The NXSM Recombinant Inbred Strains of Mice: Genetic Profile for 58 Loci Including the *Mtv* Proviral Loci

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ABSTRACT

We report the construction of 17 recombinant inbred (RI) strains of mice derived from the progenitor strains NZB/BlNRe and SM/J and the typing of this RI strain set, designated NXSM, for 58 loci distributed on 16 autosomes and the *X* chromosome. Two backcrosses involving NZB/BlNJ and SM/J were constructed to confirm chromosomal assignments and determine gene orders suggested from NXSM RI strain data. From these results we recommend that chromosomal assignments and gene orders suggested from analyses of RI strain sets be confirmed using data obtained by other means. We also typed NZB/BlNJ and SM/J for mammary tumor proviral (*Mtv*) loci. Both strains share three previously described *Mtv* loci: *Mtv-7*, *Mtv-14* and *Mtv-17*. In addition, NZB/BlNJ contains the previously described *Mtv-3* and *Mtv-9* loci and two new *Mtv* proviral loci: *Mtv-27* located on chromosome (Chr) *1* and *Mtv-28* located on the *X* chromosome. SM/J contains the previously described loci *Mtv-6* and *Mtv-8*. Four LTR, mink cell focus-forming murine leukemia viral loci were identified and mapped: *Ltrm-1* on Chr *12*, *Ltrm-2* on Chr *16*, *Ltrm-3* on Chr *5*, and *Ltrm-4* on Chr *13*. The *Tgn* locus was positioned proximal to the *Ly-6* locus on Chr *15*.

RECOMBINANT inbred (RI) strains of mice are one of the most useful systems in mammalian genetics for rapidly identifying chromosomal assignments of newly discovered loci (TAYLOR 1978). The only criterion necessary for utilizing RI strains for gene mapping is that the two progenitor strains differ in allelic composition for the loci of interest. The first set of RI strains, designated CXB, were constructed from the inbred strains BALB/cBy (C) and C57BL/6By (B) by BAILEY (1971). The number of mouse RI strain sets now available or in the process of construction exceeds 20 and some RI strain sets contain as many as 26 strains (see listing in TAYLOR 1989).

In 1974 we began the construction of a set of RI strains using NZB/BlNRe (NZB) and SM/J (SM) as progenitor strains. Mice of the NZB strain develop an autoimmune disease similar to the human disease systemic lupus erythematosis (SLE) (see review by THEOFILOPOULOS and DIXON 1985). We reasoned that having a set of RI strains available involving NZB might aid in unraveling the genetics of this heritable disorder. SM/J provided an ideal companion strain for constructing a set of RI strains involving NZB because this combination would maximize the genetic differences available and, thus, would aid in determining the number and chromosomal locations of genes involved in the NZB autoimmune disorder.

Although RI strains have been and will continue to be a valuable tool for facilitating gene mapping in mice, pitfalls can be encountered when using this mapping method. For example, genes that appear linked from analysis of RI strain data may not show linkage when backcross data is analyzed. Conversely, two genes that appear unlinked may, in fact, show linkage when backcross data is analyzed. These problems are especially evident when a limited number of RI strains is analyzed. Establishing gene order using RI strain data can be problematic. Normally, one establishes the order of three loci on the assumption that each single-recombinant class is more frequent than the double-recombinant class. However, this assumption is not valid when analyzing RI strain data because what appear as "doubles" are more likely two independent, single-recombination events. For these reasons, whenever possible we used backcross data to confirm linkages suggested from the NXSM RI strain set.

We report here details concerning the construction of the NXSM RI strain set and the strain distribution patterns for 58 loci. In addition, we identified the *Mtv* (mouse mammary tumor virus) loci present in the SM/J and NZB/BlNJ inbred strains and found two new *Mtv* proviral loci, *Mtv-27* and *Mtv-28*, and what may be a deleted *Mtv-8* locus in the NXSM-L/Ei strain. Finally, we positioned the thyroglobulin (*Tgn*) locus proximal to the lymphocyte antigen-6 (*Ly-6*) locus on chromosome (Chr) *15*.

MATERIALS AND METHODS

Origin of NXSM RI strains: We began construction of the NXSM RI strain set in 1974. The initial mating involved an NZB/BlNRe female and an SM/J (*a/a*) male. The Jackson

Laboratory pedigree number and generation of the SM/J male was 3862 and F_{74}, respectively. The NZB female was the granddaughter of a pedigreed pair of NZB/BlNRe mice (pedigree numbers 112 and 113, F_{77}) obtained from ELIZABETH RUSSELL (The Jackson Laboratory). (Note that when RUSSELL transferred breeding stock of her NZB strain, F_{70}, to The Foundation Stocks of The Jackson Laboratory, the Re holder designation was dropped and the J added to the strain designation. All NZB mice used as controls in experiments reported here are from the NZB/BlNJ strain.) From the F_1 offspring produced, two separate matings were made to obtain F_2 offspring, which were then sib-mated to produce the F_3 generation. At the F_3 generation of inbreeding, 26 separate lines were begun, designated NXSM-A/Ei, NXSM-B/Ei, NXSM-C/Ei, etc. (hereafter designated A, B, C, etc.), and sister by brother matings were continued within each line thereafter. Lines A, B, C, D, J, K, L, M, N, O, P, U, V, W and Z were derived from one F_1 mating, whereas lines E, F, G, H, I, Q, R, S, T, X and Y were derived from the other F_1 mating. The T line was split into two lines, T1 and T2, at generation F_8.

A total of 17 NXSM RI strains is reported here. Strains C, D, E, F, I, L, N, P, Q, T1, T2, U, W, X and Z are presently breeding. Strains A and V became extinct at generation F_{36} and F_{34}, respectively. DNA was prepared from the spleen of a number of strain A and strain V mice and is available for analysis. In addition, a number of isozyme loci and lymphocytic antigen loci were typed for strains A and V before they became extinct.

Loci used for mapping: Loci referred to or typed in the NXSM RI strain set are: non-agouti (*a*); α_1-antitrypsin (*Aat*); alkaline phosphatase-1 (*Akp-1*); carbonic anhydrase-1 (*Car-1*); carbonic anhydrase-2 (*Car-2*); complement component factor h (*Cfh*); gamma crystalline (*Cryg*); dilute (*d*); DNA segment, Chr *12*, NYU-3 (*D12Nyu3*); DNA segment, Chr *12*, NYU-10 (*D12Nyu10*); DNA segment, Chr *13*, University of Washington-70 (*D13Was70*); DNA segment, Chr *17*, Roswell Park-17 (*D17Rp17*); DNA segment, Chr *17*, Lehrach-66E (*D17Leh66E*); DNA segment, Chr *17*, Lehrach-119I (*D17Leh119I*); DNA segment, Chr *17*, Lehrach-119II (*D17Leh119II*); endogenous ecotropic MuLV-1 (*Emv-1*); endogenous ecotropic MuLV-15 (*Emv-15*); avian erythroblastosis oncogene B (*Erbb*); ecotropic viral integration site-2 (*Evi-2*); galactose-1-phosphate uridyl transferase (*Galt*); glycerolphosphate dehydrogenase-1 (*Gdc-1*); growth hormone (*Gh*); glutamic-pyruvic transaminase-1, soluble (*Gpt-1*); beta-glucuronidase-structural (*Gus-s*); major histocompatibility complex (*H-2*); hemoglobin α-chain complex (*Hba*); hemoglobin α-4, pseudogene (*Hba-4ps*); hemoglobin β-chain complex (*Hbb*); hemolytic complement (*Hc*); isocitrate dehydrogenase -1 (*Idh-1*); immunoglobulin heavy-chain variable region (*Igh-V*); immunoglobin κ gene complex (*Igk*); LTR, mink cell focus-forming murine leukemia virus-1 (*Ltrm-1*); *Ltrm-2*; *Ltrm-3*; *Ltrm-4*; major liver protein-1 (*Lvp-1*); lymphocyte antigen-2 (*Ly-2*); *Ly-6*; *Ly-7*; *Ly-15*; myelin basic protein (*Mbp*); malic enzyme, supernatant (*Mod-1*); malic enzyme, mitochondrial (*Mod-2s*); Moloney sarcoma oncogene (*Mos*); modified polytropic murine virus-17 (*Mpmv-17*); major urinary protein-1 (*Mup-1*); neuraminidase-1 (*Neu-1*); peptidase-3 (*Pep-3*); phosphoglucomutase-1 (*Pgm-1*); phosphoglucomutase-2 (*Pgm-2*); transcobalamin-2 (*Tcn-2*); *t*-complex protein-1 (*Tcp-1*); thyroglobulin (*Tgn*); tyrosine hydroxylase (*Th*); and T cell antigen receptor alpha chain (*Tcra*). In addition, the following mammary tumor proviral loci were typed in the SM/J, NZB/BlNJ, and NXSM RI strains or are discussed in this paper: *Mtv-3*, *Mtv-6*, *Mtv-7*, *Mtv-8*, *Mtv-9*, *Mtv-11*, *Mtv-13*, *Mtv-14*, *Mtv-17*, *Mtv-22*, *Mtv-24*, *Mtv-27* and *Mtv-28*.

TABLE 1

Protein typing of NXSM RI strains: allelic forms and methods used

Chr	Locus	Allele NZB	Allele SM	Reference for method
1	*Idh-1*[a]	a	b	This paper
1	*Pep-3*[a]	c	b	EPPIG and EICHER (1983)
1	*Akp-1*[a]	b	a	This paper
3	*Car-1*	a	b	EICHER *et al.* (1976); this paper
3	*Car-2*	b	a	EICHER *et al.* (1976); this paper
4	*Galt*	a	b	NADEAU and EICHER (1982)
4	*Pgm-2*	a	b	EPPIG and EICHER (1983)[b]
5	*Pgm-1*[a]	b	a	EPPIG and EICHER (1983)
6	*Ly-2*	b	a	HOGARTH *et al.* (1987)
6	*Lvp-1*	a	b	WILCOX and RODERICK (1982)
7	*Mod-2s*	b	a	EICHER and COLEMAN (1977); this paper
7	*Hbb*[a]	d	s	WHITNEY (1978)
7	*Ly-15*	a	b	HOGARTH, EICHER and MCKENZIE (1986)
9	*Mod-1*[a]	b	a	EICHER and COLEMAN (1977); this paper
11	*Tcn-2*	f	s	FRATER-SCHRÖDER *et al.* (1982)
15	*Gpt-1*	b	a	EICHER and WOMACK (1977)
15	*Ly-6*[c]	a	b	HOGARTH *et al.* (1987)
17	*Neu-1*	b	a	WOMACK and EICHER (1977)

[a] Allele carried by NZB/BlNJ agrees with that suggested as the prototype allele for NZB (YONEKAWA *et al.* (1986).

[b] PGM-2 was determined using the method given for PGM-1 in EPPIG and EICHER (1983).

