Recombinant Inbred Strain and Interspecific Backcross Analysis of Molecular Markers Flanking the Murine *agouti* Coat Color Locus

Linda D. Siracusa, Arthur M. Buchberg, Neal G. Copeland and Nancy A. Jenkins

Mammalian Genetics Laboratory, BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, Maryland 21701

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ABSTRACT

Recombinant inbred strain and interspecific backcross mice were used to create a molecular genetic linkage map of the distal portion of mouse chromosome *2*. The orientation and distance of the *Ada*, *Emv-13*, *Emv-15*, *Hck-1*, *Il-1a*, *Pck-1*, *Psp*, *Src-1* and *Svp-1* loci from the *β_2-microglobulin* locus and the *agouti* locus were established. Our mapping results have provided the identification of molecular markers both proximal and distal to the *agouti* locus. The recombinants obtained provide valuable resources for determining the direction of chromosome walking experiments designed to clone sequences at the *agouti* locus. Comparisons between the mouse and human genome maps suggest that the human homolog of the *agouti* locus resides on human chromosome *20q*. Three loci not present on mouse chromosome *2* were also identified and were provisionally named *Psp-2*, *Hck-2* and *Hck-3*. The *Psp-2* locus maps to mouse chromosome *14*. The *Hck-2* locus maps near the centromere of mouse chromosome *4* and may identify the *Lyn* locus. The *Hck-3* locus maps near the distal end of mouse chromosome *4* and may identify the *Lck* locus.

THE *agouti* (*a*) coat color locus on mouse chromosome *2* controls the relative amount and distribution of hair pigments (reviewed by SILVERS 1979; GREEN 1981b). Mutations at the *agouti* locus affect several biological functions including embryonic development, fertility, obesity, and susceptibility to neoplasms (reviewed by SILVERS 1979; GREEN 1981b). Molecular probes for the *agouti* locus would be useful for studying the gene(s) responsible for these varied effects. An ecotropic provirus, *Emv-15*, was previously shown to be associated with the *lethal yellow* (A^y) mutation at the *agouti* locus (COPELAND, JENKINS and LEE 1983). Further investigations demonstrated that the *Emv-15* provirus is closely linked to the *agouti* locus but is not causally related to *agouti* locus phenotypes (SIRACUSA *et al.* 1987a, b). Unique sequence probes flanking the *Emv-15* proviral insertion site may provide a means to clone sequences corresponding to the *agouti* locus (LOVETT *et al.* 1987; SIRACUSA *et al.* 1987a, b). As a first step for cloning, it is important to know (1) the orientation as well as the distance of the *Emv-15* locus from the *agouti* locus, and (2) the orientation and distance of markers on the opposite side of the *agouti* locus. We have concentrated our analyses on molecular markers that are known to map to the *agouti* region, since molecular markers are necessary for chromosome walking experiments designed to clone the locus of interest.

The question whether any known genes are involved in producing *agouti* locus phenotypes may be addressed by determining whether these loci map at or very close to the *agouti* locus. For example, any of the protooncogene loci mapping to mouse chromosome *2* may be involved in the altered susceptibilities to neoplasms exhibited by some *agouti* mutations. Somatic cell hybrid analyses previously showed that the *Abl* protooncogene and the *Src-1* protooncogene were located on mouse chromosome *2* (GOFF *et al.* 1982; SAKAGUCHI *et al.* 1984). *In situ* hybridization studies placed the *Abl* locus at band *2B* (THREADGILL and WOMACK 1988). Since analyses of translocation breakpoints placed the *agouti* locus at band *2H1* (SEARLE *et al.* 1979; reviewed by SEARLE 1981), it is unlikely that the *Abl* locus is involved in *agouti* locus phenotypes. However, the *Src-1* locus appears to map within the vicinity of the *agouti* locus. Previous analysis of the BXD recombinant inbred (RI) strains placed the locus identified by a v-*src* probe (BLATT *et al.* 1984; HARPER *et al.* 1984) 2.2 ± 1.6 cM from the *parotid secretory protein* (*Psp*) gene (HJORTH and NIELSEN 1980), which is close to the *agouti* region (see below). The *Src*-related protein tyrosine kinase gene family currently has eight members: *Fgr*, *Hck*, *Lck*, *Lyn*, *Src*, *Syn/Slk*, *Tkl* and *Yes* (reviewed by HUNTER and COOPER 1985; HANKS, QUINN and HUNTER 1988). One of these loci, *HCK*, maps close to the *SRC-1* locus in humans; the *HCK* locus is at band *20q11-12* (QUINTRELL *et al.* 1987) and the *SRC-1* locus is at band *20q12-13* (SAKAGUCHI, NAYLOR and SHOWS 1983; LE BEAU *et al.* 1984). Furthermore, the *adenosine deaminase* (*ADA*) locus most likely resides at band *20q13.1-13.2* in humans (PHILIP *et al.* 1980; MOHAN-

DAS *et al.* 1984; JHANWAR *et al.* 1987; PETERSEN *et al.* 1987). The *Ada* locus was positioned on mouse chromosome *2* between band *2C1* and the telomere by somatic cell hybrid analysis (SICILIANO, FOURNIER and STALLINGS 1984; LALLEY and DIAZ 1984). Any one of these three loci may reside at or near the *agouti* locus if the *HCK-SRC-1-ADA* linkage in humans is maintained in the mouse.

Crosses involving the *Psp* structural locus and the *agouti* locus have shown that the two loci reside within 3 ± 3 cM (OWERBACH and HJORTH 1980). RI strain analyses of the *Psp* locus (HJORTH and NIELSEN 1980; OWERBACH and HJORTH 1980) and the *Emv-13* locus (JENKINS *et al.* 1981; TAYLOR *et al.* 1985) have shown that the two loci are tightly linked. However, the orientation of the *Psp* and *Emv-13* loci with respect to the *agouti* locus was not established.

