2 [equivalent comparison-wise significance levels: 0.0183 (linear regression) and 0.008 (Cox proportional hazards)].

[4]Significant based on false discovery rate ≤ 0.2 [equivalent comparison-wise significance levels: 0.0143 (linear regression) and 0.008 (Cox proportional hazards)].

[5]There is evidence that MAXL may not be on chromosome 4 (Wang, 2003).

for analyzing the selective genotyping survival data. There was a strong linear relationship between estimates from the 2 models. An advantage of the linear regression model over the CPH model is that estimated coefficients are much easier to interpret. Estimates from linear regression are in days of survival, whereas CPH estimates are in terms of an exponential function of the odds that animals die within a short period of time following some time point given that the animals survive up to that time point. Estimation of coefficients in the CPH model is a based on the ranks of the death and censoring times, which results in the model ignoring information in the spacing between death times. The regression approach uses information in the spacing of death times, but it will be affected by the handling of censoring times. Use of the censoring time as a death time in the regression analysis was not a major issue in the current study because censoring only occurred at 140 d, and only 4.3% of the birds survived beyond 140 d.

Markers Associated with Marek's Disease Survival

Several markers were associated with length of survival in this postvaccination MD challenge using an FDR or PFP threshold of 0.2. One of these markers corresponds to a QTL identified on chromosome 2 near the region identified for MD susceptibility by Yonash et al. (1999) and Vallejo et al. (1998) (around 90 cM on the consensus map). Confirmation of QTL in multiple populations is important for eliminating false positives and demonstrating segregation of the QTL in multiple populations. In the current study, no QTL were found in regions on chromosomes 1, 4, 7, 8, 12, and 17, which were identified as possibly harboring QTL by Vallejo et al. (1998), Yonash et al. (1999), and Bumstead (1998). These discrepancies could be due to lack of segregation of these same QTL between the lines used in the present study, insufficient power, or false positives in the other 3 studies. Discrepancies between the studies might also have arisen because a recent vv+ field isolate of the MD virus was used in the current study, whereas the other studies used less virulent strains. The lines of birds used here are resistant to the commonly used older laboratory strains of the MD virus. Previous studies also did not identify the strong QTL identified in the present study on chromosome Z. This may be due to the fact that in the previous studies, this chromosome was only surveyed with a single marker. In an F_2 cross of highly inbred chicken lines, however, Zhou et al. (2003) identified a QTL for antibody response kinetics on chromosome Z, near the QTL for MD resistance identified in the present study. The growth hormone receptor gene is also located on chromosome Z near the same position. The *GH1* gene (on chromosome 1) has been associated in previous studies with MD resistance (Kuhnlein et al., 1997; Liu et al., 2001a,b). Interactions between markers near the *GH1* and the growth hormone receptor genes affecting MD resistance were not significant (data not shown).

For the 17 markers identified in the present study to have an association with MD resistance with a CWER ≤ 0.10, 12 showed allele effects in the expected direction, and the favorable allele originated from the more resistant line 2. These QTL, therefore, explain part of the difference

