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Linkage of loci associated with two pigment mutations on mouse chromosome 13

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Summary

Progeny from one intra- and two inter-specific backcrosses between divergent strains of mice were typed to map multiple markers in relation to two pigment mutations on mouse chromosome 13, beige (*bg*) and pearl (*pe*). Both recessive mutants on a C57BL/6J background were crossed separately with laboratory strain PAC (*M. domesticus*) and the partially inbred *M. musculus* stock PWK. The intra- and inter-specific F_1 hybrids were backcrossed to the C57BL/6J parental strain and DNA was prepared from progeny. Restriction fragment length polymorphisms were used to follow the segregation of alleles in the backcross offspring at loci identified with molecular probes. The linkage analysis defines the association between the *bg* and *pe* loci and the loci for the T-cell receptor γ-chain gene (*Tcrg*), the spermatocyte specific histone gene (*Hist1*), the prolactin gene (*Prl*), the Friend murine leukaemia virus integration site 1 (*Fim-1*), the murine Hanukuh Factor gene (*Muhf/Ctla-3*) and the dihydrofolate reductase gene (*Dhfr*). This data confirms results of prior chromosomal mapping studies utilizing *bg* as an anchor locus, and provides previously unreported information defining the localization of the prolactin gene on mouse chromosome 13. The relationship of multiple loci in relation to *pe* is similarly defined. These results may help facilitate localization of the genes responsible for two human syndromes homologous with *bg* and *pe*, Chediak–Higashi syndrome and Hermansky–Pudlak syndrome.

1. Introduction

Intra- and inter-specific backcrosses among genetically divergent species of *Mus* provide a powerful tool for the analysis of genetic linkage (Roberts *et al.* 1985; Avner *et al.* 1988; Seldin *et al.* 1989). It appears that there are not large differences in overall chromosomal organization despite the very high frequency of restriction fragment length polymorphisms (RFLPs) between different strains (Stephenson *et al.* 1988; Mullins *et al.* 1988), though small potential rearrangements of segments of chromosomes 4 and 17 have been proposed (Nadeau *et al.* 1986; Hammer *et al.* 1988). Furthermore, the inclusion of mouse mutants of unknown aetiology has facilitated the identification of specific genes involved in the phenotypic defect (Chabot *et al.* 1988; Ryder-Cook *et al.* 1988). In this study, one intra-specific backcross and two inter-specific backcrosses are utilized to define linkage in relation to two pigment mutations on chromosome 13, beige (*bg*) and pearl (*pe*). The locations of several genes known to be linked to *bg*, the T-cell receptor gamma chain (*Tcrg*), spermatocyte-specific histone (*Hist1*), and the Friend murine leukaemia proviral integration site 1 (*Fim-1*) are further defined (Holcombe *et al.* 1987; Owen *et al.* 1986; Justice *et al.* 1990), and the specific chromosomal location of the prolactin gene (*Prl*) (Jackson-Grusby *et al.* 1988) is elucidated. The location of murine dihydrofolate reductase (*Dhfr*) (Killary *et al.* 1986), and the murine homologue of Hanukuh Factor (*Muhf*) (Gerschenfeld & Weissman, 1986) with respect to the pigment mutation pearl (*pe*) is described. The inclusion of the pigment loci provides a bridge between the molecular map and the historical linkage map of mouse chromosome 13. The data confirms results of a prior mapping study which utilized *bg* and *sa* as anchor loci (Justice *et al.* 1990), and provides novel information regarding the map locations with respect to *pe*. The results of this study confirm regions of homology

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between mouse chromosome 13 and human chromosomes 5, 6 and 7. Additionally, the murine mapping data provides information which may facilitate genetic mapping of two human diseases homologous to beige and pearl, Chediak–Higashi syndrome and Hermansky–Pudlak syndrome (Windhorst & Padgett, 1973; Roder & Duwe, 1979; Novak *et al.* 1985).