Additional loci previously mapped to mouse chromosome *2* and used in our analyses are the *interleukin-1a polypeptide* (*Il-1a*) locus, the cytosolic form of the *phosphoenolpyruvate carboxykinase-1* (*Pck-1*) locus, and the *seminal vesicle protein-1* (*Svp-1*) locus. The *Il-1a* locus was shown to map between the *B2m* and *a* loci by using a cDNA clone to analyze RI strains and inbred strain backcrosses (D'EUSTACHIO *et al.* 1987). The *Pck-1* locus was placed on chromosome *2* by somatic cell hybrid analysis using a rat cDNA probe (LEM and FOURNIER 1985). The *Svp-1* locus was found to be 6.9 ± 1.6 cM from the *agouti* locus by analysis of seminal vesicle protein-1 differences in RI strains (R. S. ESWORTHY, unpublished data), inbred strain backcrosses (PLATZ and WOLFE 1969; MOUTIER and BERTRAND 1983; TAYLOR *et al.* 1985) and interspecific backcrosses (ESWORTHY, GROSS and LALLEY, 1981). However, the orientation of the *Pck-1* and *Svp-1* loci with respect to the *agouti* locus was not established.

We used RI strain and interspecific backcross (IB) mice for linkage studies because each set of crosses provided unique advantages. RI strain data give an estimate of genetic distances within inbred strains (reviewed by TAYLOR 1978; BAILEY 1981). Crossovers fixed in RI strains are the result of recombinations that occurred in either male or female mice during inbreeding. The advantage of using RI strains is that many markers are already typed, thus providing reference points for new loci. In addition, the RI strain resource is unlimited as long as each strain remains viable and fertile. The advantage of using an IB is that the evolutionary distance between the two species (for example, C57BL/6J and *Mus spretus*) has allowed for accumulation of sequence differences (reviewed by AVNER *et al.* 1988); these sequence differences mean a high probability of finding a restriction fragment length polymorphism (RFLP) at any given locus using a molecular marker. Crossovers observed in IB mice are the result of recombinations that occurred in female F_1 mice, since male F_1 mice are sterile (reviewed by BONHOMME *et al.* 1984). The maximum distance between two loci that allows detectable linkage is greater in the IB than in the RI strains when equal numbers of mice are examined (TAYLOR 1978). In addition, the chances of observing rare recombinations may be greater in the IB than in the RI strains, since the number of mice that can be examined is large. Finally, the use of RI strains and IB mice enables comparisons to be made between the mapping data obtained by both methods.

MATERIALS AND METHODS

Mice: The RI strains are maintained at The Jackson Laboratory (Bar Harbor, Maine). The C57BL/6J inbred strain is maintained at the NCI-Frederick Cancer Research Facility. The *M. spretus* mice were at the F_7, F_9, F_{10} or F_{12} generation of inbreeding and were a gift from E. M. EICHER [The Jackson Laboratory (Bar Harbor, Maine)]. The [(C57BL/6J × *M. spretus*)F_1 × C57BL/6J] IB and the C57BL/6J-*a*/*a* × C57BL/6J-A^y/*a* backcross (or the reciprocal) were performed at the NCI-Frederick Cancer Research Facility.

Probes: The pADA5-29 probe for the *adenosine deaminase* (*Ada*) gene is a full length 1.5-kb mouse cDNA cloned in pBR322 (YEUNG *et al.* 1985); the pADA5-29 probe was a gift from R. E. KELLEMS [Baylor College of Medicine (Houston, Texas)]. The g2B2mdIIIB probe for the *β_2-microglobulin* (*B2m*) gene is a 1.6-kb *Hin*dIII-*Bam*HI fragment cloned in pGemini II that contains exons II and III (PARNES and SEIDMAN 1982); the g2B2mdIIIB probe was a gift from T. V. RAJAN [Albert Einstein College of Medicine (Bronx, New York)]. The pEmv-13 *Sst*I probe for the *Emv-13* locus, the site of integration of the *Akv-3* (*Emv-13*) provirus, is a 1.15-kb *Sst*I construct of genomic DNA located both 5′ and 3′ to the *Emv-13* viral integration site subcloned in pBR325 (COPELAND *et al.* 1984). The p15.4 probe for the *Emv-15* locus is a 1.1-kb *Eco*RI genomic fragment located 3′ to the *Emv-15* viral insertion site (SIRACUSA *et al.* 1987a). The pHK24 probe for the *hematopoietic cell kinase-1* (*Hck-1*) gene is a 1.95-kb human cDNA cloned in a pUC vector (ZIEGLER *et al.* 1987); the pHK24 probe was a gift from R. M. PERLMUTTER [Howard Hughes Medical Institute (Seattle, Washington)]. The pIL1 1301 probe for the *interleukin-1 α-polypeptide* (*Il-1a*) gene is a 2.0-kb mouse cDNA cloned in pBR322 (LOMEDICO *et al.* 1984); the pIL1 1301 probe was a gift from H. YOUNG [National Cancer Institute (Frederick, Maryland)]. The pPCK10 probe for the *phosphoenolpyruvate carboxykinase-1* (*Pck-1*) gene is a 2.6-kb rat cytosolic cDNA clone (YOO-WARREN *et al.* 1983); the pPCK10 probe was a gift from R. W. HANSON [Case Western Reserve University (Cleveland, Ohio)]. The HhaI-Psp probe for the *parotid secretory protein* (*Psp*) gene is a *Hha*I fragment that starts in exon II and covers the CAP site cloned in pSP6 (SHAW and SCHIBLER 1986); the HhaI-Psp probe was a gift from P. H. SHAW [Institute of Pathology (Lausanne, Switzerland)]. The pN1.8 probe for the *Src-1* protooncogene is a 1.8-kb mouse brain c-*src* cDNA cloned in pUC18 (MARTINEZ *et al.* 1987); the pN1.8 probe was a gift from R. MARTINEZ and D. BALTIMORE [Whitehead Institute for Biomedical Research (Cambridge, Massachusetts)]. The pSV-008 probe for the *seminal vesicle protein-1* (*Svp-1*) gene is a 0.5-kb mouse Svp-1 cDNA clone (ESWORTHY, GROSS and LALLEY 1981); the

TABLE 1

Segregation of alleles mapping to mouse chromosome 2 in the BXH RI strains[a]