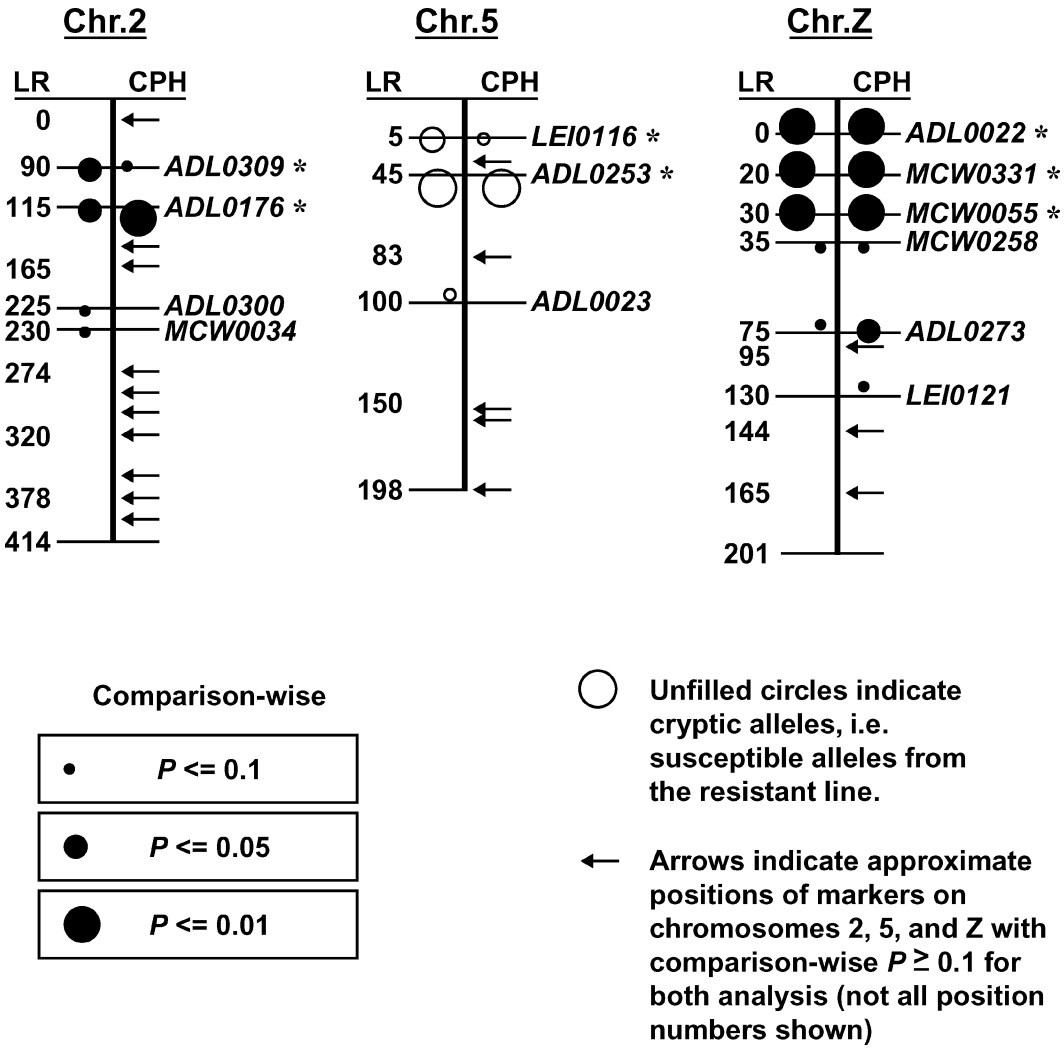

Figure 3. Markers associated with Marek's disease resistance (comparison-wise $P \leq 0.1$) on chromosomes 2, 5, and Z. Markers are indicated on the right side of each chromosome and their positions (cM) on the left. The stringency of the threshold is indicated by the size of the circle. Unfilled circles indicate cryptic alleles. The model [linear regression (LR) or Cox's proportional hazards (CPH)] in which a marker was found to be associated with Marek's disease resistance is indicated by the location of the circle on the left or right side of the chromosome, respectively. Asterisks (*) indicate markers exceeding probability of false positives or false discovery rate threshold of 0.2. Arrows indicate approximate positions of markers on chromosomes 2,5, and Z with comparison-wise $P \geq 0.1$ for both analyses (not all position numbers shown).

in MD resistance between the 2 lines. However, favorable QTL alleles were also identified as originating from the less resistant line (i.e., cryptic alleles).

MHC

The MHC has been shown to be associated with resistance to many diseases in poultry, including MD (Bacon et al., 1981; Bacon, 1987; Steadham et al., 1987; Lamont, 1989, 1998; Bacon and Witter, 1994; Lakshmanan et al., 1997). The current study did not, however, find an association between the MHC and MD survival. In the current study, the MHC-associated blood group alleles present were *B2* and *B15*, and the blood group genotypes in the backcross offspring were *B2/B2* or *B2/B15*. If, for the experimental population in the current study, the *B2* allele was completely dominant over the *B15* allele, a difference in survival between the 2 MHC genotypes would not be expected. Epistatic interactions between the MHC and background genes that were not linked to a marker used in this study, which could mask an MHC effect, might also explain the lack of association between blood group alleles and MD survival. The strain of the MD virus used in the current study (vv+ 648A) might have also affected the role of the MHC for affecting survival.