2. Materials and methods

(i) *Crosses*

Mice from a laboratory mouse stock, homozygous for the recessive coat colour mutation beige (*bg*) on chromosome 13, and the sash mutation at the dominant spotting locus (W^{sh}) on chromosome 5, were crossed to the wild-derived inbred *M. domesticus* stock PAC. The F_1 hybrids were crossed with the C57BL/6J.*bg* congenic strain to produce intra-specific backcross offspring that were informative at both pigment loci. The same laboratory stock (homozygous for the sash and beige genes) was crossed to the partially inbred *M. musculus* stock PWK derived from wild trappings in Eastern Europe. The F_1 hybrids were then crossed to the same beige congenic strain to establish inter-specific backcross offspring. A second inter-specific backcross was established to follow segregation of alleles at the pearl (*pe*) locus, a recessive coat colour mutation mapping to the distal end of mouse chromosome 13. In this cross, the *M. musculus* stock (PWK) was crossed to a C57BL/6J.*pe* congenic strain and the F_1 hybrid backcrossed to the same *pe* congenic strain. The progeny consisted of 115 mice from crosses of (*bg* × PAC)F_1 to C57BL/6J.*bg*, 286 mice from crosses of (*bg* × PWK)F_1 to C57BL/6J.*bg*, and 56 mice from crosses of (*pe* × PWK)F_1 to C57BL/6J.*pe*. Segregation of the pigment genes was scored at dissection. The sash heterozygote has a distinctive broad white band across the back (Lyon & Glenister, 1982), and therefore scoring the segregation of alleles in the backcross offspring was unambiguous. Both beige and pearl reduce the pigment intensity of the eumelanin (black pigment), diluting the coat coloration to grey. Segregation of alleles at the agouti (*A*, *a*) locus has a subtle effect on the phenotype by virtue of the presence or absence of phaeomelanin (yellow pigment) but this does not preclude accurate scoring of the recessive *bg* or *pe* phenotypes.

(ii) *Southern analysis*

High molecular weight DNA from the kidneys of each mouse was prepared according to standard procedures as described previously (Mullins *et al.* 1988). Restriction endonucleases were obtained from either New England Biolabs or Bethesda Research Laboratories and used according to the manufacturer's recommendations. Fragments were resolved by electrophoresis of the DNA through a 0·8 or 1% agarose gel, denatured, and transferred to nitrocellulose or nylon (Genescreen, DuPont) as described previously (Holcombe *et al.* 1987; Mullins *et al.* 1990). Hybridization with ^{32}P probes labelled by nick-translation or random priming was performed overnight at 42 °C in a 10% dextran/formamide hybridization solution, followed by two washes at room temperature in 2x SSC/0·1% SDS, and two washes at 55 °C in 0·2x SSC/0·1% SDS. Bands were visualized by autoradiography against Kodak X-Omat AR film.

(iii) *Molecular probes and biochemical analysis*

The *Tcrg* probe is a 2·1 kb genomic fragment comprised of constant region exons II and III of the murine T-cell receptor γ-chain gene (supplied by Dr Ilonna Rimm). The *Prl* probe is a murine-derived cDNA probe (Linzer *et al.* 1985), the *Fim-1* probe is a 1·5 kb genomic *Pvu* II fragment (Sola *et al.* 1986), the *Dhfr* probe is a 0·6 kb cDNA fragment (supplied by Dr David Wilson), and the *Muhf* probe is a cDNA probe homologous to human CTLA-3 (Gerschenfeld *et al.* 1988). Biochemical analysis of testes specific histones were performed as previously described (Zweidler, 1984). Testes were isolated from male *bg* backcross offspring and homogenized before Triton-electrophoresis and determination of protein polymorphism pattern. Some of the males in the PWK crosses were sacrificed before the appearance of spermatocyte specific histones and therefore could not be typed.

(iv) *Statistical analysis*

Standard error for recombination fractions was calculated according to the formula $[(r)(1-r)/N]^{\frac{1}{2}}$ where r = recombination fraction; N = total number tested. Chi-squared analysis was performed for each group of loci in a 2 × 2 table comparing observed frequencies against 1:1 segregation. To support analysis of distances between loci based upon recombination fractions, distances and confidence intervals were also determined based on the maximum LOD score and LOD score distributions calculated separately for each set of recombination events between two loci, $\theta = 0{\cdot}50$, according to the following equation:

$$F(x) = \log_{10}[(x)^R(1-x)^{N-R}/(0{\cdot}50)^N],$$

where R = number of recombinants; N = total number tested; x = recombination fraction (Lathrop *et al.* 1984).

