A Review of the Development of Chicken Lines to Resolve Genes Determining Resistance to Diseases

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ABSTRACT The resolution of genes that determine resistance to disease is described using chicken lines maintained at the Avian Disease and Oncology Laboratory (ADOL). This description includes a summary 1) of existing selected and inbred lines differing for resistance to viral-induced tumors, i.e., Marek's disease (MD) and lymphoid leukosis (LL), and of the use of inbred and line crosses to define relevant disease-resistant genes, e.g., *TV*, *ALVE*, *B*, *R*, *LY4*, *TH1*, *BU1*, and *IGG1*; 2) of the development of *TVB*/ALVE* congenic lines to establish the affects of endogenous virus (EV) expression on resistance to avian leukosis virus (ALV), and methods to detect *ALVE* expression; 3) of the development of *B* congenic lines to define the influence of the *MHC* on MD resistance and vaccinal immunity, for producing B antisera, and for evaluating DNA sequences of Class I and II genes; and 4) of the current development of 6C.7 recombinant congenic strains (RCS) to define the role of non-*MHC* genes influencing susceptibility to MD and LL tumors, immune competence, and epistatic effects of genes. The procedures of pedigree mating, to avoid or maintain inbreeding, and of blood-typing, to ensure genetic purity of the lines, are also described.

(*Key words*: *B* congenic, endogenous virus, disease resistance, inbred, recombinant congenic)

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INTRODUCTION

The resolution of genes controlling disease resistance in chickens is a somewhat daunting, but ultimately rewarding, task because, in most breeding schemes, disease resistance is not a high priority trait (Albers, 1993); possibly because heritability estimates of mortality are often less than 10%. However, the heritability of resistance to a specific disease is generally higher (Gavora, 1990), and, when major genes affecting resistance to a specific disease are identified, e.g., the *MHC* or *B* complex effects on Marek's disease (MD), they are frequently evaluated by poultry breeders. The effect of a major disease resistance gene is frequently variable because of other genes in the chicken genome, and, therefore, evaluations for a gene must be made within breeding strain combinations. Subsequently, if a disease is influencing a strain's market share, and a gene is shown to reduce that disease, then selection for the resistance gene is generally considered beneficial and profitable.

This paper is narrowly focused, describing special types of selected, inbred, and congenic lines maintained at the Avian Disease and Oncology Laboratory (ADOL) that may prove valuable for the identification and clarification of the role of genes that influence disease resistance as well as other traits. To obtain a broader description of methods for identifying genes that confer disease resistance, the reader should refer to reviews that include heritability analyses in field populations (Gavora, 1990), selection for disease or correlated traits (Gavora and Spencer, 1983), or genome mapping procedures (Crittenden, 1993).

DEVELOPMENT AND USE OF INBRED LINES

In 1975, Howard Stone published a technical bulletin that described the selection and inbreeding of lines of chickens for the identification of genes influencing resistance to avian tumors at the Regional Poultry Research Laboratory (now the ADOL) (Stone, 1975). The development of the selected and inbred lines was initiated and led by Nelson Waters from 1939 to 1960; followed by Lyman Crittenden, who introduced use of individual

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Abbreviation Key: ADOL = Avian Disease and Oncology Laboratory; ALV = avian leukosis virus; BC = bursal cells; BCM = backcross mating; *env* = envelope; EV = endogenous virus; HA = hemagglutination; LL = lymphoid leukosis; MD = Marek's disease; MDV = MD virus; RBC = red blood cells; RCS = recombinant congenic strains; REV = reticuloendotheliosis; Rfp = restriction fragment polymorphism; RFLP = restriction fragment length polymorphism; TC = thymic cells.

cages, artificial insemination, and brother-sister matings during the 1960s; and then Howard Stone, who completed establishment of specific pathogen-free breeders in the 1970s. The avian viral-induced tumors were resolved into lymphoid (LL) and myeloid leukosis induced by avian leukosis viruses (ALV) (Payne and Fadly, 1997), reticuloendotheliosis (REV) tumors induced by an unrelated retrovirus (Witter, 1997), and MD induced by MD viruses (MDV) (Calnek and Witter, 1997). Stone (1975) summarized the initiation and development of 15 lines that were gradually inbred during selection for resistance or susceptibility to tumors. During development, one group of pedigree hatched chicks in each line was kept in quarantine isolation and used for line reproduction. Another group was exposed to suspensions of tumor material. The inoculated birds provided an estimate of the resistance or susceptibility of their unexposed sibs. Furthermore, sibs within families in susceptible Line 15 were grown either in isolation (15I) or while intermingled with other families. In some families, sibs grown in isolation were free of tumors, whereas the sibs grown intermingled with other families developed tumors. This finding led to the conclusion that LL-type tumors developed because of vertical transmission of a virus in some families, but, if families lacked vertical transmission of the virus, their chicks could become exposed to horizontal transfer of the virus present in chicks of other families. Thus, the first LL-free infected chickens were identified in susceptible families (Waters and Prickett, 1944). During development of the lines, they were gradually inbred, and, by 1952, the lines were over 95% inbred (Waters and Fontes, 1960). Three of the original lines or their sublines are presently maintained. These basic inbred lines include Line 6, which was selected for tumor resistance and is resistant to MD and LL, and Lines 7 and 15, which were selected for tumor susceptibility. Line 7 is susceptible to MD (see subsequent information for genetically important ALV differences), and Line 15 is susceptible to LL and MD. The lines have also been useful in defining a spectrum of resistance to REV-induced tumors. The F_2 and backcross studies involving Lines 6, 7, or 15 produced evidence for major genes that influence resistance to tumors (see subsequently). Five gene systems influencing resistance to tumors will be briefly documented.