Significance Tests

To account for multiple testing, FDR and PFP thresholds were used. Seven and 6 markers, repectively, exceeded FDR and PFP threshold of 0.2 using linear regression and 5 and 5, respectively, using the CPH model. For PFP, a threshold of 0.2 results in the expectation that 80% of the tests exceeding this threshold are true positives (Fernando et al., 2004). Interpretation of a 0.2 threshold for FDR is more difficult to define but is similar, although somewhat more conservative, depending on the number of true effects present in the dataset.

Another issue to consider regarding experiment-wise thresholds is that 56 of the markers used in the current study were selected from previous analyses of DNA pools for 117 markers on the same experimental population. Reported results assumed that the 56 markers represented a random set of markers, regardless of the early pools results (i.e., on the assumption that the pooling analyses were not at all indicative of the results of the current analyses). If the pooling analyses were highly predictive of results of the current study, all 117 markers used in the pooling analyses would need to be considered when determining experiment-wise significance levels using FDR and PFP, resulting in only 3 markers exceeding the 0.2 threshold (the same 3 markers would exceed the FDR and PFP threshold of 0.2 for the CPH and linear models). This, along with the results reported, represents the 2 extremes (i.e., the upper and lower bounds of the experiment-wise thresholds to be used in the current study). A comparison of CWER probability values from individual genotyping for the 56 markers that were selected based on the pools, however, showed rather low correlations, ranging from 0.11 to 0.21, with probability values from the pooling analyses, in which the pools were created within B blood group genotype. This finding indicates that statistical tests based on the pooling analyses were not very predictive of the results of statistical tests based on individual genotyping, and, therefore, our assumption was correct and the experiment-wise thresholds need not be corrected for preselection of markers from the pooling analysis. Note that the problem of preselection does not arise for the 25 markers selected based on pool analyses of the reciprocal backcross because these were based on different individuals and data.

The large discrepancy between results of the statistical tests from the pooling analysis and the selective genotyping analysis are likely due to several reasons, including accuracy of the pools and differences in traits considered and in methods for statistical analyses. Although the correlation between frequencies of alleles estimated from pools and the actual frequency of alleles in the individuals that contributed to the pools was high (approximately 0.90), it was not 1.00, and parental lines were not fixed for alternate alleles for 44 of the 117 markers, which could lead to errors from the pooling analyses. The trait analyzed in pooling analyses was also different from the trait analyzed in selective genotyping analyses. For the pooling analyses, pools were formed within blood group genotype, and the number of tumors was considered as an additional variable when selecting individuals, whereas only length of survival was considered as the phenotype in the current analyses. In addition, individuals that did not have tumors and that survived to the end of the study were not included in the pooling analysis but were included in the selective genotyping analysis. Because these individuals were the most extreme, they were likely the most informative in the selective genotyping study and, therefore, contributed to the discrepancy between the pooling and individual genotyping analyses. Finally, the statistical models used for pool analysis and those used for analysis of the individual genotyping results were also quite different.

Implications

Identification of and subsequent selection upon QTL affecting MD resistance will be useful to the poultry industry to reduce losses caused by MD virus infection (Vallejo et al., 1998). Improving genetic resistance to MD can also improve vaccine efficacy (Lamont et al., 2002) and possibly increase the length of time that vaccines are useful before the virus mutates to become resistant. The QTL identified in the current experiment are starting points for more intensive studies to precisely locate the QTL positions for utilization in marker-assisted selection or for identification of the genes responsible for phenotypic variation. Phenotypic selection for MD requires exposure and costly challenge of relatives of selection candidates with the pathogenic agent to obtain phenotypic data on resistance or susceptibility (Arthur and Albers, 2003). Direct selection on genes that affect resistance to MD, or on linked markers, does not require a direct disease challenge of immediate relatives, although challenge studies are needed to identify initial associations. Direct selection uses genetic information on selection candidates rather than their relatives and can be implemented almost immediately upon hatching, thereby potentially shortening the generation interval. Therefore, identification of genetic regions affecting MD resistance is of great value to the poultry industry. The current study is the first reported QTL scan for MD resistance in commercial layers. However, the identification of the QTL on chromosome 2 in both the current study and in studies using experimental lines also shows the usefulness of experimental populations in identifying QTL that may also be segregating in commercial populations. Current availability of the draft of the complete chicken genome sequence and a 2.8 million single nucleotide polymorphism map will facilitate future QTL and causative gene identification (Hillier et al., 2004; Wong et al., 2004).