[c] *Ly-6* was also determined using a DNA probe (see Table 2).

Isozyme and protein variant methods: The isozyme and protein variants typed for the NXSM RI strains, including references for the methods used, are listed in Table 1. Because the method we used for typing AKP-1 has not been previously published, this method, kindly supplied by RICHARD FOX (The Jackson Laboratory) is included. In addition, we include an improved method for determining CAR-1, a correction in the electrophoretic method for determining CAR-2, and a method for determining the IDH-1, MOD-1 and MOD-2 genotype of individual mice on a single cellulose acetate electrophoresis plate.

To determine the AKP-1 genotype of each mouse, a single kidney was homogenized in dH_2O (1:1, v/v) and the supernatant collected after centrifugation at 27,713 × *g* for 30 min. Electrophoresis was conducted on Titan III cellulose acetate plates (Helena Laboratories) using the Helena Zip Zone electrophoresis apparatus. Electrophoresis was conducted anode to cathode for 40 min at 200 V in a pH 8.2 Tris-citrate buffer [10.5 g Trizma base (Sigma T-1503); 3.0 g citric acid, monohydrate (Fisher Scientific, A-105/1000 ml] that was also used to presoak the Titan III plates before loading the samples. Under these conditions, the AKP-1A isozyme migrates more rapidly than the AKP-1B isozyme. The staining mixture consisted of 250 μl β-naphthyl Na phosphate (100 mg/ml, Sigma N-1132), 100 μl fast blue BB salt (saturated solution, Sigma F-0250), 50 μl $MgSO_4 \cdot 7H_2O$ (1 mg/10 ml), 50 μl $MnCl_2 \cdot 4H_2O$ (3.15 g/100 ml) and 2 ml dH_2O. (All of these chemical solutions can be stored frozen until needed.) Staining was accomplished using an agar overlay. The reaction was allowed to proceed (for approximately 15 min) at 37° in the dark until the AKP-1 bands developed. Because the AKP-1 enzyme appears to be unstable, we determined the AKP-1 pheno-

types of individual mice before using the supernatants to determine the genotype of other isozyme loci.

The revised method for CAR-1 consists of conducting the electrophoresis cathode to anode at 300 V for 30 min on Titan III plates using a pH 8.9 Tris-glycine (5.16 g Trizma base, 3.48 g glycine/1000 ml) buffer. Best results are obtained when a total of 1.6 cm length of packed frozen RBCs (collected in a non-heparin treated hematocrit tube) are lysed in 0.1 ml hemolysate solution (1 mg ethylenediamine tetraacetic acid, tetrasodium salt, per ml dH_2O) before use.

Inadvertently, the published method for CAR-2 (EICHER *et al.* 1976) contained an error, in that the electrophoresis buffer must be diluted (1 part buffer with 4 parts dH_2O).

IDH-1, MOD-1, and MOD-2 were determined using supernatants prepared from kidney or heart homogenates (see method above). Electrophoresis was conducted cathode to anode at 200 V for 40 min on Titan III Plates using a Tris-citrate buffer (one part 0.1 M Tris, adjusted to pH 7.6 with citric acid monohydrate, diluted one part buffer to 4 parts dH_2O). Staining for MOD-1 and MOD-2 was accomplished using the method of EICHER and COLEMAN (1977). Because this particular citric acid (Fisher Scientific, A-105) contains isocitric acid, the IDH-1 genotype of each mouse can be directly read on the MOD stained gels without having to specifically stain for IDH. (The order of the three isozymes on the gel is, from origin: MOD-1, IDH-1, MOD-2). If the IDH typing is not clear, confirmation of the IDH genotype can be obtained by pipetting a solution of isocitric acid (100 mg/ml) onto the agar overlay and allowing it to diffuse through the agar to the cellulose acetate gel surface.

Probes used for typing: A list of the probes used, including references, and the loci they recognize are listed in Table 2.

DNA extraction, Southern blotting, probe labeling and hybridization conditions: Genomic DNA was extracted from the frozen spleen of individual mice using the method of JENKINS *et al.* (1982). Ten micrograms of genomic DNA were digested with the appropriate restriction enzyme using conditions recommended by the manufacturer (BRL) and the DNA fragments were separated by size in 1% agarose (Seakem, FMC Corp.) gels at 30 V for 18–24 hr. DNA was blotted to nylon membranes (Zeta-Probe, Bio-Rad Laboratories) with 0.4 M NaOH (REED and MANN 1985). The filters were washed at 65° for one hr in 0.1 × SSC, 0.5% sodium dodecyl sulfate (SDS) before prehybridization.

Hybridization conditions for all probes, except pMC5.04 (*Hc*), were as follows: The filters were prehybridized at 65° for 3–4 hr in 4 × SSCP (1 × SSCP = 121 mM NaCl, 15 mM Na_2 citrate, 15 mM Na_2HPO_4, 5 mM NaH_2PO_4), 10 × Denhardt's solution (0.2% Ficoll, 0.02% polyvinylpyrollidone, 0.2% bovine serum albumin) and 1.0% SDS. The filters were hybridized at 65° for 18 hr with 1–5 × 10^6 cpm/ml of denatured, ^{32}P-labeled probe in a solution of 4 × SSCP, 0.2% Denhardt's solution, 1.0% SDS and 0.1 mg/ml denatured sonicated salmon sperm DNA. The filters were then washed extensively at 65° in 0.1–4 × SSC, 0.1% SDS, with the concentration of SSC used determined experimentally or by published recommendation (references for probes are provided in Table 2).

Filters hybridized with the pMC5.04 probe were prehybridized in 5 × SSCP, 10 × Denhardt's solution, 0.5% SDS and 50% formamide at 42° for 4 hr. Hybridization was conducted in the same solution with the addition of 1.5 × 10^6 cpm/ml denatured labeled probe, 0.1 mg/ml denatured sonicated salmon sperm DNA and 10% dextran sulfate (Oncor, Inc.). After hybridization overnight at 42°, the filters were washed extensively with 0.5 × SSC, 0.1% SDS at 65° before exposure to X-ray film.

Autoradiography was performed using Kodak XAR-5 X-ray film and DuPont Cronex intensifying screens at −70° for exposure times of 1–7 days, depending on the probe used.

Restriction fragment sizes were determined by comparison with the migrating distances of λ *Hin*dIII fragments using the Gel Pad Program (K&H Biosoft, Inc.) and a HiPad digitizer (Houston Instrument) on an Apple IIe computer.

Most filters containing appropriately digested DNA were reused by stripping labeled probe with two 20 min washes of 0.1 × SSC, 0.1% SDS solution preheated to 95°. The stripped filters were then prehybridized and hybridized with a new labeled probe.

Probes p15.4, VhQ52, α-φ4, Bam.7 and CI-3-LTR were labeled with [α-^{32}P]dCTP (3000 Ci/mM) to a specific activity of >1 × 10^9 cpm/μg using the T4 DNA Polymerase Labeling System (BRL) Probes v-*erb*B, pRT57 and pKLy6E.1-2R were oligo-labeled with [α-^{32}P]dCTP to a specific activity of >1 × 10^9 cpm/μg using the Multiprimer DNA Labeling System (Amersham). M13φ20-1 was labeled using a modification of the M13 dideoxy sequencing method described in EICHER *et al.* (1989).

All other probes were nick-translated to a specific activity >2 × 10^8 cpm/μg with [α-^{32}P]dCTP by the method of RIGBY *et al.* (1977).

Identification of *Mtv* loci: Because most endogenous mouse mammary tumor viruses (MMTV) contain one *Eco*RI site, hybridization of an MMTV long terminal repeat (LTR) probe to Southern blots containing *Eco*RI digested DNAs generates two fragments for each provirus, a 5′ virus-cell junction fragment and a 3′ virus-cell junction fragment. The MMTV LTR probe used was MMTV 8-29, a pBR322 subclone containing a 1.45-kb MMTV LTR *Pst*I fragment isolated from an integrated provirus of a C3H-MMTV infected rat cell line (MAJORS and VARMUS 1981). In addition we used a MMTV *env* clone, MMTV 8-21 (MAJORS and VARMUS 1981), to probe DNA from the progenitor strains and the NXSM RI strain set. The criterion used to identify *Eco*RI fragments derived from a single NZB or SM *Mtv* proviral locus was that the fragments cosegregated in the NXSM RI strain set, *i.e.*, had identical strain distribution patterns (SDP). This approach, of course, can lead to false conclusions if two MMTV proviral loci are closely linked or if the number of RI strains analyzed is small. Therefore, we also compared the sizes of the *Eco*RI fragments obtained with the published *Eco*RI fragment sizes of MMTV proviral loci and we compared the linkage data obtained with known linkage information for MMTV loci (see review by KOZAK *et al.* 1987). In addition, because different electrophoretic and gel conditions can lead to different estimates of DNA fragment length, we compared the patterns and sizes of *Mtv*-derived *Eco*RI fragments obtained on Southern blots containing DNAs from NZB/BlNJ, SM/J and DBA/2J mice.

MCF LTR probe used: We used an LTR sequence cloned from a mink cell focus-forming (MCF) murine leukemia virus as a probe to search for restriction fragment length variants between NZB/BlNJ and SM/J. This LTR, hereafter designated CI-3-LTR, was isolated from a C3H IdUrd induced virus designated CI-3 (RAPP *et al.* 1983) and is the same LTR used by PHILLIPS *et al.* (1982) to identify retroviral related sequences on the mouse Y chromosome. The loci identified by CI-3-LTR are designated as LTR, mink cell focus-forming murine leukemia virus (*Ltrm*), with a number defining the specific locus.