Tumor-Virus Susceptibility Genes

From inbred line crosses, important evidence was defined for dominant ALV susceptibility (*TV*S*) genes coding for viral receptors. Resistance to viral replication was demonstrated in cell culture that eliminated immune system effects (Crittenden et al., 1963). Several subgroups of ALV were differentiated based on biological and serological characteristics (Vogt and Ishizaki, 1965), e.g., ALVA, ALVB, ALVC, and ALVD; exogenous viruses; and ALVE EV (reviewed by Crittenden, 1975 and 1991; Gavora, 1990; Weiss, 1993). The ALVA, ALVB, and, most recently, ALVJ, are the only exogenous ALV identified in commercial chickens in North America. Chickens were identified that contained receptors and were susceptible (*TV*S*), or that lacked receptors and were resistant (*TV*R*), to ALVA and ALVB tumor viruses (Waters and Burmester, 1961; Crittenden et al., 1963, 1964b; Vogt and Ishizaki, 1965). For example, the genes at the *TVA* locus were denoted *TVA*S* or *TVA*R*, and genotypes were *TVA*S/*S*, *TVA*S/*R*, or *TVA*R/*R*; susceptibility was dominant (Crittenden, 1968; Crittenden et al., 1996). Similar evidence was also defined for other ALV subgroups (Crittenden, 1991), except for ALVJ, where all chickens appear to be susceptible (Payne and Fadly, 1997). Alleles of one locus were shown to determine susceptibility to ALVB and ALVE (Crittenden and Motta, 1975; Bacon et al., 1996b). The *TVB*S1* gene codes for receptors for both ALVB and ALVE, the *TVB*S3* gene codes for receptors for ALVB but not for ALVE, and the *TVB*R* gene codes for a lack of receptors for ALVB and ALVE. No gene has been identified for susceptibility to ALVE but resistance to ALVB. A shorthand notation was developed to abbreviate the susceptibility phenotype to a subgroup of ALV, e.g., the resistance of a chicken to an ALVA virus was abbreviated as C/A (Vogt and Ishizaki, 1965). In shorthand, the resistance of several sublines to ALVA, ALVB, ALVC, and ALVE is annotated: Line 6_3 = C/0, Line 7_2 = C/ABE, Line $15I_5$ = C/C, and Line 15B1 = C/0. Thus, important genes determining the first level of resistance, i.e., virus infectivity, for ALVA- or ALVB-induced LL tumors were defined in the ADOL lines (Table 1).

Endogenous Viruses

The ADOL inbred lines were also beneficial for the identification of ALVE EV and the characterization of *ALVE* endogenous genomic proviral genes at the DNA level that code for them. In this paper, when an ALVE provirus is integrated into genomic DNA to form a gene, the *ALVE* term is italicized. The proviral DNA in each line, and in line crosses, was characterized using restriction fragment length polymorphism (RFLP) analyses employing several DNA restriction endonucleases (Smith, 1986; Crittenden, 1991). By evaluating the DNA fragments of chickens of each line, or line-cross, the identification of fragments caused by each *ALVE* gene and the total number of *ALVE* genes in each line were defined and tabulated (Table 1; column 4). The lines were valuable for defining the influence of different *ALVE* genes on resistance to exogenous ALVA-induced LL tumors. Basically, four categories of *ALVE* expression were identified. First, the complete *ALVE* expresses three genes; i.e., the envelope (*env*), the capsid (group-specific antigen or *gag*), and the polymerase. Examples of complete *ALVE* include *ALVE2*, *ALVE10*, and *ALVE21*. Second, incomplete *ALVE* were defined that expressed only the gene for *env*, e.g., *ALVE6* or *ALVE9*. Third, *ALVE* were found that expressed the *env* gene and *gag* genes, e.g., *ALVE3*. Importantly, if *TVB*S1* chickens possess *ALVE env* genes such as *ALVE6*, *ALVE9*, or *ALVE3*, the expressed *env* will block (or interfere with) the ALVE receptor. This interference provides a unique method for *TVB*S1* chickens to resist infection

TABLE 1. Characteristics of Avian Disease and Oncology Laboratory (ADOL) inbred chicken lines in 2000

Line	*MHC B**[1]	IgG *G1**[2]	ALV[3] *C/?*[4]	ALV[3] *ALVE*[5]	RSV (RAV-1) Rous sarcoma[6]	MDV JM strain[7]
Rh-C	*12*	*G*	C/AE	1, 7, 10	Virus resistant	Susceptible
6_3	*2*	*E*	C/0*	3	Regress	Resistant
7_2	*2*	*A*	C/ABE	1, 2	Virus resistant	Susceptible
7_1	*2*	*A*	Undefined	Undefined	Undefined	Susceptible
$15I_5$	*15*	*A*	C/C*	1, 6, 10, 15	Progress	Susceptible

[1]*MHC B* haplotype. The * indicates the gene (haplotype), and the number identifies the allele; e.g., the Rh-C *B* haplotype is 12.

[2]Immunoglobulin G locus *G1* allele. The * indicates the gene, and the letter identifies the allele.

[3]ALV = avian leukosis virus.

[4]ALV susceptibility phenotype. C/? is a chicken resistant to the subgroup defined by the "?"; e.g., C/E is a chicken resistant to ALV subgroup E. Although chickens of Lines 6_3 and $15I_5$ have receptors for ALVE, their cells resist ALVE infection because of expression of *ALVE3* or *ALVE6*.

[5]Endogenous virus loci; e.g., Rh-C contains an endogenous virus at loci 1, 7, and 10.

[6]Lines resistant to Rous sarcoma virus (RSV) (RAV-1) fail to develop tumors. Susceptible lines develop tumors that regress or progress.

[7]All chickens are susceptible to infection with Marek's disease virus (MDV) (JM strain), but Line 6_3 is resistant to tumors.

by a complete ALVE, i.e., in reality the chickens or their cells act as if they lack subgroup E cellular receptors (Table 1) (Weiss, 1969; Hanafusa et al., 1970; Crittenden, 1991). Fourth, there are some *ALVE* that are incomplete and are not expressed, e.g., *ALVE15*, or that are rarely expressed, e.g., the complete *ALVE1*, which is often present in chickens but possesses inhibitory cytidine methylation (Cooper and Silverman, 1978; Smith, 1986).

After *ALVE* gene characterization in the ADOL lines, it was possible to evaluate the importance of these genes on resistance to ALVA-induced LL. Several studies led to the conclusion that *ALVE env* expression induces tolerance to ALVA and that this enhances the development of LL tumors (Crittenden et al., 1982; 1984; Crittenden and Fadly, 1985). Additional compelling evidence concerning LL susceptibility awaited the development of *ALVE* congenic lines (see subsequent). In contrast, after REV infection, neither *ALVE3* or *ALVE2* had an influence on tumors or antibody development, but data indicate that *ALVE2* may influence viremia (Crittenden et al., 1982). Moreover, *ALVE* genes did not influence MD tumors after injection of MDV (Crittenden, 1991).