ACKNOWLEDGMENTS

This research was partly supported by a USDA National Needs Fellowship in Animal Biotechnology (JM), by Hatch and State of Iowa funds (SJL, JCMD) Project 6680, Project 6674, and USDA NRICGP award 99-03307 (HHC and MS). The authors acknowledge Laurie Molitor for her technical assistance.

REFERENCES

Abbassi, H., F. Coudert, Y. Cherel, G. Dambrine, J. Brugere-Picoux, and M. Maciri. 1999. Renal cryptosporidiosis (*Cryptosporidium baileyi*) in specific-pathogen-free chickens experimentally coinfected with Marek's disease virus. Avian Dis. 43:738–744.

ADSA. 1988. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. ADSA, Savoy, IL.

Arthur, J. A., and G. A. A. Albers. 2003. Industrial perspective on problems and issues associated with poultry breeding.

Pages 1–12 in Poultry Genetics, Breeding and Biotechnology. W. M. Muir and S. E. Aggrey, ed. CABI Publ., Cambridge, MA.

Bacon, L. D. 1987. Influence of the major histocompatibility complex on disease resistance and productivity. Poult. Sci. 66:802–811.

Bacon, L. D., and R. L. Witter. 1994. *B* haplotype influence on the relative efficacy of Marek's disease vaccines in commercial chickens. Poult. Sci. 73:481–487.

Bacon, L. D., R. L. Witter, L. B. Crittenden, A. Fadly, and J. Motta. 1981. *B*-haplotype influence on Marek's disease, Rous sarcoma, and lymphoid leukosis virus-induced tumors in chickens. Poult. Sci. 60:1132–1139.

Beckmann, J. S., and M. Soller. 1983. Restriction fragment length polymorphisms in genetic improvement: Methodologies, mapping and costs. Theor. Appl. Genet. 67: 35–43.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. J. R. Stat. Soc. B 57:289–300.

Biggs, P. M., P. L. Long, S. G. Kenzy, and D. G. Rootes. 1968. Relationship between Marek's disease and coccidiosis. II. The effect of Marek's disease on the susceptibility of chickens to coccidial infection. Vet. Rec. 83:284–289.

Bumstead, N. 1998. Genomic mapping of resistance to Marek's disease. Avian Pathol. 27:S78-S81.

Cox, D. R. 1972. Regression models in life tables (with discussion). J. R. Stat. Soc. B 34:187–220.

Darvasi, A., and M. Soller. 1992. Selective genotyping for determination of linkage between a marker locus and a quantitative trait locus. Theor. Appl. Genet. 85:353–359.

Darvasi, A., and M. Soller. 1994. Selective DNA pooling for determination of linkage between a molecular marker and a quantitative trait locus. Genetics 138:1365–1373.

Dekkers, J. C., and F. Hospital. 2002. The use of molecular genetics in the improvement of agricultural populations. Nat. Rev. Genet. 3:22–32.

Fernando, R. L., D. Nettleton, B. R. Southey, J. C. Dekkers, M. F. Rothschild, and M. Soller. 2004. Controlling the proportion of false positives in multiple dependent tests. Genetics 166:611–619.

Groenen, M. A., H. H. Cheng, N. Bumstead, B. F. Benkel, W. E. Briles, T. Burke, D. W. Burt, L. B. Crittenden, J. Dodgson, J. Hillel, S. Lamont, A. Ponce de Leon, M. Soller, H. Takahashi, and A. Vignal. 2000. A consensus linkage map of the chicken genome. Genome Res. 10:137–147.