Ascertainment of linkage: To ascertain linkage assignments suggested from analysis of the NXSM RI strain set

TABLE 2

Probes used to determine RFLPs in NXSM RI strains: allelic forms of loci and methods used

Chr	Locus	Probe	Enzyme	Size (kb)[a] NZB	Size (kb)[a] SM	Reference for probe
1	*Cryg*	pMγ1Crl	*Bam*HI/*Bgl*II	2.3	2.7	SHINOHARA *et al.* (1982)
1	*Cfh*	pMH.8	*Hin*dIII	2.6	3.2, 1.5	KRISTENSEN and TACK (1986)
1	*Mtv-27*	MMTV 8-29	*Eco*RI	11.4		MAJORS and VARMUS (1981)
		MMTV 8-21	*Eco*RI	11.4		MAJORS and VARMUS (1981)
2	*Hc*	pMC5.04	*Hin*dIII	6.0, 4.6	2.7	WETSEL, OGATA and TACK (1987)
2	*Emv-15*[b]	p15.4	*Hin*dIII	5.0	2.4	SIRACUSA *et al.* (1987)
4	*Mos*	pMSH	*Taq*I	5.3	4.6	PROPST *et al.* (1989)
4	*Mup-1*	p1057	*Hin*dIII	10.8, 6.5, 4.0	3.8, 2.7	KUHN *et al.* (1984)
5	*Emv-1*	Unnamed[c]	*Pvu*II		4.3	CHATTOPADHYAY *et al.* (1980)
5	*Ltm-3*	CI-3-Ltr	*Eco*RI		2.9	RAPP *et al.* (1983)
5	*Gus-s*	pGUS-1	*Hin*dIII	3.2	3.6	PALMER *et al.* (1983)
6	*Mtv-8*	MMTV 8-29	*Eco*RI		7.7, 6.3	MAJORS and VARMUS (1981)
		MMTV 8-21	*Eco*RI		6.3	MAJORS and VARMUS (1981)
6	*Igk*	LX1X 27'b'	*Hin*dIII	3.4, 1.9		SCHIFF *et al.* (1983)
7	*Th*	pHR3.0	*Taq*I	5.3	3.4	BRILLIANT, NIEMANN and EICHER (1987)
11	*Erbb*	v-*erb*B	*Pst*I	5.3	5.5	ONCOR, Inc., Gaithersburg, Maryland
11	*Hba*	α-φ3	*Eco*RI	13.1	14.9	LEDER *et al.* (1981)
11	*Evi-2*	pXS1.9	*Eco*RI	8.0	7.3	BUCHBERG *et al.* (1988)
11	*Gh*	pRGH 1	*Hin*dIII	4.2	7.9	SEEBURG *et al.* (1977)
11	*Mtv-3*[d]	MMTV 8-29	*Eco*RI	19.3, 6.7		MAJORS and VARMUS (1981)
		MMTV 8-21	*Eco*RI	6.7, 0.9		MAJORS and VARMUS (1981)
12	*D12Nyu10*	pUCφ43G14	*Msp*I	4.4	13.3	BLANK *et al.* (1988)
12	*D12Nyu3*	M13φ20-1	*Eco*RI	7.9	6.9	D'EUSTACHIO (1984)
12	*Ltrm-1*	CI-3-LTR	*Eco*RI	2.5		RAPP *et al.* (1983)
12	*Mtv-9*	MMTV 8-29	*Eco*RI	9.7, 7.4		MAJORS and VARMUS (1981)
		MMTV 8-21	*Eco*RI	9.7		MAJORS and VARMUS (1981)
12	*Aat*	pG3.5	*Eco*RI	9.0, 2.4	6.0, 5.5	D'EUSTACHIO (1984)
12	*Igh-V*	VhQ52	*Eco*RI	8.8, 4.7, 4.3	20.0, 3.5	BRODEUR and RIBLET (1984)
13	*D13Was70*	70-38	*Eco*RI	6.5		DISTECHE *et al.* (1985)
13	*Ltrm-4*	CI-3-LTR	*Eco*RI		3.7	RAPP *et al.* (1983)
14	*Tcra*	pHDS58	*Eco*RI	11.1, 2.2		SAITO *et al.* (1984)
15	*Tgn*	pRT57	*Taq*I	4.8	6.6	MUSTI *et al.* (1986)
15	*Ly-6*[e]	pKLy6E.1-2R	*Eco*RI	9.0, 3.1	5.2	LECLAIR *et al.* (1986)
15	*Gdc-1*	c8	*Pvu*II	3.2	3.0	KOZAK and BIRKENMEIER (1983)
16	*Ltrm-2*	CI-3-LTR	*Eco*RI		2.8	RAPP *et al.* (1983)
16	*Mtv-6*	MMTV 8-29	*Eco*RI		16.7	MAJORS and VARMUS (1981)
		MMTV 8-21	*Eco*RI		16.7	MAJORS and VARMUS (1981)
17	*D17Leh119I*	p119AR	*Msp*I	2.6	4.4	HERRMANN *et al.* (1986)
17	*D17Leh66E*	p66M-RT	*Bam*HI	3.6	21.5	HERRMANN *et al.* (1986)
17	*D17Leh119II*	p119AR	*Bam*HI	5.8, 3.0	6.7, 4.3	HERRMANN *et al.* (1986)
17	*D17Rp17*	pMK174	*Bam*HI	6.4	6.1	MANN, SILVER and ELLIOTT (1986)
17	*Tcp-1*	pB1.4	*Taq*I	3.0	1.1	WILLISON, DUDLEY and POTTER (1986)
17	*Hba-4ps*	α-φ4	*Taq*I	3.4	5.2	LEDER *et al.* (1981)
17	*H-2*[f]	b7	*Hin*dIII	14.3, 2.6, 1.9	17.2, 5.9, 3.2, 2.8	SCHULZE *et al.* (1983)
18	*Mbp*	pSP-GM1	*Pst*I	4.5	2.2	TAKAHASHI *et al.* (1985)
X	*Mtv-28*	MMTV 8-29	*Eco*RI	5.8		MAJORS and VARMUS (1981)
		MMTV 8-21	*Eco*RI	5.8		MAJORS and VARMUS (1981)

[a] For those DNA probes that hybridize to two or more fragments, all or some of which may differ between NZB/BlNJ and SM/J, we have designated the fragment or fragments we used to distinguish the strain specific alleles.

[b] Probe p15.4 is a cellular sequence flanking the *Emv-15* locus, a proviral locus closely linked to the *a* locus on Chr *2* (SIRACUSA *et al.* 1987).

[c] *Emv-1* was typed using a 0.4-kb ecotropic-specific probe subcloned from clone 623 of AKR MuLV DNA (CHATTOPADHYAY *et al.* 1980). To our knowledge this probe has never been given an official designation.

[d] Note that the NZB/BlNJ-derived *Mtv Eco*RI fragments we define as the *Mtv-3* locus were formerly defined as *Mtv-24* (KOZAK *et al.* 1987).

[e] *Ly-6* was also typed using an antibody method (see Table 1).

[f] Probe b7 hybridizes to a number of restriction fragments derived from the MHC complex.

and to determine gene order, we produced two sets of backcross mice. The first backcross set, referred to as the NZB backcross, consisted of 108 females produced by mating (NZB/BlNJ × SM/J)F_1 females and males to NZB/BlNJ males and females, respectively. The second backcross set, referred to as the SM backcross, consisted of 37 females produced by mating (NZB/BlNJ × SM/J)F_1 females to SM/J males. The methods used to type genotypes of the back-

cross mice were the same as those used to analyze mice of the NXSM RI strains. Genetic distances between loci are presented as percent recombination ± standard error.

RESULTS AND DISCUSSION

Strain distribution pattern for loci: The NXSM RI SDP for the 58 loci reported here is shown in Table 3. These loci are distributed on 16 autosomes with the number of loci for each chromosome ranging from one locus each on Chrs *9*, *14* and *18* to 8 loci on Chr *17*. One *Mtv* locus was assigned to the *X* chromosome. Because most of the genes used in this study have been assigned to and positioned on a chromosome using standard backcross data, we will not comment on their chromosomal assignment or position unless necessary.

Identification of *Mtv* loci in SM/J and NZB/BlNJ mice As shown in Table 4 and Figure 1, we identified five previously defined *Mtv* proviral loci in the genome of SM/J mice: *Mtv-6*, *Mtv-7*, *Mtv-8*, *Mtv-14* and *Mtv-17*. To our knowledge, this represents the first typing of the SM/J strain for MMTV proviruses.

As shown in Table 4 and Figure 1, five previously defined *Mtv* proviral loci were identified in the genome of NZB/BlNJ mice: *Mtv-3*, *Mtv-7*, *Mtv-9*, *Mtv-14* and *Mtv-17*. In addition, we identified two new proviral loci in NZB/BlNJ, *Mtv-27* and *Mtv-28* (provisional gene symbols). Our typing of *Mtv* loci in the NZB/BlNJ strain is in partial agreement with that reported by PETERS *et al.* (1986) for another NZB strain, NZB/Icrf, maintained by the Imperial Cancer Research Fund (ICRF) Animal Breeding Unit of Great Britain. PETERS and collaborators observed the presence of the *Mtv-9* and *Mtv-17* proviral loci and detected an 11.0-kb *Eco*RI fragment derived from the 3′ end of a provirus that lacked a 5′ end. As will be more fully discussed below, we believe that the 11.0-kb *Eco*RI fragment constitutes a new *Mtv* proviral locus, designated *Mtv-27*. PETERS and co-workers also detected a 20-kb *Eco*RI 5′ junction fragment and a 6.6-kb *Eco*RI 3′ junction fragment derived from a single MMTV proviral locus provisionally designated *Mtv-24* (KOZAK *et al.* 1987). Our evidence (see below) suggests that these fragments constitute a previously described *Mtv* locus, *Mtv-3*. Finally, PETERS and collaborators described a 17-kb *Eco*RI 5′ junction fragment and an 11.5-kb *Eco*RI 3′ junction fragment in NZB/Icrf mice that were provisionally designated in KOZAK *et al.* (1987) as constituting the *Mtv-22* locus. We believe that these fragments represent the previously defined *Mtv-7* locus (see below).

We found two additional *Mtv* loci in NZB/BlNJ that were not found in NZB/Icrf by PETERS *et al.* (1986): *Mtv-14*, represented by a 1.7-kb *Eco*RI fragment, and *Mtv-28*, a new MMTV provirus represented by a 5.8-kb *Eco*RI fragment (see Table 4 and Figure 1B). Several factors could account for our additional findings. The first possibility is that one of the NZB strains was contaminated during its propagation, as has been reported for a number of NZB sublines (YONEKAWA *et al.* 1986). We do know that the isozyme SDP we obtained for NZB/BlNJ strain agrees with the one YONEKAWA and co-workers suggested as the NZB prototype SDP (see Table 1). However, a more likely possibility for the differences noted between these two NZB sublines is that the NZB/BlNJ strain acquired two new MMTV proviral loci. Additional evidence for this suggestion is provided by ROBBINS *et al.* (1986) who also noted the presence of a 1.7-kb *Eco*RI fragment in the NZB/BlNJ strain. We have also typed the NZB/Icr strain and obtained the same MMTV profile as we obtained for NZB/BlNJ (data not shown).

A more detailed discussion of the *Mtv* proviral loci present in NZB/BlNJ and SM/J is now presented. To facilitate this presentation, we use the standardized MMTV proviral fragment sizes provided in KOZAK *et al.* (1987) when describing the work of other laboratories. The actual fragment sizes obtained by various laboratories are available in the original references.