B *Genes*

The ADOL lines were evaluated by L. W. Johnson and W. E. Briles for the *B* and other blood-group genotypes in the 1960s and 1970s (Table 1) (Crittenden et al., 1964a; Stone, 1975; Abplanalp et al., 1979). One of two studies that first demonstrated a dramatic affect of the *B* genotype on Rous sarcoma regression was conducted by Collins and coworkers at the University of New Hampshire using crosses of Lines 6_1 (*B*2*) and 15_1 (*B*5*) (Collins et al., 1977). The *B*2/*2* and *B*2/*5* chickens regressed most sarcomas rapidly, whereas sarcomas progressed and metastasized and killed most *B*5/*5* chickens. Concurrently, Briles and coworkers demonstrated an equally clear, and more commercially relevant, influence of the *B* genotype on resistance to MD using noninbred Cornell Strain N (*B*21/*21*) and P (*B*13* and *B*19*) chickens developed by Cole (Cole, 1968) selected, respectively, for MD resistance or susceptibility. Lines N and P were introduced to the ADOL in 1972, and, in F_2 and backcross chickens, the *B*21* allele was associated with MD resistance (Briles et al., 1977) (Table 2). Based on these and other data, the development of *B* congenic lines was proposed to define further the effects of *B* genes on disease resistance (see subsequent).

Lymphocyte Alloantigen Loci

The highly inbred Lines 6_3, 7_2, $15I_5$, and Reaseheath C (Rh-C) were shown to be histocompatible within lines (Crittenden et al., 1964a; Stone, 1975; Bacon and Motta, 1982), with the exception of prolonged rejections caused by the female histoantigen in Lines 6_3 and Rh-C (Bacon and Craig, 1969). Therefore, it was conceivable to produce antibodies to lymphocyte antigens by reciprocal immunizations between Lines 6 and 7 that had the same MHC haplotypes, i.e., (*B*2*). When chickens of Lines 6 and 7 were reciprocally immunized with lymphoid cells, the thymic (TC) and bursal (BC) lymphoid cells were shown to possess alloantigens that differed in each strain. Two loci have been identified for the TC alloantigens, i.e., *TH1* (Gilmour et al., 1976) and *LY4* (Fredericksen et al., 1977), and one locus has been identified for BC alloantigens (*BU1*) (Gilmour et al., 1976). Using F_2 and backcross chickens, alleles at the *TH1* and *LY4* loci were shown to interact to affect regression of Rous sarcoma tumors (Gilmour et al., 1983) and resistance to MD (Fredericksen et al., 1982). Moreover, resistance to LL was associated with alleles at the *TH1* but not alleles of the *LY4* or *BU1* loci (Bacon et al., 1985).

Immunoglobulin Genes

Immunoglobulin allotypes have been defined for the ADOL inbred lines (Table 1) (Benedict, 1979). Genes de-

TABLE 2. Characteristics of noninbred Avian Disease and Oncology Laboratory (ADOL) chicken lines in 2000

Line	*MHC B**[1]	ALV[2] C/?[3]	ALV[2] *ALVE*[4]	*ALVE* expression[5]	MDV JM strain[6]
0	*21*	C/E	None	No	Undefined
15B1	*5, 15*	C/0	*1*	No	Susceptible
Cornell N	*21*	Undefined	*1, 3, 6*	Variable	Resistant
Cornell P	*19*	Undefined	Undefined	Undefined	Susceptible

[1]*MHC B* haplotype. The * indicates the haplotype, and the number identifies the allele.

[2]ALV = avian leukosis virus.

[3]ALV susceptibility phenotype. C/? is a chicken resistant to the subgroup defined by the "?"; e.g., C/E is a chicken resistant to ALV subgroup E.

[4]Endogenous virus loci; e.g., 15B1 contains an endogenous virus at locus 1.

[5]Expression of endogenous virus.

[6]All chickens are infected with Marek's disease virus (MDV) (JM strain), but Line N is resistant to tumors.

termining immunoglobulin G heavy chains were associated with a recessive resistance to B-cell lymphomagenesis (LL) in F_3 chickens of Lines 100 and 6_3 , but there was no influence on MD tumor development or on ability to regress Rous sarcoma tumors (Bacon et al., 1986).

DEVELOPMENT OF CONGENIC LINES

7-VB Congenic Lines

After Line 6 was shown to be susceptible to infection by ALVA and ALVB, whereas Line 7 was resistant to these viruses, efforts were made to develop Line 7-type chickens susceptible to these viruses. Thus, Line 100 was developed as the first congenic line at the ADOL, which involved crossing Line 6 (C/0) with Line 7 (C/ABE), then backcrossing for four generations while selecting ALV C/0 phenotypes (Stone, 1975). Therefore, Line 100 C/0 chickens had 97% of their genes in common with Line 7_2, and Line 100 was used in the 1980s (Bacon et al., 1986). Subsequently, selection for susceptibility to Subgroup A was discontinued, but selection for susceptibility to ALVB was easily continued by selecting males of this line expressing the R2 antigen. Thus, *TVB*S1/*R* males expressing R2 antigen were backcrossed yearly to 7_2 to provide C/A chickens heterozygous for *TVB*S1/*R* that expressed *ALVE2* present in Line 7_2. In this report, congenic lines are identified using nomenclature established in mice (Lyon, 1979), i.e., abbreviations are given for the background line followed by a period, then the line from which a gene was introduced followed by a hyphen, then a designation for the introduced gene that is italicized. This may be followed by a number in parentheses, indicating the number of backcross generations. Thus, the C/A version of Line 100 is designated 7.6-*VB*S1*(38) indicating that the line was developed in inbred Line 7_2 by introducing the *TVB*S1* allele from Line 6 and then selecting the backcross chickens for 38 generations for expression of *ALVE2* that exists in Line 7_2; detection was based on R2 antibody agglutination or detection of gag antigen (Crittenden et al., 1971; Crittenden and Motta, 1975; Bacon et al., 1996b). Therefore, 7.6-*VB*S1* chickens are 99.9% identical to very highly inbred Line 7_2 chickens but are *TVB*S1/*R* (i.e., susceptible to *ALVE*) and express *ALVE2* (also termed RAV-0, the prototype ALVE), whereas 7_2 chickens are *TVB*R/*R* and do not express quantities of ALVE2 (Table 3) (Smith et al., 1974; Crittenden, 1991). Lines 7 and 7.6-*VB*S1* are probably the most highly developed existing congenic chicken lines, and, recently, they have been very beneficial for developing an assay to detect expression of ALVE in the serum of chickens (Bacon, 2000).