Groves, P. 1995. Some epidemiological aspects of Marek's disease in the Australian poultry industry. Pages 27–25 in Epidemiol. Chapter Proc. Austr. College Vet. Sci., Sydney, Australia.

Hanson, M. P., G. R. J. Law, and J. N. Van Zandt. 1967. Differences in susceptibility to Marek's disease in chickens carrying two different *B* locus blood group alleles. Poult. Sci. 46:1268. (Abstr.)

Heifetz, E. M. 2004. Fine mapping of QTL, methods and results. Ph.D. Thesis. Hebrew Univ. Jerusalem, Jerusalem, Israel.

Hillier, L. W., W. Miller, E. Birney, W. Warren, R. C. Hardison, C. P. Ponting, P. Bork, D. W. Burt, M. A. Groenen, M. E. Delany, J. B. Dodgson, A. T. Chinwalla, P. F. Cliften, S. W. Clifton, K. D. Delehaunty, C. Fronick, R. S. Fulton, T. A. Graves, C. Kremitzki, D. Layman, V. Magrini, J. D. McPherson, T. L. Miner, P. Minx, W. E. Nash, M. N. Nhan, J. O. Nelson, L. G. Oddy, C. S. Pohl, J. Randall-Maher, S. M. Smith, J. W. Wallis, S. P. Yang, M. N. Romanov, C. M. Rondelli, B. Paton, J. Smith, D. Morrice, L. Daniels, H. G. Tempest, L. Robertson, J. S. Masabanda, D. K. Griffin, A. Vignal, V. Fillon, L. Jacobbson, S. Kerje, L. Andersson, R. P. Crooijmans, J. Aerts, J. J. van der Poel, H. Ellegren, R. B. Caldwell, S. J. Hubbard, D. V. Grafham, A. M. Kierzek, S. R. McLaren, I. M. Overton, H. Arakawa, K. J. Beattie, Y. Bezzubov, P. E. Boardman, J. K. Bonfield, M. D. Croning, R. M. Davies, M. D. Francis, S. J. Humphray, C. E. Scott, R. G. Taylor, C. Tickle, W. R. Brown, J. Rogers, J. M. Buerstedde, S. A. Wilson, L. Stubbs, I. Ovcharenko, L. Gordon, S. Lucas, M. M. Miller, H. Inoko, T. Shiina, J. Kaufman, J. Salomonsen, K. Skjoedt, G. K. Wong, J. Wang, B. Liu, J. Yu, H. Yang, M. Nefedov, M. Koriabine, P. J. Dejong, L. Goodstadt, C. Webber, N. J. Dickens, I. Letunic, M. Suyama, D. Torrents, C. von Mering, E. M. Zdobnov, K. Makova, A. Nekrutenko, L. Elnitski, P. Eswara, D. C. King, S. Yang, S. Tyekucheva, A. Radakrishnan, R. S. Harris, F. Chiaromonte, J. Taylor, J. He, M. Rijnkels, S. Griffiths-Jones, A. Ureta-Vidal, M. M. Hoffman, J. Severin, S. M. Searle, A. S. Law, D. Speed, D. Waddington, Z. Cheng, E. Tuzun, E. Eichler, Z. Bao, P. Flicek, D. D. Shteynberg, M. R. Brent, J. M. Bye, E. J. Huckle, S. Chatterji, C. Dewey, L. Pachter, A. Kouranov, Z. Mourelatos, A. G. Hatzigeorgiou, A. H. Paterson, R. Ivarie, M. Brandstrom, E. Axelsson, N. Backstrom, S. Berlin, M. T. Webster, O. Pourquie, A. Reymond, C. Ucla, S. E. Antonarakis, M. Long, J. J. Emerson, E. Betran, I. Dupanloup, H. Kaessmann, A. S. Hinrichs, G. Bejerano, T. S. Furey, R. A. Harte, B. Raney, A. Siepel, W. J. Kent, D. Haussler, E. Eyras, R. Castelo, J. F. Abril, S. Castellano, F. Camara, G. Parra, R. Guigo, G. Bourque, G. Tesler, P. A. Pevzner, A. Smit, L. A. Fulton, E. R. Mardis, and R. K. Wilson. 2004. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. Nature 432: 695–716.