3. Results

(i) *Genetic polymorphism or variation between parental stocks*

Allele-specific restriction fragment length polymorphisms (RFLPs) for the molecularly defined loci

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Table 1. *Restriction fragment length polymorphisms for PAC and PWK vs. c57BL/6J*

	Tcrg		*Prl*		*Fim1*		*Dhfr*		*Muhf*	
Enzyme	A	W	A	W	A	W	A	W	A	W
*Eco*R V	+	+(*)	−	+(*)	−	−	−	−	−	−
Bgl II	ND	ND	−	+	−	−	−	−	−	+(*)
Xba I	ND	ND	ND	ND	ND	ND	−	+(*)	−	−
*Eco*R I	ND	ND	−	−	−	−	−	+	−	−
*Bam*H I	ND	ND	−	−	−	+(*)	−	−	−	−
Hind III	ND	ND	−	−	−	−	−	+	−	+
Pst I	ND	ND	−	+	−	−	ND	ND	ND	ND
Hinc II	ND	ND	ND	ND	ND	ND	−	−	ND	ND
Tag I	ND	ND	ND	ND	ND	ND	−	+	−	−
Rsa I	ND	ND	ND	ND	ND	ND	−	−	−	−
Pvu II	ND	ND	ND	ND	ND	ND	−	−	−	−
*Bst*E II	ND	ND	ND	ND	ND	ND	−	−	−	+

A, PAC; W, PWK; +, polymorphism detected *vs.* C57BL/6J; −, no polymorphism detected *vs.* C57BL/6J; ND, not determined; (*), polymorphism utilized in this study.

(*Tcrg*, *Prl*, *Fim-1*, *Dhfr*, *Muhf*) were ascertained by digesting parental DNAs with a series of restriction endonucleases (Table 1). Variation between C57BL/6J and PAC was identified for *Tcrg* only whereas C57BL/6J and PWK were variant for all five genes studied. Moreover, C57BL/6J and PWK differed from each other with 3/6 enzymes for *Prl*, 1/6 for *Fim-1*, 4/11 for *Dhfr*, and 3/10 for *Muhf*. It is possible that other restriction endonucleases identified the same deviations in these different genes, but even if that were the case, C57BL/6J and PAC differed for only one of the molecularly defined genes whereas PWK and C57BL/6J were variant for all five. These findings are consistent with the expected higher degree of variation between *Mus* species than within *Mus* species (Avner *et al.* 1988).

Fig. 1(*a–e*) depicts the restriction endonucleases and specific polymorphisms used in this study to follow segregation of alleles at the *Tcrg*, *Prl*, *Fim-1*, *Dhfr*, and *Muhf* loci in the PWK backcrosses and also the *Tcrg* polymorphism in the PAC parent. The restriction endonuclease *Eco*R V defines a polymorphism for *Tcrg* in both the PAC and PWK stocks. *Eco*R V was also used to follow the segregation of alleles at the *Prl* locus, though other restriction endonucleases were equally informative. Segregation of alleles for *Fim-1*, *Dhfr*, and *Muhf* were determined by digestion with restriction endonucleases *Bam*H I, *Xba* I, and *Bgl* II respectively. Also shown in Fig. 1 is the protein polymorphism in the testis-specific histone (*Hist1*) used to follow the segregation of alleles at this locus in the male offspring from the [C57BL/6J.*bg* × PWK]F_1 × C57BL/6J.*bg* backcross. No protein polymorphism was detected in the testis-specific histone between the inbred strain C57BL/6J and the PAC stock.

(ii) *Gene order*

A total of 43 backcross offspring (18 females and 25 males) from the PWK cross segregating alleles at the *bg* locus were simultaneously characterized at all the molecularly defined loci. An additional 25 offspring were studied at the loci in close proximity to *bg*. This represents a sample size sufficient to establish the linkage of loci mapping an estimated 30 cM apart at the 99% probability level. The data obtained in this analysis, that minimizes the number of multiple crossover events, is presented in Tables 2 and 3. Male and female data are presented separately in order to incorporate the analysis of the segregation of alleles at the *Hist1* locus. The segregation of alleles of 68 backcross offspring were determined for four loci closely linked to *bg*. Since 61 of the 68 offspring showed coincident segregation at the *bg*, *Tcrg*, *Prl*, and *Fim-1* loci ($\chi^2 = 25{\cdot}5$ assuming independent segregation), their linkages are more than adequately defined. Similarly, the linkage and relative position of the *Hist1* locus is also well defined given the observation that it cosegregates with both its nearest proximal and distal markers 30 out of 31 times ($\chi^2 = 17{\cdot}0$ assuming independent segregation). To further confirm the location of *Hist1* relative to *Tcrg* and *Prl*, 15 additional male backcross offspring were typed for each of these loci. There is coincident segregation in 43 of the 46 mice ($\chi^2 = 21{\cdot}5$, data not shown). Any gene order other than the one presented in Table 2 would require at least one double recombinational event, an unlikely occurrence over the postulated map distance of less than 9 centiMorgans (cM).