0-VB-ALVE Semicongenic Lines

A special line of chickens was developed that lacked *ALVE* genes based on RFLP analyses, i.e. Line 0. Line 0 was developed by producing F_1 chickens from a Line 7_2 × SPAFAS Line 11 mating and then backcrossing to Line 11 and selecting chickens lacking *ALVE* based on RFLP analysis of DNA (Astrin et al., 1979). Line 0 was subsequently introduced to the ADOL and selected for susceptiblility to all ALV subgroups except ALVE while avoiding inbreeding, i.e., the *TVB* genotype of Line 0 is *TVB*S3/*S3* and its phenotype is C/E (Crittenden, 1991). Several semicongenic lines were developed in noninbred

TABLE 3. Characteristics of *ALVE/TVB* congenic lines at Avian Disease and Oncology Laboratory (ADOL) in 2000

Line	*MHC B**[1]	ALV phenotype[2]	*ALVE* genes[3]	*ALVE* expression[4]
7_2	*2*	C/ABE	*ALVE1, 2*	No
7.6-*VB*S1*	*2*	C/A	*ALVE1, 2*	Yes
0	*21*	C/E	*ALVE0*	No
0.44-*VB*S1*	*21*	C/0	*ALVE0*	No
0.44-*EV21*	*21*	C/E	*ALVE21*	No
0.44-*VB*S1-EV21*	*21*	C/0	*ALVE21*	Yes
0-*VA6*[5]	*21*	C/AE	*ALVA6*	No

[1]*MHC B* haplotype. The * indicates the haplotype, and the number identifies the allele.

[2]Avian leukosis virus (ALV) susceptibility phenotype; e.g., C/E is a chicken resistant to ALV subgroup E.

[3]Endogenous virus loci; e.g., Line 7_2 contains an endogenous virus at loci at 1 and 2.

[4]Expression of *ALVE* endogenous virus.

[5]Transgenic Line 0 chickens that express ALVA envelope but no *ALVE* genes.

Line 0 to evaluate the effects of *ALVE* gene expression on various traits. *ALVE21* and *TVB*S1* genes were introduced by mating a SPAFAS Line 44 slow-feathering male to Line 0 hens and selecting males expressing *ALVE21* (Smith and Crittenden, 1988). Males heterozygous for the *ALVE21/0* locus and *TVB*S1/*S3* alleles have been backcrossed to Line 0 for 13 generations to provide four types of semicongenic chickens. For each generation, approximately one-half of the offspring are slow-feathering and heterozygous for *ALVE21* based on RFLP tests (Bacon et al., 1988). Of these, approximately one-half are heterozygous for *TVB*S1/*S3* [identified by reactivity of red blood cells (RBC) with R2 antibody, or gag ELISA tests, which detect expression of *ALVE21*], and one-half are *TVBS*3/*S3* chicks that lack ALVE expression and reactivity by these assays. The other one-half of the offspring are rapid feathering and, therefore, lack *ALVE21*. They are analyzed for their *TVB* genotype based on the ability of their RBC to bind serum containing ALVE as detected by flow cytometry, i.e., the *TVB*S1/*S3* chicks' RBC bind ALVE, whereas the *TVB*S3/*S3* chicks' RBC do not (Bacon, 2000). The four types of chickens are considered semicongenic as Line 0 is not inbred. They include (see Table 3): Line 0, which has ALVE resistance (*TVB*S3/*S3*) and lacks *ALVE*; Line 0.44-*VB*S1*, which has ALVE susceptibility (*TVB*S1/*S3*) but lacks *ALVE*; Line 0.44-*EV21*, which has ALVE resistance (*TVB*S3/*S3*) and little *ALVE21* expression; and Line 0.44-*VB*S1-EV21*, which has ALVE susceptibility (*TVB*S1/*S3*) and expresses *ALVE21*. The various types of *0-TVB-ALVE* semicongenic chickens have been valuable in numerous studies, identifying the effects of *ALVE* gene expression on resistance to ALV (Smith and Crittenden, 1988; Smith and Fadly, 1988; Crittenden, 1991). Currently, they are being used to demonstrate an effect of *ALVE* gene expression on antibody response and eradication of the ALVJ virus (Williams et al., 1999). Three additional ALVE Line 0 semicongenic lines were developed for *ALVE3*, *6*, and *12* and were used to study ALVE effects on productivity (Gavora et al., 1991; Crittenden, 1991). The *ALVE6* semicongenic line was crossed to semicongenic Line 0.44-*VB*S1-V21* to study the interaction of *ALVE6* and *ALVE21* (Smith et al., 1990). The Line 0 chickens semicongenic for *ALVE3*, *ALVE6*, and *ALVE12* are not currently maintained, but frozen semen is stored that may rejuvenate the lines when artificially inseminated to Line 0 hens.