Islam, A. F. M. F., C. W. Wong, S. W. Walkden-Brown, I. G. Colditz, K. E. Arzey, and P. J. Groves. 2002. Immunosuppressive effects of Marek's disease virus (MDV) and herpesvirus of turkeys (HVT) in broiler chickens and the protective effect of HVT vaccination against MDV challenge. Avian Pathol. 31:449–461.

Kaplan, E. L., and P. Meier. 1958. Nonparametric estimation from incomplete observations. J. Am. Stat. Assoc. 53:457–481.

Kuhnlein, U., L. Ni, S. Weigend, J. S. Gavora, W. Fairfull, and D. Zadworny. 1997. DNA polymorphisms in the chicken growth hormone gene: Response to selection for disease resistance and association with egg production. Anim. Genet. 28:116–123.

Lakshmanan, N., J. S. Gavora, and S. J. Lamont. 1997. Major histocompatibility complex class II DNA polymorphisms in chicken strains selected for Marek's disease resistance and egg production or for egg production alone. Poult. Sci. 76: 1517–1523.

Lamont, S. J. 1989. The chicken major histocompatibility complex in disease resistance and poultry breeding. J. Dairy Sci. 72:1328–1333.

Lamont, S. J. 1998. Impact of genetics on disease resistance. Poult. Sci. 77:1111–1118.

Lamont, S. J., M. G. Kaiser, and W. Liu. 2002. Candidate genes for resistance to *Salmonella enteritidis* colonization in chickens as detected in a novel genetic cross. Vet. Immunol. Immunopathol. 87:423–428.

Lande, R., and R. Thompson. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics 124:743–756.

Lander, E. S., and D. Botstein. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199.

Larsen, R. J., and M. L. Marx. 1990. Statistics. Prentice Hall, Englewood Cliffs, NJ.

Lebowitz, R. J., M. Soller, and J. S. Beckmann. 1987. Trait-based analyses for the detection of linkage between marker loci and quantitative trait loci in crosses between inbred lines. Theor. Appl. Genet. 73:556–562.

Legendre, A. M. 1805. Appendix to Nouvelles Méthodes pour la Détermination des Orbites des Comètes: Sur la Méthode des Moindres Quarrés. Libr. Math., Paris.

Lipkin, E., M. O. Mosig, A. Darvasi, E. Ezra, A. Shalom, A. Friedmann, and M. Soller. 1998. Quantitative trait locus mapping in dairy cattle by means of selective milk DNA pooling