***Mtv* proviral loci shared by NZB/BlNJ and SM/J:** The *Mtv-7* locus, located on Chr *1*, is defined by a 16.7-kb *Eco*RI (5′) and an 11.7-kb *Eco*RI (3′) fragment (TRAINA, TAYLOR and COHEN 1981); the *Mtv-14* locus, chromosomal assignment unknown, is defined by a 1.7-kb *Eco*RI fragment (MACINNES *et al.* 1984); and the *Mtv-17* locus, located on Chr *4*, is defined by a 10.0-kb *Eco*RI (5′) and an 8.3-kb *Eco*RI (3′) fragment (TRAINA, TAYLOR and COHEN 1981; MACINNES *et al.* 1984) (see review by KOZAK *et al.* 1987). As seen in Figure 1 (see also Table 4), NZB/BlNJ and SM/J share *Eco*RI fragments of sizes similar to those that define these three MMTV proviral loci and thus they probably contain these loci. A few further comments are relevant to the *Mtv-7* locus. As previously mentioned, PETERS *et al.* (1986) suggested that the 17.0-kb *Eco*RI and 11.5-kb *Eco*RI fragments present in the NZB/Icrf strain were derived from a new *Mtv* proviral locus. As seen in Figure 1, however, SM/J and NZB/BlNJ contain 16.7-kb and 12.0-kb *Eco*RI fragments that comigrate with the DBA/2J-derived *Eco*RI fragments constituting the *Mtv-7* locus. In addition, these fragments co-segregate in the NXSM RI strains and in the NZB and SM backcross mice. We suggest from this evidence that these fragments constitute the previously defined *Mtv-7* locus.

***Mtv* proviral loci present in SM/J:** *Mtv-6*, characterized by a single 16.7-kb *Eco*RI fragment (COHEN and VARMUS 1979), is located in a central position on Chr *16* (CALLAHAN, GALLAHAN and KOZAK 1984; REEVES *et al.* 1987). *Mtv-6* has been described as an incomplete proviral unit that hybridizes strongly to the MMTV LTR probe and weakly to the MMTV *env* probe (PAULEY, PARKS and POPKO 1984). We

TABLE 3

Loci typed for NXSM RI Strains

Chr	Locus	RI Strains																
		A	C	D	E	F	I	L	N	P	Q	T1	T2	U	V	W	X	Z
1	*Idh-1*	N	N	S	S	N	N	N	S	N	N	N	N	S	S	N	S	N
1	*Cryg*	N	N	S	S	S	N	N	S	N	N	N	N	S	S	N	S	N
1	*Pep-3*	N	S	S	S	N	S	N	N	N	S	N	N	N	N	N	N	N
1	*Cfh*	N	S	S	S	N	S	N	N	N	S	N	N	N	N	N	N	N
1	*Mtv-27*	N	S	N	S	N	S	N	S	S	N	N	N	N	S	S	N	N
1	*Akp-1*	N	N	N	S	N	N	N	S	S	N	N	N	N	S	S	N	N
2	*Hc*	S	N	N	S	N	N	S	N	N	S	S	N	N	S	N	N	N
2	*Emv-15*[a]	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	*Car-1*	S	N	S	S	S	N	S	N	N	N	S	S	S	S	S	S	S
3	*Car-2*	S	N	S	S	S	N	S	N	N	N	S	S	S	S	S	S	S
4	*Mos*	S	N	S	N	S	S	S	N	S	N	N	N	N	N	S	N	N
4	*Galt*	S	S	N	S	S	N	S	S	S	N	N	S	N	S	S	N	N
4	*Mup-1*	S	S	N	S	S	N	S	N	S	N	S	S	N	S	S	S	N
4	*Pgm-2*	S	S	N	N	S	S	S	N	N	N	S	N	N	S	S	N	N
5	*Emv-1*	N	N	N	S	N	S	N	S	N	N	S	S	N	N	N	S	S
5	*Ltrm-3*	S	N	N	S	N	S	N	S	N	S	S	S	N	S	N	N	S
5	*Pgm-1*	S	S	N	S	N	S	N	S	N	S	S	S	N	S	N	N	S
5	*Gus-s*	S	S	N	N	N	S	N	S	S	S	S	S	N	N	S	S	N
6	*Mtv-8*[b]	N	S	S	S	S	S	"S"	N	N	N	S	S	S	N	N	N	S
6	*Igk*	N	S	S	S	S	S	S	N	N	N	S	S	S	N	N	N	S
6	*Ly-2*	N	S	S	S	S	S	S	N	N	N	S	S	S	N	N	N	S
6	*Lvp-1*	N	S	S	S	S	S	S	N	N	N	S	S	S	N	N	N	S
7	*Mod-2s*	S	N	S	S	N	S	S	S	S	N	N	N	S	N	S	N	S
7	*Hbb*	S	N	S	S	N	S	N	S	N	N	N	S	N	N	S	N	S
7	*Ly-15*	S	N	S	S	N	N	N	S	N	N	S	S	N	S	S	N	S
7	*Th*	N	S	S	S	N	N	S	N	N	S	S	S	N	S	S	N	S
9	*Mod-1*	S	N	N	S	S	S	N	N	S	S	N	N	N	N	N	N	S
11	*Tcn-2*	S	S	N	S	S	S	N	N	S	N	S	S	S	N	S	S	N
11	*Erbb*	S	S	S	S	S	S	N	N	S	S	S	S	S	S	S	S	N
11	*Hba*	N	N	N	N	S	S	N	N	S	S	S	S	S	N	S	S	N
11	*Evi-2*	S	N	N	S	N	S	N	N	N	S	N	N	S	S	S	N	N
11	*Gh*	S	N	N	S	N	S	N	N	N	N	S	S	S	N	S	N	N
11	*Mtv-3*	S	N	N	S	N	S	N	N	N	S	S	S	S	N	S	N	N
12	*D12Nyu10*	N	N	S	S	S	N	S	S	N	N	S	S	S	S	S	N	N
12	*D12Nyu3*	N	S	S	S	S	S	S	N	N	S	S	S	S	S	N	N	N
12	*Ltrm-1*	N	S	S	S	S	S	S	N	N	S	S	S	S	S	N	N	N
12	*Mtv-9*	S	N	S	S	S	S	S	S	S	N	S	S	S	S	N	S	N
12	*Aat*	N	N	S	S	S	S	N	S	S	N	S	S	S	S	S	S	N
12	*Igh-V*	N	N	N	S	S	S	S	N	S	S	S	S	S	S	N	S	N
13	*D13Was70*	N	N	N	N	S	S	S	S	S	S	S	S	S	N	N	S	N
13	*Ltrm-4*	N	N	N	N	S	S	S	N	S	S	S	S	S	N	N	S	N
14	*Tcra*	N	N	N	S	S	N	N	S	N	S	S	S	N	S	N	N	N
15	*Tgn*	N	N	S	N	N	S	N	N	N	N	S	S	S	N	N	S	N
15	*Ly-6*	N	N	S	N	N	S	N	N	S	N	S	S	S	N	S	S	N
15	*Gpt-1*	S	S	N	N	N	S	S	N	S	N	S	S	N	N	S	S	N
15	*Gdc-1*	N	N	S	N	S	S	N	S	S	S	S	S	S	N	S	S	N
16	*Ltrm-2*	N	N	S	N	S	N	S	N	N	S	N	N	S	S	S	N	S
16	*Mtv-6*	N	N	N	N	S	S	S	N	N	S	S	S	S	N	S	S	S
17	*D17Leh119I*	S	N	N	N	S	S	S	N	N	S	S	S	S	N	S	S	N
17	*D17Leh66E*	S	N	N	N	S	S	S	N	N	S	S	S	S	N	S	S	N
17	*D17Leh119II*	S	N	N	N	S	S	S	N	N	S	S	S	S	N	S	S	N
17	*D17Rp17*	S	N	N	N	N	S	S	N	N	S	S	N	S	N	S	S	S
17	*Tcp-1*	S	N	N	N	N	S	S	N	N	S	S	N	S	N	S	S	S
17	*Hba-4ps*	S	N	N	N	S	S	N	N	N	S	N	N	S	N	N	S	S
17	*Neu-1*	S	N	N	N	S	S	S	S	N	S	S	N	S	N	N	S	S
17	*H-2* complex	S	N	N	N	S	S	S	S	N	S	S	N	S	N	N	S	S
18	*Mbp*	S	S	N	S	N	S	S	S	S	S	S	S	N	S	N	S	S
X	*Mtv-28*	N	N	S	N	S	N	N	N	N	N	N	N	N	N	N	S	N

[a] See text for comments about the apparent skewed inheritance of the *Emv-15* allele.
[b] See text for explanation of why the *Mtv-8* allelic designation for strain L is "S."

TABLE 4

Size of *Mtv* proviral *Eco*RI fragments present in NZB and SM mice

			This paper		Kozak *et al.* (1987)[a]	
					Derivation	
			Probe			
Locus	Chr	Strain	MMTV LTR	MMTV *env*	5′	3′
Mtv-3[b]	*11*	NZB	19.3, 6.7	6.7, 0.9	20.0	6.6
		SM			ND[c]	ND
Mtv-6	*16*	NZB				
		SM	16.7	16.7	ND	ND
Mtv-7[d]	*1*	NZB	16.7, 12.0	12.0	16.7	11.7
		SM	16.7, 12.0	12.0	ND	ND
Mtv-8	*6*	NZB				
		SM	7.7, 6.3	6.3	7.8[e]	6.7[e]
Mtv-9	*12*	NZB	9.7, 7.4	9.7	7.8	10.0
		SM			ND	ND
Mtv-14	?	NZB	1.7		1.7[f]	
		SM	1.7		ND	ND
Mtv-17	*4*	NZB	9.9, 7.9	7.9	10.0	8.3
		SM	9.9, 7.9	7.9	ND	ND
Mtv-27	*1*	NZB	11.4	11.4		11.0[g]
		SM			ND	ND
Mtv-28	*X*	NZB	5.8	5.8		
		SM			ND	ND

The NZB strain used in our study was NZB/BlNJ, while Peters *et al.* (1986) used NZB/Icrf in their study, the source of the majority of information about the NZB strain described in Kozak *et al.* (1987). Kozak *et al.*, however, note in their Table 1 that NZB contains a 1.7 *Eco*RI fragment (*Mtv-14*), a fragment not detected by Peters *et al.* but observed by Robbins *et al.* (1986) who also used NZB/BlNJ.

[a] Kozak *et al.* (1987) summarizes the previous gene designations and *Eco*RI fragment sizes and recommends a standardized nomenclature based on molecular cloning techniques and genetic segregation data.

[b] Designated as *Mtv-24* by Kozak *et al.* (1987).

[c] ND means not determined.

[d] These fragments were designated by Kozak *et al.* (1987) to constitute a new *Mtv* proviral locus *Mtv-22*, but our evidence suggests they are, in fact, the previously identified locus *Mtv-7*.

[e] The fragment sizes given for *Mtv-8* were determined from DNAs obtained from mice of strains other than SM/J.

[f] Not distinguished by Kozak *et al.* (1987) as to whether a 5′ or a 3′ junction fragment.