15-B Congenic Lines

The *B* haplotype is known to be a complex of several Class I (*BF*), Class II (*BL*), and Class IV (*BG*) genes (Guillemot et al., 1988). The *BF* and *BL* genes are similar to *MHC* genes defined in mammals, whereas the *BG* genes are unique to birds. Expression of the genes varies, i.e., *BF* genes are expressed on all nucleated tissues, including RBC; *BL* genes are expressed on BC cells, macrophages, and activated TC cells; and *BG* expression occurs principally on RBC. The antigenic BG products are the major epitopes identified by hemagglutination (HA) of RBC, although some antisera may also detect BF epitopes (Fulton et al., 1996b). To characterize the mechanism of the *B*-complex on MD and disease resistance, eight 15-*B* congenic lines were developed. This development involved 10 or 11 backcross generations of matings to inbred Line $15I_5$ (Figure 1) (Shen et al., 1984; Bacon et al., 1987). In each generation, male breeders heterozygous for an introduced *B* haplotype were selected by HA. After 10 to11 backcrosses, *B*-heterozygous parents were mated, and chickens homozygous for the introduced gene were selected. Since development, six males and 30 to 50 females have been used to reproduce each line for 12 generations. Each of the 15.*B* congenic lines is 99.9% identical to the inbred parental Line $15I_5$, but each is homozygous for unique genes in the *B* haplotype that are linked to the nucleolar organizer region on a microchromosome (Bloom and Bacon, 1985). Two of the 15.*B* congenic lines are homozygous for an identical *B*2* haplotype introduced from MD-resistant (Line 6_3) or MD-susceptible (Line 7_2) chickens, and the others are homozygous for *B*5*, *B*12*, *B*13*, *B*19*, or *B*21*; $15I_5$ contains *B*15*. These seven standard *B* haplotypes are commonly found in commercial White Leghorn chickens and were evaluated in the literature for MD resistance (Bacon, 1987). A summary of the *B* congenic lines and their regression of Rous sarcoma tumors (Bacon and Crittenden, 1984) and resistance to MD (Bacon and Witter, 1992, 1993, 1994) is given in Table 4. The lines have also been valuable in the development of alloantisera (Fulton et al., 1996a,b), the definition of DNA sequences of class I (Hunt and Fulton, 1998) and Class II (Pharr et al., 1998) *MHC* genes, and the development of kits to differentiate various Class II *B* alleles at the DNA level (Shuman et al., 1993).

0-B Semicongenic Lines

Noninbred Line 0 was originally segregating for several *B* genes, but was fixed for the *B*21* haplotype (Crittenden and Bacon, unpublished data). To analyze the effect of another *B* haplotype in Line 0, the *B*13* haplotype from Line 15.P-*13* was introduced, and, after five backcross generations, chickens were obtained that lacked *ALVE* genes and possessed *B*13*, i.e., Line 0.P-*13* (Table 4). To keep Line 0.P-*13* comparable with Line 0, it is reproduced each year by mating *B*13/*21* males to Line 0 hens and selecting for *B*13/*21* breeders. The *B*13/*21* males and females are mated to produce *B*13/*13*, *B*13/*21*, and *B*21/*21* experimental chicks. Lines 0-*21* and 0.P-*13* are proving important for demonstrating the role of the *B* haplotype in resistance to ALVA in chickens lacking *ALVE* in contrast to *B* effects on ALVA resistance in 15-*B* congenic lines expressing *ALVE*, i.e., lines 15.P-*13* and 15.N-*21* (Hunt et al., 1999).

Recently, a series of *Rfp* (restriction fragment polymorphism) *Y* haplotypes containing *MHC*-like Class I and Class II genes were identified in chickens (Briles et al., 1993). Although the *RfpY* and *B* haplotypes are on the same microchromosome, they appear to be unlinked because of a high frequency of meiotic recombination

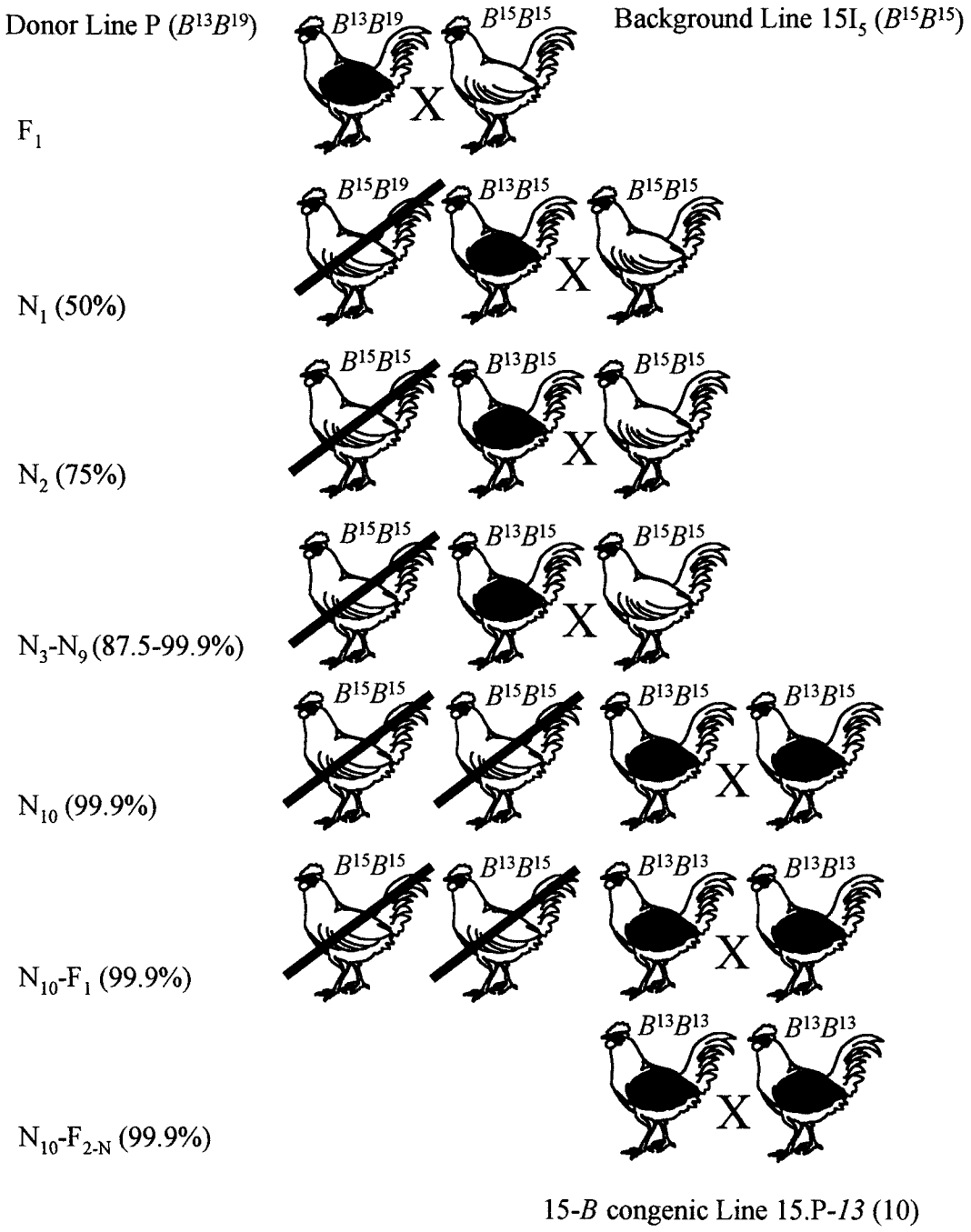

FIGURE 1. Mating scheme for production of White Leghorn 15-*B* congenic lines.