Locus	RE	Fragment sizes[b]		BXH RI strains												
		B	H	2	3	4	5	6	7	8	9	10	11	12	14	19
B2m	*Bgl*I	8.6, 1.4	9.8	B	B	B ×	H	B ×	B	H	B	H	B	H	H	B
Il-1a	*Xba*I	2.3, 1.7	2.5, 1.7, 0.8	B	B ×	H ×	H ×	H ×	B	H	B ×	H	B	H	H	B
Emv-13	*Xba*I	5.5, 5.2	8.3, 5.0	B	H	B	B	B ×	B	H	H	H ×	B	H	H	B
Psp	*Xba*I	7.2, 6.8, 4.7, 1.9	7.2, 6.8, 1.9, 1.1	B	H	B	B	H	B	H	H	B	B	H	H	B
a				B	H	B	B	H	B	H	H	B	B	H	H	B
Emv-15	*Hin*dIII	5.0	2.4	B	H	B	B	H	B	H	H	B	B	H	H	B
Src-1	*Hin*dIII	~12, 5.0	9.3, 5.0	B ×	H	B	B	H	B	H	H	B	B	H	H	B
Svp-1	*Msp*I	5.3	4.6	H	H	B	B	H	B	H	H	B	B	H	H	B

[a] The BXH RI strains were typed as "B" if they exhibited the C57BL/6J allele or "H" if they exhibited the C3H/HeJ allele. The "×" denotes a crossover. The "RE" is the restriction endonuclease used to detect the RFLP. The typing for BXH-5 was obtained by examination of two outcrossed mice produced from a cross of [(C57BL/6J × BXH-5)F_1 × BXH-5]. The SDPs for *B2m*, *Il-1a* and *Svp-1* agree with those previously found (CHORNEY *et al.* 1982; D'EUSTACHIO *et al.* 1987; R. S. ESWORTHY, unpublished data). The SDPs for *a* and *Emv-15* were previously determined (MARTIN *et al.* 1984; LOVETT *et al.* 1987; SIRACUSA *et al.* 1987b).

[b] The fragment sizes are listed in kilobases.

pSV-008 probe was a gift from R. S. ESWORTHY [City of Hope National Medical Center (Duarte, California)] and K. W. GROSS [Roswell Park Memorial Institute (Buffalo, New York)].

Southern blot analyses: High molecular weight genomic DNA was extracted from mouse spleen, liver or kidney as described (JENKINS *et al.* 1982). Preparation of DNA from mouse tails, conditions for restriction endonuclease digestions, and Southern blot analyses were as described (SIRACUSA *et al.* 1987a) with the exceptions listed. The membrane used for Southern blot analyses was Zetabind (Cuno, Inc.). Blots were stripped by washing in 0.1 M NaOH, 0.1X SSC, 0.1% SDS at 65° for 30 min, followed by two rinses in distilled-deionized water and equilibration in 4X SSCP, 1% SDS.

Statistical analyses: Recombination frequencies for the RI strains were calculated as described (SILVER 1985). Recombination frequencies for the IB data were calculated as described (GREEN 1981a) using the computer program SPRETUS MADNESS developed by D. DAVE [Data Management Services, Inc. (Frederick, Maryland)] and A. M. BUCHBERG. A maximum likelihood estimate for the weighted averages on linkage data between the RI strain and IB data was calculated using an algorithm as established by B. A. TAYLOR [The Jackson Laboratory (Bar Harbor, Maine)].

RESULTS

Initial screen: RFLPs among the BXH and CX8 RI strain progenitors or among the parents of the IB were detected by Southern blot analyses. The genomic DNAs tested for RFLPs were BALB/cWtEi, C58/J, C3H/HeJ, C57BL/6J and *M. spretus*. The restriction endonucleases used were *Bam*HI, *Bgl*I, *Eco*RI, *Hin*dIII, *Kpn*I, *Msp*I, *Pst*I, *Taq*I and *Xba*I. The RFLPs used for mapping are listed in Tables 1, 2 and 4. The *agouti* locus was typed by observation of coat color. The loci used as anchors for mouse chromosome 2 were the *B2m* locus and the *a* locus. The *B2m* locus and the *a* locus are believed to reside 46 cM and 62 cM distal to the centromere, respectively (DAVISSON *et al.* 1988).

RI strain analysis: The BXH and CX8 RI strains were chosen for most of the RI strain analyses because both RI strains segregated for *agouti* alleles. In addition, previous results indicated that a crossover occurred between the *a* locus and the *Emv-15* locus in the CX8-I RI strain (SIRACUSA *et al.* 1987b). Mapping of loci on either side of the *a* and *Emv-15* loci should establish the orientation of the *Emv-15* locus with respect to the *a* locus.

The results of the initial screen showed that seven of the ten probes detected RFLPs between the progenitors of the BXH RI strains, and seven of the ten probes detected RFLPs between the progenitors of the CX8 RI strains. The CXB, LXPL and NX129 RI strains were also included in the analysis, but only to obtain additional mapping data for the *Psp* and *Emv-13* loci. These three sets of RI strains had previously been typed for segregation of *a* and *Emv-15* alleles (LOVETT *et al.* 1987; SIRACUSA *et al.* 1987b).

Tables 1 and 2 show the RFLPs and the strain distribution patterns (SDPs) in the BXH and CX8 RI strains, respectively. Table 3 shows the ordering of the loci and the recombination distance between each pair of loci based on the combined data from RI strain analyses; Figure 1A shows the orientation and distance of the loci examined on mouse chromosome 2. The most proximal locus mapped is the *B2m* locus, followed by the *Il-1a* locus, which is consistent with previous reports (D'EUSTACHIO *et al.* 1987). The most

TABLE 2

Segregation of alleles mapping to mouse chromosome *2* in the CX8 RI strains[a]

Locus	RE	Fragment sizes[b]		CX8 RI strains							
		C	8	B	C	D	G	I	M	N	LT/Sv[c]
Il-1a	*Msp*I	7.6, 6.8	6.6, 6.2	8	8 ×	C ×	C	8	C	8	8
Hck-1	*Eco*RI[d]	~25	~22	8	C	8	C	8	C	8	8
Emv-13	*Xba*I	8.3, 5.0	5.5, 5.2	8	C	8	C	8	C	8	8
Psp	*Xba*I[e]	7.2, 1.1	~15	8	C	8	C	8	C	8	8
a				8	C	8	C	8 ×	C	8	8
Emv-15	*Hin*dIII	2.4	5.0	8	C	8	C	C	C	8	8
Src-1	*Xba*I	~16	~14	8	C ×	8	C	C ×	C	8	8
Svp-1	*Msp*I	5.3	4.6	8	8	8	C	8	C	8	8

[a] The CX8 RI strains were typed as "8" if they exhibited the C58/J allele or "C" if they exhibited the BALB/cWtEi allele. The "×" denotes a crossover. The "RE" is the restriction endonuclease used to detect the RFLP. The SDPs for *a* and *Emv-15* were previously published (SIRACUSA *et al.* 1987b).