Analysis of the segregation of alleles at the loci for *Dhfr*, *Muhf*, *Tcrg*, and *bg* shows no evidence for a

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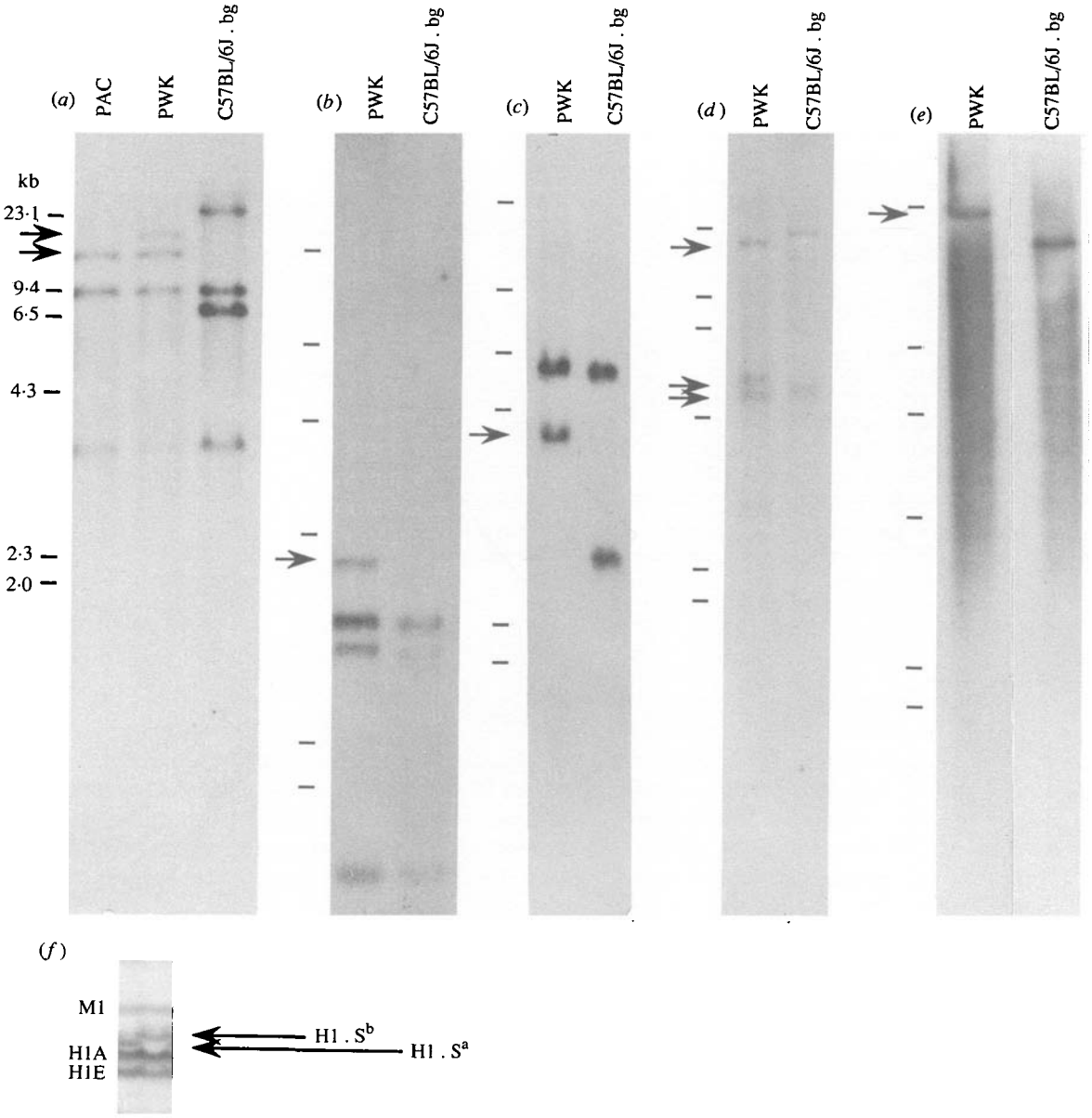