(Miller et al., 1996). Three *RfpY* haplotypes were identified in Lines N and P; one was common to each line (Pharr et al., 1997). Lines 6 and 7 were also shown to contain unique *RfpY* haplotypes that differed from lines N and P. Tests at ADOL indicate that these *RfpY* genes do not influence MD resistance (Bacon et al., 1996c; Vallejo et al., 1997), in contrast to results by Wakenell et al. (1996) who used other chicken lines. Semen and cells are stored from chickens homozygous for variant *RfpY* haplotypes, but no sublines or semicongenic lines exist for *RfpY* genes at the ADOL.

DEVELOPMENT OF 6C.7 RECOMBINANT CONGENIC STRAINS

Highly inbred selected chickens of Lines 6 and 7 differ dramatically for resistance to MD and ALV susceptibility (Table 1). With regard to ALV, Line 6 is C/0, but Line 7 is C/ABE and can only be infected with subgroup C ALV. However, both lines are susceptible to MDV infection, but Line 6 is resistant to MD tumors compared with Line 7 (Crittenden et al., 1972; Stone, 1975). Importantly, both of these lines have the same *MHC B**2 haplotype (Lamont

et al., 1990; Hunt and Fulton, 1998; Pharr et al., 1998), so differences in resistance must be attributable to non-*MHC* genes. Lines 6 and 7 also differ dramatically in the size of their primary lymphoid organs, i.e., the lobes of the thymus and the bursa of Fabricius are smaller in Line 6 (Lee et al., 1981; Powell et al., 1982). Moreover, lymphoproliferation traits are higher in Line 7_2 than in Line 6_3, i.e., graft vs. host response in vivo (Pazderka et al., 1975), in vitro response of lymphoctyes to mitogens (Lee and Bacon, 1983), or mixed lymphoctye cultures (Bacon and Lee, 1981). A number of additional traits, including immunoglobulin G allotypes, *RfpY* genes, body weight, and behavioral traits, also differ in Lines 6 and 7 (see previous and Stone, 1975). To resolve the effects of the known gene differences and to define other non-*MHC* genes that may determine differences in resistance to LL, MD, or other traits, congenic line development was considered between Lines 6 and 7. However, rather than develop congenic lines for each trait, as was done for *B* haplotypes in Lines $15I_5$ and 0 and *ALVE* genes in Lines 7_2 and 0, the development of recombinant congenic strains (RCS) was considered more feasible to address the number and complexity of traits.

Recombinant congenic strains were first proposed and developed in mice by Demant et al. (1989) as a tool for analyzing complex genetic traits determined by more than one gene. Recombinant congenic strains are produced by an F_1 and limited backcross matings (BCM) between two strains followed by full-sib matings for about 20 generations. In the end, 15 to 20 RCS are developed, and given two BCM each, RCS will possess a unique random ≈12.5% of the donor genome in the genetic background of the recurrent parent. This genetic background allows the transformation of a multigenic trait into a series of single gene traits that can be easily mapped if the gene for the donor yields a measurable phenotype. At ADOL, RCS development was initiated using Line 6_3 as the recurrent parent. Line 6_3 has somewhat better fitness traits than Line 7_2 (Stone, 1975), and it was suggested that equal resolution of the genes should be obtained using the resistant line instead of the susceptible line as the recurrent parent (Peter Demant, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands, personal communication). Thus, pooled semen from five Line 7_2 males was used to inseminate six Line 6_3 hens (Figure 2). One F_1 male from each of the six 6_3 hens was backcrossed to one Line 6_3 hen to produce a BCM_1 male breeder. Each of six BCM_1 males was backcrossed again to four 6_3 hens to produce 10 to 15 BCM_2 chicks per hen. In each of the 24 dam families, a single RCS (6C.7A–6C.7X) was generated by brother-sister mating one male to about seven sisters. The sixth generation of full-sib mating was achieved for 19 of the 6C.7 RCS in 1999.

To evaluate the inheritance and the degree of homozygosity developing for Line 7 genes in the 6C.7 RCS, the chickens were analyzed for three blood-group systems that differed between Lines 6_3 and 7_2 (Table 5). In the fifth full-sib generation, six of 19 RCS were segregating for *E* locus antigens, nine were segregating "*J*" locus antigens (W. E. Briles, Department of Biological Sciences, Northern Illinois University, Dekalb, Illinois 60115, personal communication), and six were segregating and one was apparently homozygous for *TVB*R*, as the chickens failed to express R2 antigens and were similar to Line 7_2. Thus, more lines were still segregating for expression of Line 7_2 alleles than perhaps expected, and only one RCS had become homozygous for a Line 7_2 allele. Eventually, we expect that an average of about two of the 19 lines should become homozygous for each Line 7_2 allele. These data indicate that it may indeed take 20 or so generations before most individual loci become fixed for alleles introduced from Line 7_2. The current data on blood-group antigens are supported by initial analyses at the DNA level using DNA from parents for the fifth generation (Yonash et al., 1998). The DNA from each individual chicken was amplified using microsatellite markers roughly spaced throughout the genome. As expected, the

TABLE 4. Characteristics of Avian Disease and Oncology Laboratory (ADOL) *B* congenic lines in 2000