[b] The fragment sizes are listed in kilobases.

[c] The LT/Sv strain was included because it is derived from the C58 strain outcrossed to the BALB/c strain prior to inbreeding (STAATS 1980).

[d] Several common bands were detected (data not shown).

[e] Several common bands were detected as well as two additional bands that did not map to mouse chromosome *2* (data not shown).

TABLE 3

Summation of RI strain analyses for loci mapping to mouse chromosome *2*[a]

Locus	BXH	CX8	CXB	LXPL	NX129	AKXD	Totals	$\hat{r}$	95% confidence limits
B2m	2/13	—	0/7[b]				2/20	2.94	0.31–15.11
Il-1a	5/13	2/8	3/7[c]			5/25[c]	15/53[d]	12.29	5.61–29.03
Hck-1	—	0/8					0/8	0.00	0.00–20.71
Emv-13	2/13	0/8	0/7	0/5	0/6	0/27[e]	2/66	0.79	0.09–3.12
Psp	0/13	0/8	0/7	0/5	0/6		0/39	0.00	0.00–2.61
a	0/13	1/8	0/7	0/5	0/6		1/48[f]	0.54	0.01–3.32
Emv-15	0/13	0/8					0/21	0.00	0.00–5.31
Src-1	1/13	2/8					3/21	4.55	0.80–19.97
Svp-1									

[a] The data are listed as the number of recombinants over the total number of RI strains analyzed. The "—" indicates that no RFLP was found between the progenitors of these RI strains. The "$\hat{r}$" represents an estimate of the percent recombination in a single meiosis. The "$\hat{r}$" and 95% confidence limits were calculated as described (SILVER 1985). Although *Hck-1* shows no recombination with *a*, *Emv-13*, *Emv-15* and *Psp* in the CX8 RI strains, *Hck-1* has been placed proximal to *Emv-13* based on the IB data (Figure 2). Although *Emv-15* shows no recombination with *Src-1* in the BXH and CX8 RI strains, *Emv-15* has been placed proximal to *Src-1* based on the IB data (Figure 2). The CXB, LXPL, and NX129 RI SDPs for *Emv-13* and *Psp* are the same as those previously published for *a* and *Emv-15* (LOVETT *et al.* 1987; SIRACUSA *et al.* 1987b).

[b] The CXB RI SDP for *B2m* was previously published (MICHAELSON 1983).

[c] The CXB and AKXD RI SDPs for *Il-1a* were previously published (D'EUSTACHIO *et al.* 1987).

[d] This number represents the recombinants found between *Il-1a* and *Emv-13*.

[e] The 27 AKXD RI strains were previously typed for *Emv-13* and *Psp* (JENKINS *et al.* 1981; TAYLOR *et al.* 1985; B. A. TAYLOR, personal communication); previous analysis had shown that one strain, AKXD-13, was recombinant between *Emv-13* and *Psp*. However, Southern blot analysis using the pEmv-13 *Sst*I probe showed that the AKXD-13 RI strain is not recombinant between *Emv-13* and *Psp*.

[f] This total includes two BXJ RI strains and seven SWXL RI strains previously published (SIRACUSA *et al.* 1987b).

distal locus mapped is the *Svp-1* locus. The results show that four loci are <9 cM (upper 95% confidence limit) from the *a* locus (Table 3). No crossovers were detected between the *Hck-1* and *Emv-13* loci, the *Psp* and *a* loci, and the *Emv-15* and *Src-1* loci.

IB analysis: The results of the initial screen showed

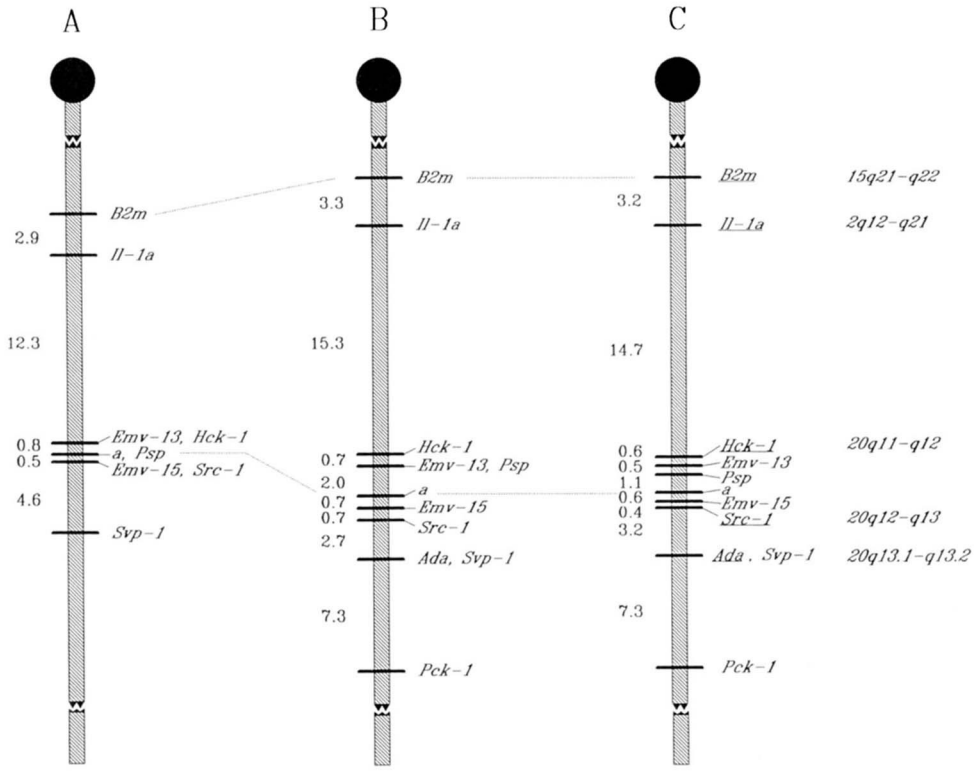

FIGURE 1.—A molecular genetic linkage map of the distal portion of mouse chromosome 2. The loci mapped are listed to the right of each chromosome. The recombination distances (cM) are listed to the left of each chromosome. A, Map obtained using the data from the RI strains. B, Map obtained using the data from the IB. Figure 1C is the map obtained using a weighted average of the data from the RI strains and the IB. Loci that have been mapped in humans are underlined. Listed to the right of Figure 1C is the chromosomal location of homologous loci mapped in humans [*B2M*: FABER *et al.* (1976); GOODFELLOW *et al.* (1975); SHEER *et al.* (1983); *IL-1a*: MODI *et al.* (1988); *HCK-1*: QUINTRELL *et al.* (1987); *SRC-1*: SAKAGUCHI, NAYLOR and SHOWS (1983); LE BEAU *et al.* (1984); *ADA*: PHILIP *et al.* (1980); MOHANDAS *et al.* (1984); JHANWAR *et al.* (1987); PETERSEN *et al.* (1987)].