[g] This 11.0 *Eco*RI fragment, noted by Peters *et al.* (1986) as a 3′ fragment, was not mentioned by Kozak *et al.* (1987).

detected a 16.7-kb *Eco*RI fragment in SM/J that met both criteria for the *Mtv-6* proviral locus. The size of this SM/J-derived fragment with that observed for the *Mtv-6*-derived fragment present in the DBA/2J strain is identical (see Figure 1). Although this proviral fragment co-migrated with the 16.7-kb *Eco*RI 5′ fragment of the *Mtv-7* locus present in NZB/BlNJ and SM/J, we were able to score the segregation of this fragment in the NXSM RI strain set by its greater hybridization intensity (indicating the presence of two proviral fragments) using the MMTV LTR probe (Figure 1, A and B). In addition, the segregation pattern of this SM/J-positive 16.7-kb *Eco*RI fragment was confirmed using the MMTV *env* probe, which detects only the *Mtv-6*-derived 16.7-kb *Eco*RI fragment and not the same sized 5′ fragment derived from the *Mtv-7* locus (Figure 1A). Analysis of the NXSM RI strains indicated that this fragment was not linked to any of the other loci typed in these RI strains, including the *Ltrm-2* locus carried on Chr *16* (see below). Until confirmation experiments can be conducted, we tentatively designate this SM/J locus as *Mtv-6*.

Mtv-8, located on Chr *6*, is characterized by a 7.8-kb *Eco*RI 5′ viral-cell junction fragment and a 6.7-kb *Eco*RI 3′ viral-cell junction fragment (Cohen and Varmus 1979; Robbins *et al.* 1986). Analysis of the SM/J genome with the MMTV LTR probe revealed a 7.7-kb *Eco*RI and a 6.3-kb *Eco*RI fragment (Figure 1B). The MMTV *env* probe detected a 6.3-kb *Eco*RI fragment in SM/J. These fragments co-segregated in the NXSM RI strain set and were linked to *Igk* and other Chr *6* loci (Table 3) indicating they were derived from the *Mtv-8* locus. Further verification that these fragments represent the *Mtv-8* locus was found by typing the NZB backcross mice for this *Mtv* proviral locus and two loci known to be closely linked, *Igk* and *Lvp-1* (Table 5). Of 107 backcross mice analyzed, no recombinants were observed between the *Mtv* locus and *Igk*, whereas 5 recombinants were found between these loci and *Lvp-1*, placing these loci 4.7 ± 2.0 map units from *Lvp-1* on Chr *6* in the region known to contain *Mtv-8* (Yang *et al.* 1987). We conclude that these fragments constitute the *Mtv-8* locus.

One interesting exception noted was strain L, which lacked the *Eco*RI fragments that define the *Mtv-8* locus derived from SM/J but contained a novel 5.4-kb *Eco*RI MMTV LTR hybridizing fragment not present in the SM/J or NZB/BlNJ strains or the other NXSM RI strains (Figure 2). Further investigation of DNAs derived from an earlier generation of strain L mice revealed that the pair of L mice sampled at generation F_{34} strain were segregating for the fragments representing *Mtv-8* and the novel 5.4-kb *Eco*RI fragment, as the male contained both SM/J-derived *Mtv-8* fragments whereas the female lacked both fragments but contained the 5.4-kb *Eco*RI fragment. By contrast, we found that the pair of strain L mice tested at generation F_{46} contained neither the 7.7-kb nor the 6.3-kb *Eco*RI *Mtv-8*-derived fragments but contained the 5.4-kb *Eco*RI fragment. Both the F_{34} and F_{46} generation pairs of mice sampled were ancestral pairs of mice leading to the present L strain mice. From these results we conclude that the strain L originally contained the *Mtv-8* locus, however, a mutation occurred before generation F_{34} that resulted in the apparent loss of the *Mtv-8* locus and this mutation was fixed in this strain by generation F_{46}. We further

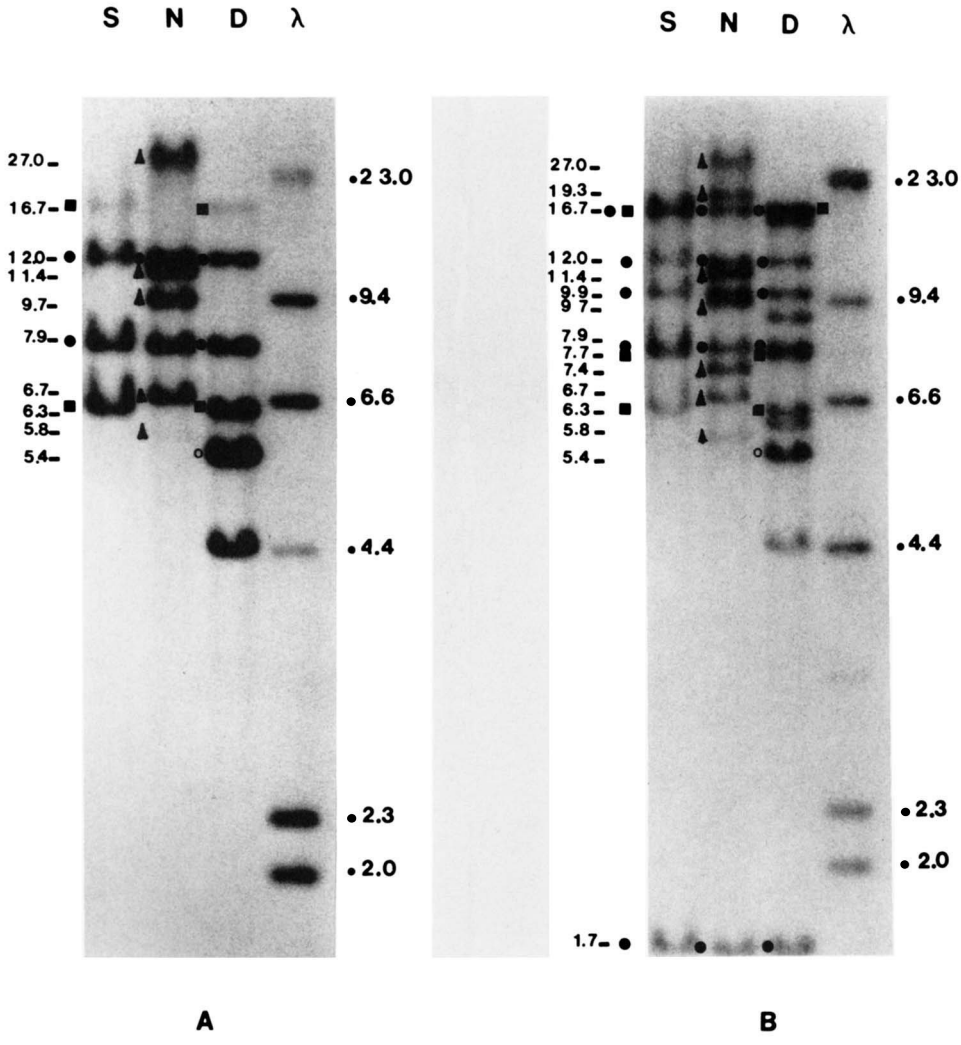

FIGURE 1.—Comparison of MMTV proviral loci in SM/J (S), NZB/BlNJ (N) and DBA/2J (D) inbred strains. A, *Eco*RI-digested male genomic DNAs hybridized with the MMTV *env* probe. B, The same filter stripped of probe and hybridized with the MMTV LTR probe. Dots denote each proviral fragment common to all three strains. Squares denote those fragments shared by the SM/J and DBA/2J strains. Triangles mark fragments unique to the NZB/BlNJ strain. (The *Mtv-3* derived 0.9-kb *Eco*RI fragment identified in NZB/BlNJ DNA with the MMTV *env* probe was not observed on this blot but was observed on other blots.) An open circle denotes the *Mtv-11* and *Mtv-13* derived 5.4-kb *Eco*RI fragment unique to DBA/2J. (The remaining unmarked fragments present in DBA/2J DNA are not relevant to this study.) Note that the 16.7-kb *Eco*RI fragment represents two comigrating proviral sequences: 1) *Mtv-6*, shared by SM/J and DBA/2J and 2) the 5′ cellular junction fragment of *Mtv-7* shared by all three strains. Autoradiography was performed for 3 days at −70°. Sizes of fragments are given at the left of each panel.

conclude that the novel 5.4-kb *Eco*RI fragment appeared in strain L before generation F_{34} and was fixed in this strain by generation F_{46}. We hypothesize that this novel 5.4-kb *Eco*RI fragment represents a deleted *Mtv-8* locus and is derived from ~4.2-kb flanking cellular sequences of an unoccupied *Mtv-8* proviral site (PETERS *et al.* 1986) plus a single 1.4-kb LTR (MAJORS and VARMUS 1981) of the *Mtv-8* locus. We further hypothesize that this fragment was generated by the excision of *Mtv-8* proviral sequences, possibly by the mechanism of precise homologous recombination described by COPELAND, HUTCHISON and JENKINS (1984) to explain their finding that a single LTR of the *Emv-3* ecotropic provirus remains when the autosomal recessive mutation *d* mutates to a wild-type (+) allele. The observation that the 5.4-kb *Eco*RI fragment hybridizes to the LTR probe but not the *env* probe supports our hypothesis.

***Mtv* proviral loci present in NZB/BlNJ:** *Mtv-3*, originally described by NUSSE *et al.* (1980), contains two internal *Eco*RI sites that release three fragments: 17.4-kb (5′), 6.9-kb (3′) and 0.9-kb (MACINNES *et al.* 1984). Analysis of NZB/BlNJ using the MMTV LTR probe revealed a 19.3-kb *Eco*RI and a 6.7-kb *Eco*RI fragment (Figure 1B), a finding in agreement with PETERS *et al.* (1986) who analyzed the NZB/Icrf strain. Analysis of the NZB/BlNJ genome using the MMTV *env* probe indicated it contained a 6.7-kb *Eco*RI 3′ viral-cell junction fragment (Figure 1A) and an additional 0.9-kb *Eco*RI fragment (data not shown) not noted by PETERS *et al.* The 6.7-kb and 0.9-kb *Eco*RI MMTV *env* hybridizing fragments co-segregated in the NXSM RI strain set with the 19.3-kb and 6.7-kb *Eco*RI fragments detected by the MMTV LTR probe, indicating these fragments are derived from a single MMTV provirus. Analysis of the SDP in the NXSM RI strain set suggested that this *Mtv* proviral locus was located at the distal end of Chr *11* (Table 3) and analysis of the SM backcross set confirmed that this proviral locus was located 5.4 ± 3.7 map units distal to the *Gh* locus (see Table 6) which is located distal to the *Evi-2* locus on Chr *11* (ELLIOTT, LEE and EICHER 1990). Because this *Mtv* proviral locus 1) contains three *Eco*RI fragments that are consistent in size to

TABLE 5

Segregation of Chr *6* loci *Mtv-8*, *Igk* and *Lvp-1* in NZB backcross

Genes			No. backcross offspring	Region of recombination
Mtv-8	*Igk*	*Lvp-1*	NZB[a]	
N	N	N	48	None
S	S	S	54	
N	S	S	0	
×				*Mtv-8–Igk*
S	N	N	0	
N	N	S	3	
	×			*Igk–Lvp-1*
S	S	N	2	
N	S	N	0	
×		×		*Mtv-8–Igk–Lvp-1*
S	N	S	0	
Total			107	

Percent recombination ± SE = (*Mtv-8–Igk*) − 4.7 ± 2.0–*Lvp-1*

An N refers to the NZB/BlNJ-derived allele and an S to the SM/J-derived allele.