using dinucleotide microsatellite markers: Analysis of milk protein percentage. Genetics 149:1557–1567.
Liu, H. C., H. H. Cheng, V. Tirunagaru, L. Sofer, and J. Burnside. 2001a. A strategy to identify positional candidate genes conferring Marek's disease resistance by integrating DNA microarrays and genetic mapping. Anim. Genet. 32:351–359.
Liu, H. C., H. J. Kung, J. E. Fulton, R. W. Morgan, and H. H. Cheng. 2001b. Growth hormone interacts with the Marek's disease virus SORF2 protein and is associated with disease resistance in chicken. Proc. Natl. Acad. Sci. USA 98:9203–9208.
Liu, H.C., M. Niikura, J. Fulton, and H.H. Cheng. 2003. Identification of chicken stem lymphocyte antigen 6 complex, locus E (*LY6E*, alias *SCA2*) as a putative Marek's disease resistance gene via a virus-host protein interaction screen. Cytogenet. Genome Res. 102:304–308.
Miller, I., and M. Miller. 1999. John E. Freund's Mathematical Statistics. Prentice Hall, Upper Saddle River, NJ.
Morrow, C., and F. Fehler. 2004. Marek's disease: a worldwide problem. Pages 49–61 in Marek's Disease, An Evolving Problem. F. Davison and V. Nair, ed. Elsevier Ltd., Oxford, UK.
Ronin, Y .I., A. B. Korol, and J. I. Weller. 1998. Selective genotyping to detect quantitative trait loci affecting multiple traits: interval mapping analysis. Theor. Appl. Genet. 97:1169–1178
Schat, K. A., R. L. Taylor, Jr., and W. E. Briles. 1994. Resistance to Marek's disease in chickens with recombinant haplotypes to the major histocompatibility (*B*) complex. Poult. Sci. 73:502–508.
Schierman, L. W., and W. M. Collins. 1987. Influence of the major histocompatibility complex on tumor regression and immunity in chickens. Poult. Sci. 66:812–818.
Schmid, M., I. Nanda, M. Guttenbach, C. Steinlein, M. Hoehn, M. Schartl, T. Haaf, S. Weigend, R. Fries, J. M. Buerstedde, K. Wimmers, D. W. Burt, J. Smith, S. A'hara, A. Law, D. K. Griffin, N. Bumstead, J. Kaufman, P. A. Thomson, T. Burke, M. A. Groenen, R. P. Crooijmans, A. Vignal, V. Fillon, M. Morisson, F. Pitel, M. Tixier-Boichard, K. Ladjali-Mohammedi, J. Hillel, A. Maki-Tanila, H. H. Cheng, M. E. Delany, J. Burnside, and S. Mizuno. 2000. First report on chicken genes and chromosomes 2000. Cytogenet. Cell Genet. 90:169–218.
Smith, P. J. 2002. Analysis of Failure and Survival Data. Chapman & Hall/CRC, Boca Raton, FL.
Soller, M., and J. S. Beckmann. 1983. Genetic polymorphisms in varietal identification and genetic improvement. Theor. Appl. Genet. 67:25–33.
Steadham, E. M., S. J. Lamont, I. Kujdych, and A. W. Nordskog. 1987. Association of Marek's disease with Ea-B and immune response genes in subline and F2 populations of the Iowa State S1 Leghorn line. Poult. Sci. 66:571–575.
Vallejo, R. L., L. D. Bacon, H. C. Liu, R. L. Witter, M. A. Groenen, J. Hillel, and H. H. Cheng. 1998. Genetic mapping of quantitative trait loci affecting susceptibility to Marek's disease virus induced tumors in F2 intercross chickens. Genetics 148:349–360.
Wang, J. 2003. Interval mapping of QTL with selective DNA pooling data. Ph.D. Diss. Iowa State Univ., Ames, IA.
Weller, J. I., J. Z. Song, D. W. Heyen, H. A. Lewin, and M. Ron. 1998. A new approach to the problem of multiple comparisons in the genetic dissection of complex traits. Genetics 150:1699–1706.
Witter, R. L. 1985. Principles of vaccination. Pages 203–250 in Marek's Disease. L. N. Payne, ed. Martinus Nijhoff Publishing, Boston.
Witter, R. L. 1997. Increased virulence of Marek's disease virus field isolates. Avian Dis. 41:149–163.
Wong, G. K., B. Liu, J. Wang, Y. Zhang, X. Yang, Z. Zhang, Q. Meng, J. Zhou, D. Li, J. Zhang, P. Ni, S. Li, L. Ran, H. Li, R. Li, H. Zheng, W. Lin, G. Li, X. Wang, W. Zhao, J. Li, C. Ye, M. Dai, J. Ruan, Y. Zhou, Y. Li, X. He, X. Huang, W. Tong, J. Chen, J. Ye, C. Chen, N. Wei, L. Dong, F. Lan, Y. Sun, Z. Yang, Y. Yu, Y. Huang, D. He, Y. Xi, D. Wei, Q. Qi, W. Li, J. Shi, M. Wang, F. Xie, X. Zhang, P. Wang, Y. Zhao, N. Li, N. Yang, W. Dong, S. Hu, C. Zeng, W. Zheng, B. Hao, L. W. Hillier, S. P. Yang, W. C. Warren, R. K. Wilson, M. Brandstrom, H. Ellegren, R. P. Crooijmans, J. J. van der Poel, H. Bovenhuis, M. A. Groenen, I. Ovcharenko, L. Gordon, L. Stubbs, S. Lucas, T. Glavina, A. Aerts, P. Kaiser, L. Rothwell, J. R. Young, S. Rogers, B. A. Walker, A. van Hateren, J. Kaufman, N. Bumstead, S. J. Lamont, H. Zhou, P. M. Hocking, D. Morrice, D. J. de Koning, A. Law, N. Bartley, D. W. Burt, H. Hunt, H. H. Cheng, U. Gunnarsson, P. Wahlberg, L. Andersson, E. Kindlund, M. T. Tammi, B. Andersson, C. Webber, C. P. Ponting, I. M. Overton, P. E. Boardman, H. Tang, S. J. Hubbard, S. A. Wilson, J. Yu, and H. Yang. 2004. A genetic variation map for chicken with 2.8 million single-nucleotide polymorphisms. Nature 432:717–722.
Yonash, N., L. D. Bacon, R. L. Witter, and H. H. Cheng. 1999. High resolution mapping and identification of new quantitative trait loci (QTL) affecting susceptibility to Marek's disease. Anim. Genet. 30:126–135.
Zhou, H., H. Li, and S. J. Lamont. 2003. Genetic markers associated with antibody response kinetics in adult chickens. Poult. Sci. 82:699–708.