Line	*MHC B**[1]	Rous sarcoma tumor[2]	Type of MDV/type of MD vaccine[3]				
			JM/none	Md5/none	Md5/HVT	Md5/SB1	Md5/Rispens
$15I_5$	*15*	Progress	s[4]	s	Mod. r	Mod. r	Mod. r
15.6-*2*	*2*	Regress	s	. . .	. . .	. . .	. . .
15.7-*2*	*2*	Regress	s	s	s	Mod. s	Mod. s
15.15I-*5*	*5*	Progress	s	s	Mod. r	Mod. r	Mod. s
15.C-*12*	*12*	Mod. regress	s	. . .	Mod. r	. . .	. . .
15.P-*13*	*13*	Progress	s	s	s	s	Mod. s
15.P-*19*	*19*	Progress	s	. . .	s	. . .	. . .
15.N-*21*	*21*	Mod. regress	r	s	Mod. s	Mod. s	Mod. r
0	*21*	Mod. regress	Undefined	Undefined	Undefined	Undefined	Undefined
O.P-*13*	*13*	Undefined	Undefined	Undefined	Undefined	Undefined	Undefined

[1]*MHC B* haplotype. The * indicates the haplotype and the number identifies the allele.

[2]All lines develop tumors after infection with Rous sarcoma virus (RSV) (RAV-1). Tumors regress or progress as shown. Mod = moderate.

[3]JM = virulent Marek's disease virus (MDV); Md5 = very virulent MDV; HVT = serotype 3 vaccine; SB1 = serotype 2 vaccine; Rispens = serotype 1 vaccine; and mod. = moderate.

[4]All chickens are susceptible (s) to MDV, and the resistance (r) to tumor progression is given.

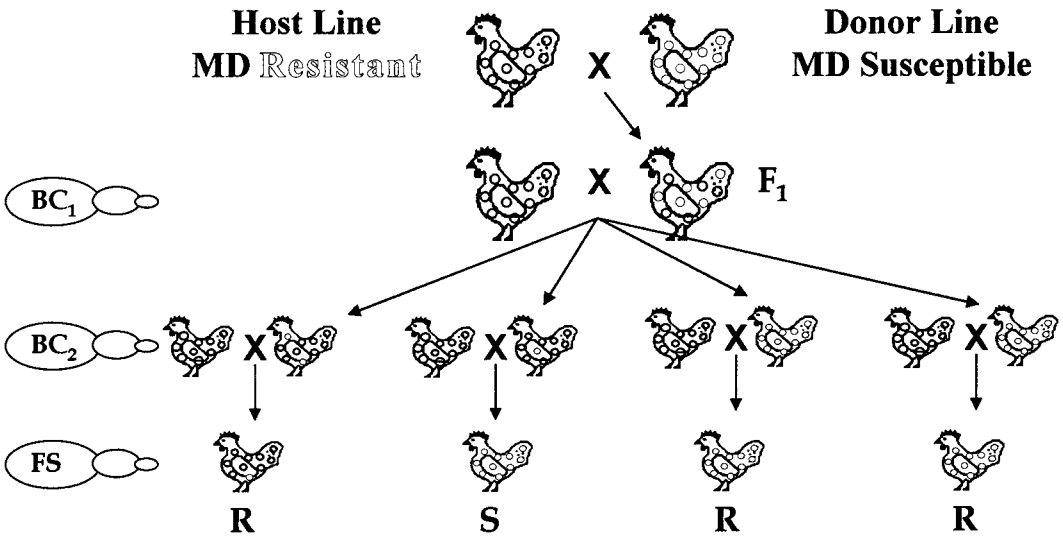

FIGURE 2. Mating scheme for production of 6C.7 recombinant congenic strains. MD = Marek's disease; R = resistant; and S = susceptible.

RCS are becoming inbred, and about two to four strains carry Line 7_2 alleles for any part of the genome (Chromosome 1 results are shown in Figure 3), i.e., only two to four strains have dark hatch boxes at most horizontal microsatellite marker positions.

Initial research with the RCS has investigated susceptibility to MD, and the data suggest the RCS will be useful in defining genes that influence the various traits that differ between inbred Lines 6 and 7. In the second full-sib generation, the 6C.7 RCS and Line 7_2 control chicks were challenged with 2000 plaque forming units of the JM strain of MDV at 1 wk of age. The chickens were observed for 10 wk, and several RCS were found with elevated MD susceptibility (Bacon et al., 1996a). In 4 subsequent yr, RCS 6C.7M, -P, and -W were repeatedly more susceptible to MD than Line 6_3 chickens (Table 4) (Yonash et al., 1998). Previously, several genomic DNA markers were associated with susceptibility to MD in $6_3 \times 7_2$ F_2 chickens (Vallejo et al., 1998; Yonash et al., 1999). Attempts are underway to determine if those or other DNA markers are correlated with susceptibility to MD in the RCS. If so, the definition of the genes leading to this susceptibility should be achievable using fine mapping techniques. Ultimately, it is envisioned that some of the MD-resistant genes identified in these RCS may have variable alleles in some commercial breeding strains and that it will be

TABLE 5. Blood types and Marek's disease (MD) susceptibility of Lines 6_3, 7_2, and 6C.7 recombinant congenic strains

Line	Blood group system/allele[1]			MD susceptibility[2]
	*E**	*J**	*R**	
6_3	*7*	–	+	Low
7_2	*5*	+	–	High
6C.7A	Segregates	–	+	Low
6C.7B	Segregates	–	Segregates	Low
6C.7C	Segregates	Segregates	+	Low
6C.7D	Segregates	Segregates	+	Low
6C.7F	*7*	–	Segregates	Low
6C.7G	*7*	–	–	Low
6C.7I	Segregates	–	+	Low
6C.7J	*7*	–	+	Low
6C.7K	Segregates	–	+	Low
6C.7L	*7*	Segregates	+	Low
6C.7M	*7*	Segregates	Segregates	Moderate
6C.7N	*7*	Segregates	Segregates	Low
6C.7P	*7*	–	+	Moderate
6C.7R	*7*	Segregates	+	Low
6C.7S	*7*	–	+	Low
6C.7T*	*7*	–	+	Low
6C.7V	*7*	Segregates	Segregates	Low
6C.7W	*7*	Segregates	Segregates	Moderate
6C.7X	*7*	Segregates	+	Low

[1]The allelic designations for the *E* locus are shown. The * indicates the gene, and the number identifies the allele. Alleles for the *J* and *R* loci are shown by + or –.