[a] In the NZB backcross, both F_1 males and females were used to produce backcross offspring. For purposes of this paper, data are not separated by sex of the F_1 parent.

those constituting the *Mtv-3* locus and 2) resides in the region of Chr *11* known to contain *Mtv-3*, we conclude that the *Mtv-24* locus identified by PETERS *et al.* (1986) is, in fact, the *Mtv-3* locus.

The *Mtv-9* locus, originally described by COHEN and VARMUS (1979), contains a 7.8-kb *Eco*RI 5′ viral-cell junction fragment and a 10.0-kb *Eco*RI 3′ viral-cell junction fragment (PETERS *et al.* 1986; KOZAK *et al.* 1987). We also identified two similarly sized *Eco*RI fragments in NZB/BlNJ mice using the MMTV LTR probe (Figure 1B) and confirmed that the 9.7-kb *Eco*RI fragment is of 3′ origin using the MMTV *env* probe (Figure 1A). Both fragments co-segregated in the NXSM RI strains indicating they were derived from a single *Mtv* proviral locus (Tables 2, 3 and 4). *Mtv-9* was originally assigned to Chr *12* using a panel of Chinese hamster-mouse somatic cell hybrids (CALLAHAN, GALLAHAN and KOZAK 1984). The co-segregating fragments we observed appeared to be located on Chr *12* because only three of the 17 NXSM RI strains (strains A, L, W) were discordant with the *Aat* locus located on Chr *12* (Table 3). The assignment of this *Mtv* locus to Chr *12* was confirmed by typing the 37 SM backcross mice for *Aat*, *D12Nyu3*, *Ltrm-1*, and this *Mtv* proviral locus. The data in Table 7 support the assignment of the proviral locus to a position on Chr *12* between *D12Nyu3* and *Aat*, a position consistent with that reported by BLANK *et al.* (1988) for *Mtv-9*. We conclude that this MMTV proviral locus is *Mtv-9*.

We also identified a 5.8-kb *Eco*RI fragment in the NZB/BlNJ genome using the MMTV LTR and *env* probes. This fragment is the same size as the published

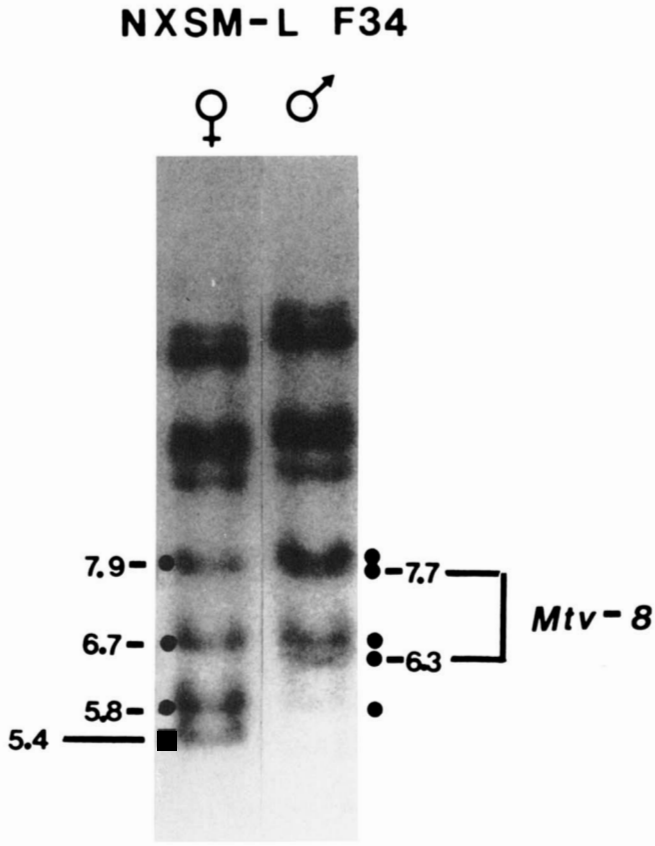

FIGURE 2.—Hybridization pattern of MMTV proviral locus *Mtv-8* in an NXSM-L strain female and male, generation F_{34}, using the MMTV LTR probe. Each lane contains 7 μg of *Eco*RI digested genomic DNA. Autoradiography was for 3 days at −70°. The sizes of the fragments constituting the *Mtv-8* proviral locus in the male DNA are given to the right. The square denotes the novel 5.4-kb *Eco*RI identified in DNA isolated from the F_{34} female and present in the both the female and male sampled at F_{46}. Notice the absence of the *Mtv-8*-derived fragments in the female DNA. The sizes of the other closely migrating fragments identified in NXSM-L are given on the left. The female and male NXSM-L DNAs were fractionated on separate gels, which accounts for the slightly different migration rates observed in the upper region of the gels.

*Eco*RI 3′ junction fragments of the *Mtv-11* locus on Chr *14* (PRAKASH, KOZAK and SARKAR 1985) and the *Mtv-13* locus on Chr *4* (MORRIS *et al.* 1979). However, the NZB/BlNJ strain did not contain either of the corresponding *Eco*RI 5′ fragments, *i.e.* the 15.0-kb derived fragment from *Mtv-11* or the 9.0-kb derived fragment from *Mtv-13*. Comparison of the size of this NZB/BlNJ-derived fragment on a Southern blot containing DNA from a DBA/2J mouse revealed that the comigrating *Mtv-11* and *Mtv-13* 3′ *Eco*RI fragments found in DBA/2J are closer to 5.4-kb in size, indicating that the NZB/BlNJ-derived 5.8-kb *Eco*RI fragment represents another proviral locus (Figure 1B). We also noted that the intensity of hybridization of the 5.8-kb *Eco*RI fragment appeared stronger in NZB/BlNJ females than males, suggesting that it could be *X*-linked. To test this hypothesis we compared the hybridization pattern of DNA isolated from F1 males

TABLE 6

Segregation of Chr *11* loci *Evi-2*, *Gh* and *Mtv-3* in SM backcross

Genes			No. backcross offspring SM	Region of recombination
Evi-2	*Gh*	*Mtv-3*		
N	N	N	12	None
S	S	S	12	
N	S	S	5	
×				*Evi-2–Gh*
S	N	N	5	
N	N	S	1	
	×			*Gh–Mtv-3*
S	S	N	1	
N	S	N	0	
×	×			*Evi-2–Gh–Mtv-3*
S	N	S	0	
Total			37	

Percent recombination ± SE = *Evi-2*–27.0 ± 7.3–*Gh*–5.4 ± 3.7–*Mtv-3*

An N refers to the NZB/BlNJ-derived allele and an S to the SM/J-derived allele. *Gh* has been previously mapped on Chr *11* distal to *Evi-2* (ELLIOTT, LEE and EICHER 1990).

TABLE 7

Segregation of Chr *12* loci *D12Nyu3*, *Ltrm-1*, *Mtv-9* and *Aat* in SM backcross

Genes				No. backcross offspring SM	Region of recombination
D12Nyu3	*Ltrm-1*	*Mtv-9*	*Aat*		
N	N	N	N	5	None[a]
S	S	S	S	23	
N	S	S	S	1	
×					*D12Nyu3–Ltrm-1*
S	N	N	N	0	
N	N	S	S	2	
×					*Ltrm-1–Mtv-9*
S	S	N	N	3	
N	N	N	S	1	
		×			*Mtv-9–Aat*
S	S	S	N	2	
Total				37	

Percent recombination ± SE = *D12Nyu3*–2.7 ± 2.7–*Ltrm-1*–13.5 ± 5.6–*Mtv-9*–8.1 ± 4.5–*Aat*

An N refers to the NZB/BlNJ derived allele and an S to the SM/J derived allele.

[a] We do not know why the number of mice receiving the N allele for all three loci is fewer than the number receiving the S allele. We did not take this skewing into account when computing the percent recombination between these loci; thus, the distances are probably overestimates.

derived from mating a NZB/BlNJ female to a SM/J male to the pattern obtained from DNA isolated from reciprocal F1 males (derived from mating an SM/J female to a NZB/BlNJ male). As noted in Figure 3, only the F1 male derived from the NZB/BlNJ female contained the 5.8-kb *Eco*RI fragment, indicating that this *Mtv* proviral locus is located on the *X* chromosome. We hereafter designate this new MMTV proviral locus *Mtv-28*.

Finally, we identified an 11.4-kb *Eco*RI fragment in NZB/BlNJ using the MMTV LTR and MMTV *env* probes (Figure 1). No other MMTV fragment cosegregated with the fragment in the NXSM RI strains. We also noted that the intensity of hybridization of the 11.4-kb *Eco*RI fragment with the MMTV LTR probe appeared stronger than that observed for the other MMTV LTR hybridizing fragments, suggesting that this fragment contains a double copy of the LTR sequence and thus may represent an intact MMTV proviral locus. Analysis of the NXSM RI strains and the SM backcross mice indicated the 11.4-kb *Eco*RI fragment is located on Chr *1* at a position 5.4 ± 3.7 map units proximal to *Akp-1* (Tables 3 and 8), which is within the same region containing another MMTV proviral locus, *Mtv-7* (TRAINA, TAYLOR and COHEN 1981). As previously mentioned, NZB/BlNJ also contains the *Mtv-7* locus, defined by 16.7-kb and 12.0-kb *Eco*RI fragments. Our reason for defining the 11.4-kb *Eco*RI fragment as a new Chr *1 Mtv* locus is that it is distinct in size from the 12.0-kb *Eco*RI fragment derived from the *Mtv-7* locus (see Figure 1) and it segregates as a discrete locus in the NXSM RI strain set. We conclude that the 11.4-kb *Eco*RI fragment derived from NZB/BlNJ represents a second *Mtv* proviral locus on Chr *1* and suggest the gene symbol *Mtv-27*.

One additional comment is required concerning *Mtv* proviral loci present in NZB/BlNJ. As noted in Figure 1, a ~27.0-kb *Eco*RI fragment was observed in DNA isolated from NZB/BlNJ mice using the MMTV *env* and MMTV LTR probes (Figure 1). Although this fragment appeared to segregate in the NXSM RI strain set, under the electrophoretic conditions used in this study we were unable to determine if the SDP was indicative of one or more proviral loci. Because the size of fragments in this region of the gel cannot be accurately determined, this fragment or fragments may, in fact, be larger. Characterization of the *Mtv* proviral locus (loci) represented by this large fragment(s) awaits further study.