Fig. 1. DNA restriction fragment length polymorphisms and *Hist1* protein polymorphism between parental C57BL/6J.*bg*, PAC (*Mus domesticus*), and PWK (*Mus musculus*). Arrows signify bands present in PAC or PWK not present in homozygous C57BL/6J.*bg*. Lambda phage cut with *Hind* III was used as a molecular weight marker. (*a*) *Eco*R V digestion; *Tcrg* constant region probe. (*b*) *Bgl* II digestion; *Muhf* probe. (*c*) *Eco*R V digestion; *Prl* probe. (*d*) *Xba* I digestion; murine *Dhfr* probe. (*e*) *Bam*H I digestion; *Fim-1* probe. (*f*) Resolution of allelic forms a and b of *Hist1* by Triton-electrophoresis for the C57BL/6J.*bg* parent and PWK parent. Entire H1 region is shown.

distortion in the segregation ratios, though there are relatively small numbers of parental classes compared with the recombinant classes (Table 3). With a frequency of recombination between *Tcrg* and *Dhfr* or *Muhf* of greater than 50%, these two loci are behaving as though they are unlinked to other loci associated with *bg* (although the recombination frequency of *Dhfr* to *Fim-1* is less than 50%). The *Dhfr* and *Muhf* loci are clearly linked to one another, however, with only 5 recombinants out of 43 backcross offspring. It is likely that *Dhfr* is centromeric to *Muhf* (and hence closer to *bg*) based on the minimizing of double recombinational events. Should the order be reversed (i.e. *Tcrg–Muhf–Dhfr*), the number of double recombinational events would rise from 0 to 5. This number of double recombinants among 43 backcross offspring cannot be entirely precluded given the distance between loci, but the empirical order is that which minimizes the number of these events (i.e. *Tcrg–Dhfr–Muhf*).

As alleles at the loci for *Dfhr* and *Muhf* appear to be segregating independently of the *bg* locus, an additional backcross, involving the *M. musculus* stock (PWK), was established segregating alleles at the *pe* locus. Indirect linkage analysis has established that the *pe* locus maps an estimated 40 cM distal to the *bg*

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Table 2. *Empirical gene order of loci associated with the pigment mutation* bg *on mouse chromosome 13 determined by following the segregation of alleles in the* M. musculus *(PWK) backcross*

	bg	*Tcrg*	*Hist1*	*Prl*	*Fim1*	Females	Males	Total
Parental	B	B	B	B	B	17	12	29
	W	W	W	W	W	17	15	32
Single	W ×	B	B	B	B	0	1	1
	B ×	W	W	W	W	1	1	2
	W	W ×	B	B	B	−*	0	0
	B	B ×	W	W	W	−*	1	1
	W	W	W ×	B	B	0	0	0
	B	B	B ×	W	W	1	0	1
	W	W	W	W ×	B	0	0	0
	B	B	B	B ×	W	1	1	2
				Grand totals		37	31	68

B, *bg* phenotype/genotype; W, PWK (wild type) phenotype/genotype; *not possible to test for *Hist1* protein polymorphism in females.

Table 3. *Empirical gene order of loci associated with the pigment mutation* bg *on mouse chromosome 13 determined by following the segregation of alleles in the* M. musculus *(PWK) backcross mice analysed with probes for* Tcrg, Dhfr *and* Muhf

	bg	*Tcrg*	*Dhfr*	*Muhf*	Females	Males	Total
Parental	B	B	B	B	4	2	6
	W	W	W	W	2	3	5
Single	W ×	B	B	B	0	1	1
	B ×	W	W	W	2	1	3
	W	W ×	B	B	5	5	10
	B	B ×	W	W	4	11	15
	W	W	W ×	B	3	0	3
	B	B	B ×	W	0	2	2
			Grand totals		18	25	43

B, *bg* phenotype/genotype; W, PWK (wild type) phenotype/genotype.