TABLE 4

Loci abbreviations and names, probes, and RFLPs used for IB mapping

Locus	Name	Probe[a]	RE[b]	Fragment size(s)[c]	
				C57BL/6J	*Mus spretus*
Ada	*Adenosine deaminase*	pADA 5-29	*Bgl*I	6.8, 5.2, 4.4, 3.5	8.2, 5.9, 4.1, 2.6
B2m	*β_2-Microglobulin*	g2B2mdIIIB	*Bgl*I	8.6, 1.4	~19
Emv-13	*Integration site of Akv-3 provirus*	pEmv-13 SstI	*Taq*I	8.6	4.1
Emv-15	*Integration site of Emv-15 provirus*	p15.4	*Kpn*I	8.3, 7.5	~12, 7.5
Hck-1	*Hematopoietic cell kinase-1*	pHK24	*Hin*dIII[d]	9.5, 3.9	3.6
Il-1a	*Interleukin-1α polypeptide*	pIL 1301	*Pst*I	8.5	7.7
Pck-1	*Cytosolic form of phosphoenolpyruvate carboxykinase-1*	pPCK-1	*Bgl*I	6.3, 5.0, 4.5, 1.9	5.9, 4.2
Psp	*Parotid secretory protein*	HhaI-Psp	*Taq*I	6.9, 5.8, 2.1, 0.5	8.1, 6.6, 3.4, 0.5
Src-1	*Src-1 protooncogene*	pN1.8	*Bgl*I	5.8, 2.1, 1.9, 1.6, 0.6	5.8, 4.1, 1.9, 0.6
Svp-1	*Seminal vesicle protein-1*	pSV-008	*Pst*I	3.7	6.6
Additional loci detected:					
Psp-2	*Parotid secretory protein-2*	HhaI-Psp	*Taq*I		4.7
Hck-2	*Hematopoietic cell kinase-2*	pHK24	*Kpn*I[e]		~14, 4.6
Hck-3	*Hematopoietic cell kinase-3*	pHK24	*Hin*dIII		5.0

[a] The references for each of the probes are listed in MATERIALS AND METHODS.
[b] RE is the restriction endonuclease used to detect each RFLP.
[c] The fragment sizes are listed in kilobases. The restriction fragments followed in the IB are underlined.
[d] Several common bands (~16, 6.2, 5.9, 2.4, 2.2, and 1.0 kb) were also observed.
[e] Only those restriction fragments identifying the *Hck-2* locus are listed.

that all of the probes exhibited RFLPs between the parents of the IB, C57BL/6J and *M. spretus*. The RFLPs used for mapping are shown in Table 4. The segregation of restriction fragments present in *M. spretus* was followed in 150 N2 progeny (Figure 2). Gene order was confirmed by the maximum likelihood analysis (BISHOP 1985). The results establish the order and recombination distance (±SE) of the markers examined as: *B2m*—3.3 ± 1.5 cM—*Il-1a*—15.3 ± 2.9 cM—*Hck-1*—0.7 ± 0.7 cM—[*Emv-13*, *Psp*]—2.0 ± 1.1 cM—*a*—0.7 ± 0.7 cM—*Emv-15*—0.7 ± 0.7 cM—*Src-1*–2.7 ± 1.3 cM—[*Ada*, *Svp-1*]—7.3 ±

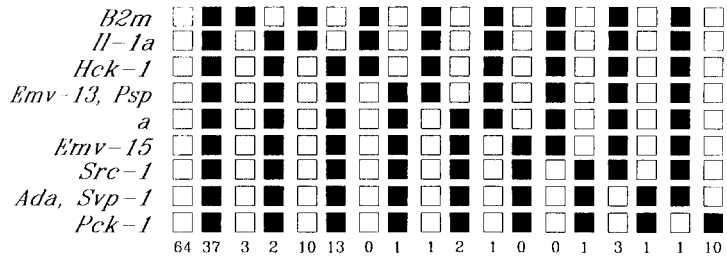

FIGURE 2.—Pedigree analysis of the N2 progeny from the interspecific backcross. The loci followed in the IB are listed on the left. Each column represents the chromosome identified in the N2 progeny that was inherited from the (C57BL/6J × *M. spretus*) F_1 parent. The open squares represent the *M. spretus* allele. The black squares represent the C57BL/6J allele. The number of N2 progeny carrying each type of chromosome is listed at the bottom. The *B2m* results for 120 N2 progeny were previously reported (BIRKENMEIER, MCFARLAND-STARR and BARKER, 1988).

TABLE 5

***G* test[a] analysis of allelic segregation in the IB**

Locus	C57BL/6J B/B	*Mus spretus* S/B	*G* value	*P* value
B2m	56	94	9.73	<0.005
Il-1a	55	95	10.80	<0.005
Hck-1	58	92	7.77	<0.010
Emv-13, Psp	59	91	6.88	<0.010
a	60	90	6.04	<0.025
Emv-15	59	91	6.88	<0.010
Src-1	60	90	6.04	<0.025
Ada, Svp-1	58	92	7.77	<0.010
Pck-1	67	83	1.71	<0.250

[a] The *G*-test was performed as described (SOKAL and ROHLF 1981).

2.1 cM—*Pck-1* (Figure 1B). The IB results did not allow determination of the order of the *Emv-13* and *Psp* loci, nor of the *Ada* and *Svp-1* loci.