Mapping of *Ltrm* loci: An LTR probe, isolated from the long terminal repeat of a mink cell focus-forming (MCF) murine leukemia virus designated CI-3 (C3H, IdUrd induced) (RAPP *et al.* 1983), hybridizes to a number of *Eco*RI fragments present in inbred strains of laboratory mice, including a 5.5-kb *Eco*RI fragment derived from the *Y* chromosome (PHILLIPS *et al.* 1982). We reasoned that this LTR probe might be a potentially useful DNA sequence for identifying restriction fragment length variants among inbred mouse strains. Probing Southern blots containing *Eco*RI digested DNAs derived from NZB/BlNJ and SM/J revealed four fragments that were present in only one strain and sufficiently isolated from other

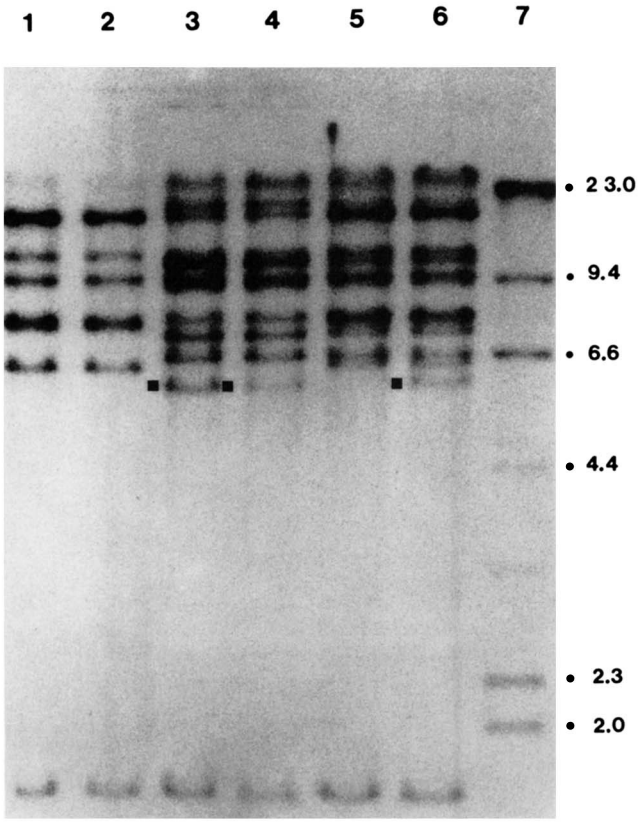

FIGURE 3.—Hybridization pattern of MMTV proviral locus *Mtv-28*. DNAs are: Lane 1, SM/J ♀; Lane 2, SM/J ♂; Lane 3, NZB/BlNJ ♀; Lane 4, NZB/BlNJ ♂; Lane 5, (SM/J ♀ × NZB/BlNJ ♂)F_1 ♂; Lane 6, (NZB/BlNJ ♀ × SM/J ♂)F_1 ♂; and Lane 7, ^{32}P-labeled *Hin*dIII digest of lambda DNA used for molecular size markers (sizes given to right). Autoradiography was for 3 days at −70°. A dot denotes the NZB/BlNJ-derived 5.8-kb *Eco*RI fragment designating the *Mtv-28* locus. Notice that this fragment hybridizes more intensely in DNA derived from a female versus a male NZB/BlNJ and that this fragment is absent in DNA isolated from an F_1 male whose female parent was SM/J but present in the reciprocal F_1 male whose female parent was NZB/BlNJ.

hybridizing fragments to allow their segregation to be followed in the NXSM RI strains or backcrosses (Figure 4). Analysis of the NXSM RI strains indicated the four *Ltrm* loci were unlinked to each other and were located on different autosomes (see Table 3): The *Ltrm-1* locus, characterized by an NZB/BlNJ-derived 2.5-kb *Eco*RI fragment, appeared to be located on Chr *12* between *D12Nyu3* and *Mtv-9*. Confirmation of this chromosomal location was obtained by analyzing the SM backcross mice where only one out of 37 mice inherited a recombinant chromosome between *D12Nyu3* and *Ltrm-1* (2.7 ± 2.7) (see Table 7). The *Ltrm-2* locus, characterized by an SM/J-derived 2.8-kb *Eco*RI fragment, did not co-segregate with the other 57 loci in the NXSM RI strain set. However, our unpublished data indicates that *Ltrm-2* is located on Chr *16* near the *Mpmv-17* locus (FRANKEL *et al.* 1990). According to the NXSM RI SDP the *Ltrm-3* locus, characterized by an SM/J-derived 2.9-kb *Eco*RI fragment, appeared to reside on Chr *5* between the

TABLE 8

Segregation of Chr *1* loci *Cfh*, *Mtv-27* and *Akp-1* in SM backcross

Genes			No. backcross offspring SM	Region of recombination
Cfh	*Mtv-27*	*Akp-1*		
N	N	N	8	None
S	S	S	12	
N	S	S	8	
×				*Cfh–Mtv-27*
S	N	N	7	
N	N	S	2	
×				*Mtv-27–Akp-1*
S	S	N	0	
N	S	N	0	
×				*Chf–Mtv-27–Akp-1*
S	N	S	0	
Total			37	

Percent recombination ± SE = *Cfh*–40.5 ± 8.1–*Mtv-27*–5.4 ± 3.7–*Akp-1*

An N refers to the NZB/BlNJ-derived allele and an S to the SM/J-derived allele.

Emv-1 and *Pgm-1* loci. Confirmation for this chromosomal assignment and position was accomplished by analyzing the NZB backcross mice for the *Emv-1*, *Pgm-1* and *Ltrm-3* loci. The results, presented in Table 9, indicate that the *Ltrm-3* locus is located between these two loci on Chr *5*, with distances being *Emv-1*–13.0 ± 3.2–*Ltrm-3*–14.8 ± 3.4–*Pgm-1*. Finally, the *Ltrm-4* locus, characterized by a 3.7-kb SM/J-derived *Eco*RI fragment, co-segregated in the NXSM RI strain set with the *D13Was70* locus (see also below). Confirmation that these two loci are linked was obtained by analyzing the NZB backcross mice. Of the 106 mice successfully typed for *Ltrm-4* and *D13Was70*, 6 inherited a recombinant chromosome. We conclude that these two loci are closely linked (5.7 ± 2.2) and, as discussed below, we hypothesize that *Ltrm-4* is located proximally on Chr *13*.

Mapping of the *D13Was70* locus: The *D13Was70* locus was originally identified by DISTECHE, GANDY and ADLER (1987) when they were investigating the chromosomal location of sequences that hybridized to probe 70-38 which was isolated from a library derived from the mouse *X* chromosome (DISTECHE *et al.* 1985). Of relevance to the NXSM RI strains is the finding that probe 70-38 hybridized to a number of multicopy fragments on Southern blots containing *Eco*RI digested mouse DNA, including a 6.5-kb *Eco*RI NZB/BlNJ-derived fragment. This fragment was also noted in the SJL/J strain but absent in five other inbred strains (DISTECHE *et al.* 1985). Analysis using hybridization *in situ* on metaphase chromosomes derived from NZB/BlNJ revealed that, in addition to the presence of 70-38 related sequences located near the centromere of the *X* chromosome, 70-38 related

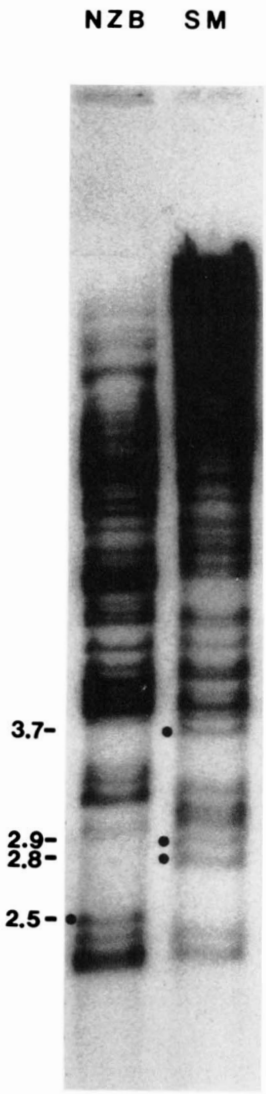

FIGURE 4.—*Ltrm* loci present in the NZB/BlNJ and SM/J strains. Autoradiography was for 24 hr at room temperature. Dots denote the fragments scorable because they were sufficiently separated from other hybridizing fragments. Fragment sizes are indicated on the left.

sequences were also located near the centromere of Chr *13* (other autosomal locations were found in two other inbred mouse strains) (DISTECHE, GANDY and ADLER 1987). When we used the 70-38 probe to search for *Eco*RI fragments that differed between SM/J and NZB/BlNJ, we noted that the 6.5-kb *Eco*RI fragment derived from the NZB/BlNJ strain hybridized in equal intensity to DNA from females and males, suggesting it was autosomal not *X* chromosomal in origin (data not shown). Verification for the autosomal origin of the 6.5-kb *Eco*RI fragment was obtained by comparing the DNA hybridization patterns in F_1 mice produced from reciprocal crosses: (1) mating a NZB/BlNJ female to an SM/J male [(N × SM)-F_1], and (2) mating an SM/J female to a NZB/BlNJ male [(SM × NZB)F_1]. We found that the (NZB × SM)F_1 and (SM × NZB)F_1 males inherited the 6.5-kb *Eco*RI NZB-derived fragment indicating that this fragment cannot be located on the *X* chromosome of the NZB/BlNJ strain but is autosomal (or pseudoautosomal). We suggest that the 6.5-kb *Eco*RI fragment is located at the centromere of Chr *13* in NZB/BlNJ because this fragment is multicopy in nature, as suggested by the intensity of hybridization on Southern blots, and, as previously mentioned, DISTECHE and coworkers (1987) showed that multiple copies of 70-38 sequences were present on Chr *13* of the NZB/BlNJ strain. Genetic confirmation of the Chr *13* assignment of this autosomal fragment, gene designation *D13Was70*, will require detecting linkage of this locus to another locus known to reside on Chr *13*. As noted above, *D13Was70* is closly linked to the *Ltrm-4* locus.

TABLE 9

Segregation of Chr *5* loci *Emv-1*, *Ltrm-3* and *Pgm-1* in NZB backcross

Genes			No. backcross offspring NZB[a]	Region of recombination
Emv-1	*Ltrm-3*	*Pgm-1*		
N	N	N	47	None
S	S	S	34	
N	S	S	6	
×				*Emv-1–Ltrm-3*
S	N	N	5	
N	N	S	5	
	×			*Ltrm-3–Pgm-1*
S	S	N	8	
N	S	N	1	
×	×			*Emv-1–Ltrm-3–Pgm-1*
S	N	S	2	
Total			108	

Percent recombination ± SE = *Emv-1*–13.0 ± 3.2–*Ltrm-3*–14.8 ± 3.4–*Pgm-1*

An N refers to the NZB/BlNJ-derived allele and an S to the SM/J-derived allele.