APPENDIX

The hazard function h(t) for a line is proportional to the conditional probability that an animal dies shortly after time t given that it survives to time t. The CPH model assumes that at any time t the hazard resulting from inheriting the line 1 allele

$$h(t) = e^{\beta}h_0(t)$$

is proportional to a baseline hazard $h_0(t)$ for inheriting the line 2 allele. At any time point t, the regression coefficient β is the natural logarithm of the relative risk that a death occurs shortly after time t for animals inheriting the line 1 allele versus those inheriting the line 2 allele, provided that the animals have survived through time t. When there is uncertainty about the allele that was inherited, the hazard is an average of the hazards for the 2 possible alleles $h_0(t)$

$$h(t) = p(L_1)e^{\beta}h_0(t) + [1 - p(L_1)]h_0(t)$$

where $p(L_1)$ is the probability of inheriting the line 1 allele. Then, a maximum partial likelihood estimator for β is obtained by maximizing

$$\prod_{i=1}^{r}\left(\frac{p(L_1)_i\exp(\beta) + [1 - p(L_1)_i]}{\sum_{j\chi R(t_i)}(p(L_1)_j\exp(\beta) + [1 - p(L_1)_j])}\right) \qquad [A1]$$

where r is the number of observed deaths, and $R(t_i)$ denotes the set of animals still alive at the time of death i. This is not a standard form of the Cox partial likelihood, and it cannot be maximized with standard statistical software. To use standard statistical software for the Cox model, an alternative estimator is obtained by using $p(L_1)_i$ as an explanatory variable in a standard Cox model. This yields

$$h(t_i) = \exp(\beta p(L_1)_i) h_0(t_i)$$

as an approximation for

$$h(t_i) = [p(L_1)_i \exp(\beta) + 1 - p(L_1)_i] h_0(t)$$

resulting in a partial likelihood

$$\prod_{i=1}^{r} \left(\frac{\exp(\beta p(L_1)_i)}{\sum_{j \chi R(t_i)} (\exp(p(L_1)_j(\beta))} \right) \qquad [A2]$$

that can be maximized with standard statistical software for the Cox model. By using simulation, it can be shown that maximizing [A2] provides an estimator of β that tends to be biased a little further away from zero than the estimator obtained by maximizing [A1]. The standard error for this estimator, however, is also slightly larger, resulting in a test of significance that maintains nearly the correct type I error level. Note that maximizing either [A1] or [A2] results in an increasingly more biased estimate of β as fewer animals with intermediate failure times are genotyped. When the null hypothesis is true ($\beta = 0$), however, standard errors of the estimators increase in a proportional manner so tests retain desired type I error levels.