The Poisson distribution was used to obtain the number of non-, single, double, and triple recombinant chromosomes expected from the IB data. The expected numbers were 108 non-, 35 single, 6 double, and 1 triple recombinant chromosomes. The observed numbers were 101 non-, 49 single, 0 double, and 0 triple recombinant chromosomes. A significant difference between the expected and observed numbers of chromosomes was found by χ^2 analysis ($\chi^2 = 13.05$, $P < 0.005$). Therefore, there appears to be some interference of multiple crossovers in this region of chromosome 2. This observation is consistent with the suggestion that chiasmata may not be randomly distributed along the entire length of mouse chromosome 2 (LYON 1976).

Composite data: Chi-square analyses showed that there are no significant differences between the RI strain and IB data; in addition, the 95% confidence intervals for both sets of data overlap. Therefore, the data from both sets of crosses were combined to establish the weighted averages of the recombination distances (±SE) as: *B2m*—3.2 ± 1.2 cM—*Il-1a*—15.3 ± 2.6 cM—*Emv-13*—0.5 ± 0.4 cM—*Psp*—1.1 ± 0.6 cM—*a*—0.6 ± 0.4 cM—*Emv-15*—0.4 ± 0.4 cM—*Src-1*—3.2 ± 1.2 cM—[*Ada, Svp-1*]—7.3 ± 2.1 cM—*Pck-1* (Figure 1C). In addition, the weighted average of the recombination distance (±SE) between the *Hck-1* and *Emv-13* loci is 0.6 ± 0.6 cM (Table 3 and Figure 2). The data from both the RI strain and IB analyses establish the order of all the loci examined except the *Ada* and *Svp-1* loci.

Transmission ratio distortion in the IB: The *G*-statistic (SOKAL and ROHLF 1981) was used to determine whether the transmission of alleles at each locus in the IB differed significantly from the 1:1 ratio expected if each allele was transmitted in a normal Mendelian fashion. The analysis showed significant differences ($P < 0.05$) from a normal Mendelian segregation for all loci examined, with the exception of *Pck-1*, the most distal marker examined (Table 5). Results using probes from the proximal half of chromosome 2 (BIRKENMEIER, MCFARLAND-STARR and BARKER 1988; L. D. SIRACUSA, C. M. SILAN, M. J. JUSTICE, S. YANG, N. G. COPELAND and N. A. JENKINS, unpublished data) indicate that the transmission ratio distortion extends to loci mapping ~20 cM proximal to the *B2m* locus.

Additional loci detected by the probes used for mapping: Two of the probes (HhaI-Psp and pHK24) used for mapping detected additional loci not present on mouse chromosome 2. The second locus detected by the HhaI-Psp probe was provisionally named *Psp-2*, the second locus detected by the pHK24 probe was provisionally named *Hck-2*, and the third locus detected by the pHK24 probe was provisionally named *Hck-3* (Tables 4 and 6).

The *Psp-2* locus was detected in both the RI strains and the IB. However, the fragments for the *Psp-2* locus were lighter in intensity relative to the fragments for the *Psp* structural locus. Since the HhaI-Psp probe starts in exon II and covers the CAP site, the *Psp-2* locus may be (1) a parotid secretory protein-related gene, (2) a parotid secretory pseudogene, or (3) a gene or sequence that has strong homology to the noncoding regions present in the HhaI-Psp probe. The BXH and CXB RI SDPs (Table 4) are the same as those found for the *purine nucleotide phosphorylase* (*Np-2*) gene, the *pancreatic ribonuclease* (*Rib-1*) gene, and the *T-cell receptor α-chain* (*Tcra*) gene on chromosome *14* (DEMBIC *et al.* 1985; ELLIOTT *et al.* 1986; B. A. TAYLOR, personal communication). The IB results confirmed that the *Psp-2* locus is tightly linked to the *Rib-1* and *Tcra* loci on chromosome *14* (J. D. CECI, L. D. SIRACUSA, N. A. JENKINS and N. G. COPELAND, unpublished data).

The members of the *Src*-related tyrosine kinase

TABLE 6

RI SDPs and RFLPs of loci detected on chromosomes other than mouse chromosome 2[a]

Locus	RE	Fragment sizes[b]		BXH RI strains													Chromosome
		B	H	2	3	4	5	6	7	8	9	10	11	12	14	19	
Psp-2	*Xba*I	3.0	3.5	H	B	H	H	B	B	H	B	B	B	B	H	H	*14*
Hck-3	*Xba*I	3.0	3.2	B	B	H	B	H	H	B	H	B	H	B	H	H	*4*

Locus	RE	Fragment sizes		CXB RI strains							Chromosome
		C	B	D	E	G	H	I	J	K	
Psp-2	*Xba*I	3.5	3.0	C	C	B	B	C	C	C	*14*

[a] The BXH RI strains were typed as "B" if they exhibited the C57BL/6J allele or "H" if they exhibited the C3H/HeJ allele. The CXB RI strains were typed as "B" if they exhibited the C57BL/6JNBy allele or "C" if they exhibited the BALB/cAnNBy allele. The "RE" is the restriction endonuclease used to detect the RFLP. The HhaI-Psp probe also detected a second locus in the other RI strains examined (data not shown).

[b] The fragment sizes are listed in kilobases. Only those restriction fragments identifying the SDPs listed are shown.

gene family are closely related in DNA sequence as well as protein function (reviewed by HUNTER and COOPER 1985; HANKS, QUINN and HUNTER 1988). Therefore, it is not unexpected that a cDNA probe for one member can detect other members as well. The IB results show that the *Hck-2* locus is closely linked to the *Mos* locus near the centromere of chromosome *4* and may identify the *Lyn* locus (PROPST *et al.* 1989; J. D. CECI, L. D. SIRACUSA, N. A. JENKINS and N. G. COPELAND, unpublished data). The BXH RI SDP (Table 5) of *Hck-3* is the same as that of *Lck* on chromosome *4* (B. A. TAYLOR, personal communication). In addition, no recombinants between the *Hck-3* locus and the *Lck* locus have been found in the IB (J. D. CECI, L. D. SIRACUSA, N. A. JENKINS and N. G. COPELAND, unpublished data). Therefore, the *Hck-3* locus may identify the *Lck* locus. These findings are not unexpected, since *Hck* was originally isolated by probing cDNA libraries with either an *Lck* probe (ZIEGLER *et al.* 1987) or a v-*src* probe (QUINTRELL *et al.* 1987).