[a] In the NZB backcross, both F_1 males and females were used to produce backcross offspring. For purposes of this paper, the data are not separated by sex of the F_1 parent.

Determining the order of three loci on Chr *15*: Analysis of the NZB and SM backcross mice allowed us to determine the relative order of the Chr *15* loci *Tgn*, *Ly-6* and *Gdc-1*. This data, presented in Table 10, indicates that the order of these loci is: *Tgn*–6.9 ± 2.1–*Ly-6*–17.4 ± 3.2–*Gdc-1*.

Segregation of *Emv-15*: On average, half of the strains in an RI set should inherit the allele derived from one progenitor strain and the other half should inherit the allele derived from the other progenitor strain unless there is selection for or against one allele. Rare exceptions are found, however, and one of these is the *Emv-15* locus. As noted in Table 3, all 17 NXSM

TABLE 10

Segregation of Chr *15* loci *Tgn*, *Ly-6* and *Gdc-1* in NZB and SM backcross

Genes			No. backcross offspring			Region of recombination
Tgn	*Ly-6*	*Gdc-1*	NZB[a]	SM	Both	
N	N	N	42	16	58	None
S	S	S	39	14	53	
N	S	S	1	2	3	
×						*Tng–Ly-6*
S	N	N	4	2	6	
N	N	S	12	2	14	
	×					*Ly-6–Gdc-1*
S	S	N	9	1	10	
N	S	N	0	0	0	
×		×				*Tng–Ly-6–Gdc-1*
S	N	S	1	0	1	
Total			107	37	144	

Percent recombination ± SE = *Tgn*–6.9 ± 2.1–*Ly-6*–17.4 ± 3.2–*Gdc-1*

An N refers to the NZB/BlNJ-derived allele and an S to the SM/J-derived allele.

[a] In the NZB backcross, both F_1 males and females were used to produce backcross offspring. For purposes of this paper, the data are not separated by sex of the F_1 parent.

RI strains inherited the NZB/BlNJ-derived *Emv-15* allele. Two possibilities may account for this skewing: (1) There is a selective disadvantage for the SM/J-derived *Emv-15* locus or a closely linked gene; or (2) A genetic change occurred in SM/J after the NXSM RI strains were initiated such that the *Emv-15* genotype of the SM/J strain today is not the same as it was in 1974, the year the NXSM RI strains were initiated. If all NXSM RI strains inherited the NZB/BlNJ-derived *Emv-15* locus owing to selective disadvantage for the SM-derived *Emv-15* allele, we might expect to observe such skewing in the SM backcrosses. However, of the 37 SM backcross mice typed for *Emv-15*, no evidence of skewing was evident as 19 inherited the SM/J-derived *Emv-15* allele (were homozygous $Emv\text{-}15^a$) and 18 inherited the NZB/BlNJ-derived *Emv-15* allele (were heterozygous $Emv\text{-}15^a/Emv\text{-}15^b$). A more rigorous test, of course, would be to analyze offspring produced from an F2 cross involving these two strains where the relative proportions of the two homozygotes could be analyzed.

We favor the second possibility for the skewed inheritance of the NZB/BlNJ-derived *Emv-15* allele. In all inbred strains of laboratory mice tested to date, save one, those that carry A^w contain the $Emv\text{-}15^a$ allele and those that carry *a* contain the $Emv\text{-}15^b$ allele (SIRACUSA *et al.* 1987). The exception is the SM/J inbred strain, which, interestingly, is maintained in a forced heterozygous state for A^w and *a* but is homozygous $Emv\text{-}15^a$. As pointed out by SIRACUSA and coworkers, the most likely explanation is that SM/J was originally of the genetic constitution A^w $Emv\text{-}15^a/a$ $Emv\text{-}15^b$. However, prior to 1987, when SM/J strain was typed for the *Emv-15* locus, a crossover occurred between *a* and *Emv-15* and the resultant $a\text{–}Emv\text{-}15^a$ crossover product was fixed. As noted in MATERIALS AND METHODS, we used a SM/J *a/a* male to initiate the NXSM RI strain set. If, in 1974, SM/J was A^w $Emv\text{-}15^a/a$ $Emv\text{-}15^b$, the NXSM RI strains would be homozygous $Emv\text{-}15^b$. Although we cannot prove this suggestion, our finding that all of the NXSM RI strains carry the $Emv\text{-}15^b$ allele is compatible with this idea.

General comments related to linkage assignments and gene order determinations using RI strain data: As noted in the Introduction, RI strains are efficient tools for determining chromosomal assignments and estimating gene order. Occasionally, however, difficulties are encountered that include apparent linkage where none exists and apparent lack of linkage where linkage exists. These difficulties are easily dealt with, however, by confirming suggested linkages using additional RI strain sets, congenic strains, somatic cell hybrid mapping panels or informative backcross mapping panels. Unfortunately, confirmation of suggestive linkages are often not pursued. For an example of apparent linkage when none exists, consider the *Mtv-28* locus. Analysis of the SDP in the NXSM RI strain set suggested that *Mtv-28* is linked to *Emv-15* on Chr *2* as only 3 of the 17 RI strains are discordant, a value indicating these loci are 6 map units apart (95% confidence interval of 1–31 map units (SILVER 1985). Analysis of the SM backcross, however, indicated that *Mtv-28* and *Emv-15* are not linked, as 20 of the 37 mice tested were recombinant. Considering that few laboratories routinely compare Southern blot hybridization patterns using female and male DNA, let alone compare the pattern observed in DNAs isolated from reciprocal F_1 males, the Chr *2* assignment for *Mtv-28* might not have been questioned until conflicting evidence surfaced, *e.g. Mtv-28* appeared linked to an *X*-linked gene or unlinked to Chr *2* loci in another RI strain set.

An example where analysis of the NXSM RI strain set did not provide information as to chromosomal assignment, whereas backcross data did, involves the *Th* locus. Analysis of the SDP of *Th* in the NXSM RI strain set detected no linkage with other loci, but analysis of the NZB backcross clearly indicated that *Th* is located on Chr *7*, gene order *Hbb*–12.0 ± 3.1–*Ly-15*–23.1 ± 4.6–*Th* (BRILLIANT, NIEMANN and EICHER 1987). This is not a surprising result because the *Th* and *Ly-15* loci lie further apart than 10 map units, which is the maximum genetic distance usually detectable with RI strain sets (TAYLOR 1978).

One of the most difficult problems encountered when analyzing RI strain data relates to determining

gene order. As an illustration consider the *Erbb*, *Hba*, and *Evi-2* loci on Chr *11*. If no other information were available, we would conclude that the order of these genes is *Hba–Erbb–Evi-2* because, as noted in Table 3, we obtain 2 "doubles" and 8 "single" recombinants with this order, whereas we obtain 3 "doubles" and 7 "singles" with the order *Erbb–Hba–Evi-2*. However, analysis of the NZB backcross data (not shown) clearly indicates that the order is *Erbb–Hba–Evi-2* because 10 of the 105 mice typed were recombinant between *Erbb* and both *Hba* and *Evi-2*, 19 were recombinant between *Evi-2* and both *Erbb* and *Hba*, and none were recombinant between *Hba* and *Erbb* or *Evi-2*. Clearly, what we considered as "double" recombinants in the NXSM RI strain set are the result of independent single-recombinant events.

Finally, the calculated gene distances using data from RI strains are suggestive, at best. To illustrate this point, consider the Chr 7 data involving *Mod-2s*, *Hbb* and *Ly-15*. If we use the method of SILVER (1985) to determine the map distances (95% confidence interval) for this region of Chr 7, we obtain *Mod-2s*–9.1 (1.9–49.6)–*Hbb*–6.0 (1.0–31.0)–*Ly-15*. However, analysis of the NZB backcross data indicates that the distances between these loci are: *Mod-2s*–3.7 ± 1.6–*Hbb*–12.0 ± 3.1–*Ly-15* (HOGARTH, EICHER and MCKENZIE 1986). What is striking is the fact that the backcross data clearly shows that *Mod-2s* is more closely linked to *Hbb* than *Hbb* is linked to *Ly-15*.

We conclude that although RI strain mapping is often a quick and efficient system for making initial chromosomal assignments, assignments must be viewed as tentative until confirmation is made. Lack of assignments should be pursued using other mapping techniques, such as analysis of congenic strains, backcrosses, segregation in somatic cell hybrids or DNA hybridization to chromosomes *in situ*. In addition, we caution that gene orders and genetic distances deduced from analysis of RI strains be considered as suggestive only.

Final comments: We have presented the genetic profile of 58 loci in 17 RI strains derived from the progenitor strains NZB/BlNRe and SM/J. The availability of this RI strain set should greatly aid in studies directed at understanding the inheritance of SLE. Previous genetic analysis has indicated that the autoimmune disease in NZB mice is not due to simple Mendelian inheritance of a dominant or recessive gene but rather it is inherited as a multifactorial disease. For example, another set of RI strains involving C58/J and NZB/Icr (NX8) has been studied by RIBLET and collaborators for a number of parameters characterizing the SLE disease of NZB mice, including B-cell abnormalities characterized by spontaneous and sheep-RBC-induced production of IgM by spleen cells (DATTA *et al.* 1982), T cell defects characterized by autologous mixed-lymphocyte reaction (BOCCHIERI, RIBLET and SMITH 1981), and production of naturally occurring thymocytotoxic and antierythrocyte antibodies (BOCCHIERI *et al.* 1982). These results indicated that the components of NZB-derived SLE are inherited as separate, unlinked defects and that none of these loci are linked to the immunologically related loci typed in this set of RI strains. Unfortunately, although the NX8 RI strain set originally contained 13 separate strains, only 6 remain, thus hindering further pursuit of the genetic components of SLE. The analysis of the NXSM RI strain set for the various components constituting SLE disease will aid in defining components of SLE that segregate as independent Mendelian loci and provide information as to their chromosomal assignment and position.

In addition, the NXSM RI strain set will complement other RI strain sets in mapping mouse loci. For example, combining the data derived from the NXSM RI strain set with data generated from the two sets of backcross mice has been instrumental in mapping *Ly-6* and *Xmmv-15* to Chr *15* (HOGARTH *et al.* 1987), assigning *Ly-15* and *Th* to the distal region Chr 7 (HOGARTH, EICHER and MCKENZIE 1986; BRILLIANT, NIEMANN and EICHER 1987), positioning *Gh* to a distal location on Chr *11* (ELLIOTT, LEE and EICHER 1990), and positioning the *Tgn* locus proximal to the *Ly-6* locus on Chr *15* (this paper).

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