[2]All lines are susceptible to MD virus (JM strain), but Line 6_3 is resistant to tumors.

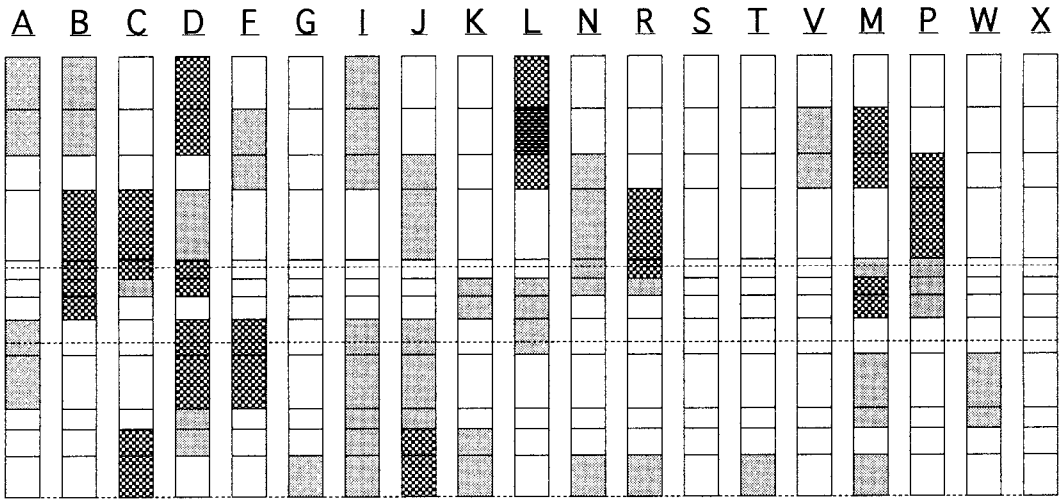

FIGURE 3. Genetic profile of chromosome 1 for 6C.7 recombinant congenic strains.

possible to identify and select for alleles that increase MD resistance.

REPRODUCTION STRATEGIES FOR VARIOUS TYPES OF LINES

A number of different types of lines are described that are maintained at the ADOL. All chicks are pedigree-hatched by hen and identified by wing bands. This procedure permits assigned matings to control inbreeding. The methods for reproduction will be briefly listed.

Noninbred Lines

In addition to inbred and congenic lines, several noninbred lines selected for disease traits are also maintained at the ADOL (Table 2). The noninbred lines are reproduced using artificial insemination of individual male's semen. Male breeders are obtained from numerous different hens having good egg production at the time of reproduction, and non-sib matings are conducted. The ADOL noninbred lines include Lines 0; Cornell Lines N and P described previously; and Line $15B_1$, which is C/0 and used to provide chick embryo fibroblasts to grow all subgroups of ALV (Table 2) (Crittenden, 1991).

Inbred Lines with Relaxed Inbreeding

After the 15-*B* congenic lines were developed (see previous), they were not further inbred as we wanted the lines to stay comparable with Line $15I_5$. Therefore, the 15-*B* congenic lines are reproduced using pooled semen, and male breeders are obtained from numerous different hens having good egg production at the time of reproduction (Table 4).

Inbred Lines with Continued Inbreeding

In the 1960s through 1979, the inbred lines were reproduced by mating primarily full-sibs within each line. However, this led to full-sib families within lines, forming further family sublines. Some sublines had different traits as drifting of nonfixed genes or mutations occurred. Subsequently, if experimental chicks were pooled from different inbred families, the pool of chicks was not uniform. To continue inbreeding but provide uniformity in the lines, the breeding plan was altered in 1980. Within each line, potential breeder males are reproduced from two of the best laying full-sib families, and female chicks are also kept from these and other hens. The two selected families might have been produced by the same or different sires the previous generation. Subsequently, single males from primarily one full-sib family are used for artificial insemination of randomly mated females. Thus, each year, essentially all male breeders are full-sibs from one or two families. After line reproduction, the semen within a line may be pooled for mass chick production. Inbred lines include Rh-C (Bacon and Motta, 1982), 6_3, 7_1, 7_2, 7.6-*VB*S1*, $15I_5$, and the 6C.7 RCS (Tables 1 and 5).

ASSURANCE OF PURITY

Two types of testing are done to ensure purity of ADOL chickens. One series of tests is done to ensure absence of 14 known poultry pathogens (Stone, 1975). The other test relevant here is to ensure genetic purity of the lines. A HA test using alloantisera is used to ensure purity (Fulton et al., 1996b). From 1978 to 1987, the purity of lines was ascertained using alloantisera specific for antigens of various blood-group systems provided by W. E. Briles (Abplanalp et al., 1979). In 1987, 82 polyvalent antisera were produced at ADOL, and from one to five selected polyvalent, or B, antisera have been used to ensure purity of each line from all other lines (L. Bacon and E. Young, unpublished data). The 6C.7 RCS are currently only tested for purity from all lines except 6_3 and other RCS, but eventually each RCS may also be distinguished from 6_3 and other RCS using a combination of blood-typing and perhaps other methods. Of course DNA technologies could now be employed to distinguish the lines. How-

ever, HA is relatively inexpensive and simple, making this the procedure of choice so long as appropriate antisera and expertise exists. Unfortunately, constant monitoring for purity is essential, e.g., when monitoring of lines was initiated, several lines had sib families that were clearly line crosses, and one line was completely crossed with another. Subsequently, from time to time, errors have been detected despite careful mating and pedigree-banding procedures. For monitoring, all males are blood-typed yearly, and all females are blood-typed biyearly; this should detect any unwanted cross in time to remove impure breeders. The testing procedure takes approximately 3 to 6 wk yearly, but it is a price one pays for assurance of pure lines.

CONCLUSION

Numerous changes have occurred in the composition of ADOL chicken lines since the report of Stone (1975). These changes relied primarily on the availability of established selected inbred lines developed at the laboratory. In the next 25 yr, analyses of special congenic lines should extend the understanding of genes affecting disease resistance in poultry.

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