DISCUSSION

The mapping results from the RI strains and the IB have provided an unambiguous orientation of the markers examined on mouse chromosome *2* (Figure 1). The fact that no contradictions were found in the placement or distance of the markers examined in the RI strains and the IB indicates that no large chromosomal rearrangements have occurred in this region of chromosome *2* between *M. spretus* and the C57BL/6J inbred strain. Several loci could not be mapped using only the RI strains due to the lack of RFLPs among RI strain progenitors. However, this difficulty was overcome by using the IB approach; more than 200 molecular probes have been examined in our laboratory and every one has detected RFLPs between the parents of the IB, C57BL/6J and *M. spretus* (MUCENSKI *et al.* 1988; BUCHBERG *et al.* 1988, 1989; A. M. BUCHBERG, J. D. CECI, D. J. GILBERT, D. M. KINGSLEY, M. J. JUSTICE, L. LOCK, C. M. SILAN, L. D. SIRACUSA, S. SPENCE, M. C. STROBEL, S. YANG, N. A. JENKINS and N. G. COPELAND, unpublished data). The map obtained (Figure 1C) is in agreement with the existing inbred strain map for mouse chromosome *2* (DAVISSON *et al.* 1988).

Potential sources of transmission ratio distortion in interspecific crosses: Transmission ratio distortion in interspecific crosses has been noted previously (BIDDLE 1987). However, the transmission ratio distortion was observed in N2 progeny from a cross of (C3H/HeHa × *M. spretus*)F_1 × *M. spretus* mice and not observed when the F_1 hybrid females were backcrossed to the inbred strain (C3H/HeHa) parent; a deficiency of N2 males carrying the intact C3H/HeHa *X* chromosome and the *M. spretus Y* chromosome was found (BIDDLE 1987). In contrast, our studies show transmission ratio distortion of autosomes in N2 progeny from a cross of (C57BL/6J × *M. spretus*)F_1 × C57BL/6J mice. Transmission ratio distortions have been noted not only for chromosome *2*, but for chromosomes *4* (J. D. CECI, N. A. JENKINS and N. G. COPELAND, unpublished data) and *10* (M. J. JUSTICE, N. G. COPELAND and N. A. JENKINS, unpublished data) as well. Several investigators have also observed transmission ratio distortions for various autosomes in their IBs involving *M. spretus* mice (J.-L. GUENET, personal communication; D. A. STEPHENSON and V. M. CHAPMAN, personal communication); the source of the *M. spretus* mice used in the various crosses is not necessarily the same. Therefore, the transmission ratio distortion appears to be a common feature of IBs involving *M. spretus* mice and does not appear to be limited to the IB performed in our laboratory. However, transmission ratio distortion is the exception and has not been observed for all autosomes in our IB

(MUCENSKI *et al.* 1988; BUCHBERG *et al.* 1988, 1989; A. M. BUCHBERG, J. D. CECI, D. J. GILBERT, D. KINGSLEY, M. J. JUSTICE, L. LOCK, C. M. SILAN, L. D. SIRACUSA, N. A. JENKINS and N. G. COPELAND, unpublished data).

The transmission ratio distortion of *M. spretus* alleles over those of C57BL/6J in the IB may have several sources. First, the uterine environment of the F_1 hybrid females may be hostile to embryos homozygous for certain C57BL/6J alleles. Second, the tramsmission ratio distortion may be a reflection of the effect(s) of various alleles themselves; the effect(s) would have to occur between meiosis in F_1 hybrid females and birth of their progeny, since comparison of the number of mice born to the number of mice weaned shows <4% loss of offspring between birth and weaning. For example, the transmission ratio distortion may be due to (1) differential oocyte survival, (2) different fertilization efficiencies, or (3) differential survival of heterozygous embyros. Isolation and polymerase chain reaction (SAIKI *et al.* 1985) analysis of individual N2 embryos prior to implantation could potentially distinguish between the first and second alternatives, and would help to further define the timing of this phenomenon. Finally, the transmission ratio distortion may be due to genomic imprinting (reviewed by CATTANACH 1986). The question whether the transmission ratio distortion is due to transmission from F1 females as opposed to F1 males cannot be tested in the IB, since F1 males are sterile (reviewed by BONHOMME *et al.* 1984).

Human-mouse homologies: The mapping of common loci in mice and humans provides insight into conservation of homologous regions and is useful for identifying mouse models of human diseases. Several loci in the distal portion of mouse chromosome *2* have been mapped in humans. Figure 1C shows that mouse chromosome *2* contains loci found on human chromosomes *2*, *15* and *20*. The maintenance of the *Hck-1-Src-1-Ada* linkage in both mice and humans enables predictions to be made about the location of certain mouse loci in the human genome. Since the *Hck-1* locus is proximal to the *a* locus and the *Src-1* locus is distal to the *a* locus, we predict that the potential human homolog of the *a* locus resides on human chromosome *20q11-13*. However, there is no evidence for a locus in humans that can produce altered hair pigmentation patterns similar to those produced in the mouse by the *agouti* locus (reviewed by SEARLE 1968). In addition, our results suggest that if human homologs exist for the regions of the mouse genome identified by the probes for the *Emv-13*, *Psp*, and *Emv-15* loci, then these regions may reside on human chromosome *20q11-13* as well. It is interesting to note that abnormalities of human chromosome *20* have been found in some patients with hematological disorders. Specifically, deletions of chromosome *20q* have been observed in patients with acute nonlymphocytic leukemia, myelodysplastic syndrome, and myeloproliferative disorders (REEVES, LOBB and LAWLER 1972; TESTA *et al.* 1978; DAVIS *et al.* 1984; reviewed by HEIM and MITELMAN 1987). It has been speculated that the *HCK* locus (QUINTRELL *et al.* 1987) or the *SRC-1* locus (LE BEAU *et al.* 1984, 1985) may be involved in the progression of these diseases. However, the presence of additional loci in the homologous region in mice leaves open the possibility that as yet unidentified gene(s) may be contributing to these malignancies.

Recombination frequency between the A^y-mutation and the *Emv-15* locus: Conventional backcross progeny from a cross of C57BL/6J-*a Emv-15^b*/*a Emv-15^b* and C57BL/6J-A^y *Emv-15^v*/*a Emv-15^b* mice (or the reciprocal) were previously analyzed to estimate more precisely the genetic distance between the *a* locus and the *Emv-15* locus (SIRACUSA *et al.* 1987b). The results of 1222 progeny analyzed showed no progeny with recombination between the *a* locus and the *Emv-15* locus (SIRACUSA *et al.* 1987b). A total of 1457 progeny have now been analyzed and still no recombinants have been found. The number of offspring from A^y/*a* females was 156; the number of offspring from A^y/*a* males was 1301. The absence of recombinant progeny in 1457 mice indicates that the *Emv-15* locus is located less than 0.2 cM (upper 95% confidence limit) distal to the *a* locus. Figure 3 shows an expanded version of the *agouti* region, with A^y proximal to *A*, as previously published (SIRACUSA *et al.* 1987b).

There is no significant difference between the recombination distance of the *a* and *Emv-15* loci obtained from the RI strains and the IB compared to the recombination distance obtained from the cross of C57BL/6J-*a Emv-15^b*/*a Emv-15^b* and C57BL/6J-A^y *Emv-15^v*/*a Emv-15^b* mice (or the reciprocal), since the 95% confidence intervals overlap. However, the probability of the values is on the borderline of significance and analysis of additional backcross mice could easily shift the values below the $P = 0.05$ level. Several explanations exist for the absence of recombinant progeny from the cross of C57BL/6J-*a Emv-15^b*/*a Emv-15^b* and C57BL/6J-A^y *Emv-15^v*/*a Emv-15^b* mice (or the reciprocal) in comparison to the two recombinants found in the RI strains and the IB reported in this study. First, an alteration in the C57BL/6J strain may inhibit recombination within this region of chromosome *2*. This alteration would have to be limited to the C57BL/6J-A^y chromosome, since higher recombination frequencies are observed when the C57BL/6J-*a* chromosome is involved (as in the RI strains and the IB). Second, since A^y/*a* males were predominantly used to generate progeny for this cross, there may be some male-specific factor(s) that result in recombina-

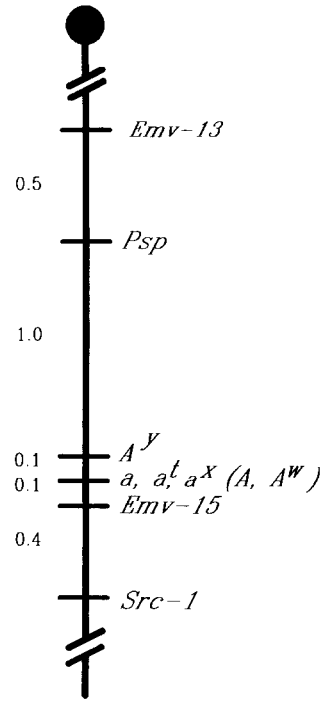

FIGURE 3.—A high resolution map of the agouti region on mouse chromosome 2. The recombination distances (cM) are listed to the left of the chromosome. The recombination distances between the *Emv-13* locus and the *Psp* locus, the *Psp* locus and the *a* locus, and the *Emv-15* locus and the *Src-1* locus are the weighted averages of the data from the RI strains and the IB. The recombination distance between A^y and *a*, a^t, a^x, *A* and A^w was previously published (SIRACUSA *et al.* 1987b). The recombination distance between the *a* locus and the *Emv-15* locus is a weighted average of the data from the RI strains, the IB, and the cross of C57BL/6J-*a Emv-15*^{b}/*a Emv-15*^{b} and C57BL/6J-A^y *Emv-15*^{v}/*a Emv-15*^{b} mice (or the reciprocal).

tion frequencies lower than those found when A^y/a females are used. In general, recombination percentages are slightly, but not significantly, lower in males than in females for most regions of the mouse genome (summarized by DAVISSON and RODERICK 1981; NADEAU and TAYLOR 1984). Third, the *Emv-15* provirus may be inhibiting recombination in the surrounding region. This explanation seems unlikely since higher recombination frequencies were observed in the stocks carrying A^y and the *Emv-15* provirus at the Oak Ridge National Laboratories (SIRACUSA *et al.* 1987b), as well as by the fact that potential recombinations were found between A^y and the *Emv-15* provirus in the YS and YBR strains (SIRACUSA *et al.* 1987a). Fourth, A^y-may have an effect on recombination frequencies, resulting in decreased recombination of nearby markers compared to recombination frequencies found with wild-type chromosomes. Some evidence for this possibility is seen in previous mapping experiments with the *brachypodism* (*bp*) mutation, which also lies distal to the *a* locus; crosses of A^y +/+ $bp^J \times +$ $bp^J/+$ bp^J mice (or the reciprocal) and crosses of A^y $bp^J/+ + \times +$ $bp^J/+bp^J$ mice (RUNNER 1959) gave recombination frequencies roughly tenfold lower than crosses of *a* $bp^H/+ + \times$ *a* bp^H/a bp^H mice (ANDREWS and PETERS 1983). This difference is similar to the differences seen in our crosses. Finally, the genetic distance between the *a* locus and the *Emv-15* locus may be closer to the lower end of the 95% confidence interval and the number of progeny examined may have been too small to detect a rare recombination in the C57BL/6J-*a Emv-15*^{b}/*a Emv-15*^{b} × C57BL/6J-A^y *Emv-15*^{v}/*a Emv-15*^{b} cross. Analyses of additional backcross mice are needed to distinguish among these possibilities.

Our mapping of molecular markers has provided the distance and orientation of several chromosome 2 loci relative to the *a* locus (Figure 3). The results demonstrate that the *Psp* locus is the next proximal locus to the *a* locus and that the *Emv-15* locus is the next distal locus to the *a* locus. The *Psp* locus is 1.1 ± 0.6 cM and the *Emv-15* locus is 0.1 ± 0.1 cM (see legend to Figure 3) from the *a* locus. These findings enable the use of molecular markers both proximal and distal in chromosome walks designed to recover sequences from the *agouti* locus. The recombinants obtained from our mapping studies are valuable resources for determining the direction of the chromosome walking experiments. As the chromosome walks expand from the flanking markers, new probes can be isolated and mapped with respect to the recombination breakpoints identified between the *Psp*, *a*, and *Emv-15* loci. The finding that a recombination breakpoint has been crossed will indicate that we have moved closer to the *a* locus and will identify the molecular direction to be taken for the remainder of the chromosome walk.

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