Table 4. *Empirical gene order of loci associated with the pigment mutation* pe *on mouse chromosome 13 determined by following the segregation of alleles in the* M. musculus *(PWK) backcross*

	Tcrg	*Dhfr*	*pe*	*Muhf*	Total
Parental	P	P	P	P	9
	W	W	W	W	7
Single	W ×	P	P	P	7
	P ×	W	W	W	5
	W	W ×	P	P	0
	P	P ×	W	W	2
	W	W	W ×	P	1
	P	P	P ×	W	2
Double	P ×	W ×	P	P	1
			Grand total		34

P, *pe* phenotype/genotype; W, PWK (wild type) phenotype/genotype.

locus on mouse chromosome 13 (Davisson & Roderick, 1981) providing a potential anchor for the linkage analysis of the *Dhfr* and *Muhf* loci. The same restriction endonucleases were used to follow the segregation of alleles at the molecularly defined loci, since in principle the same parental stocks were used. A total of 34 backcross offspring (Table 4) were characterized for the segregation of alleles at four loci (*Tcrg*, *Dhfr*, *pe*, *Muhf*). As in the previous analysis, linkage between the *Tcrg* locus and the *Dhfr* locus cannot be distinguished from independent segregation ($\chi^2 = 1{\cdot}882$) and the two sets of data are consistent with one another ($\chi^2 = 1{\cdot}403$). However, the segregation of alleles at the *Dhfr* locus were coincident with the *pe* locus in 32 of the 34 individuals typed for both loci. Similarly, allelic segregation at the *Muhf* locus was also coincident with the *pe* locus at a similar frequency (31 of 34 offspring), a pattern of segregation that deviates significantly from independence

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Table 5. *LOD scores for linked markers, all crosses*

	bg	*Tcrg*	*Hist1*	*Prl*	*Fim1*	*Dhfr*	*pe*	*Muhf*
bg	—	52·8	17·5	18·0	11·8			
Tcrg		—	9·7	37·4	14·8			
Hist1			—	10·8	6·4			
Prl				—	19·2			
Fim1					—	0·1*		
Dhfr						—	5·6	9·8
pe							—	11·9
Muhf								—

* LOD scores $\geqslant +3$ are required to define statistical linkage.

($\chi^2 = 23{\cdot}059$). The gene order *Dhfr–pe–Muhf* is defined by minimizing the number of multiple crossover events. As in the previous situation, the relative position of the *Tcrg* locus cannot be deduced on the basis of the recombination frequency. If the order were reversed (i.e. *Tcrg–Muhf–pe–Dhfr*), the number of double recombinant events would rise from 1 to 4 (a combined total of 9 including the PWK–*bg* cross). From an empirical perspective, therefore, the most likely order is as described in Table 4 (i.e. *Tcrg–Dhfr–pe–Muhf*).

To confirm postulated distances and order, individual LOD score distributions were calculated for each set of markers, utilizing data from all crosses. Analysis of the recombination frequencies at the maximum LOD scores supports the empirical order as determined above through minimization of multiple crossover events. LOD scores ranged from +5·6 to +52·8 for linked markers (Table 5). The most distal marker linked to *bg* (*Fim1*) is not statistically linked to the most proximal marker linked to *pe* (*Dhfr*), though the LOD score calculated for *Fim1* and *Dhfr* (+0·1) is the highest among non-linked marker pairs.

(iii) *Recombination frequencies*

The recombination fractions between successive loci for the three backcrosses are presented in Table 6. This table includes data from additional backcross offspring that were not exhaustively typed for all the loci. Linkage of the *bg* locus and the *Tcrg* locus includes an additional 41 offspring from the PWK backcross plus the linkage information derived from 111 typable offspring produced by the PAC backcross. Both backcrosses yielded essentially the same information ($\chi^2 = 0{\cdot}001$, with Yate's correction) making it possible to combine the two sets of data for a more definitive estimate of recombination. The recombinant fraction from the PAC backcross for the two loci is based upon the progeny from the reciprocal crosses F_1♀ × C57BL/6J.*bg*♂ and C57BL/6J.*bg*♀ × F_1♂. The frequency of recombinations does not differ with the sex of the F_1 hybrid (2/56 for the F_1♂ and 2/55 for the F_1♀). Similarly, the linkage of the *Hist1* locus and the *bg* locus is based upon the analysis of backcross offspring from reciprocal crosses. No difference in the 10 recombinants in the 92 backcross offspring from the F_1 females was observed when compared with 7 recombinants in the 36 backcross offspring from F_1

Table 6. *Combined recombinant fractions between successive loci from the three sets of backcross offspring*

		bg	*Tcrg*	*Hist1*	*Prl*	*Fim1*	*Dhfr*	*pe*	*Muhf*
bg	1♀		1/61		2/57	3/40	10/20		16/26
	1♂		2/48	17/128	6/46	6/40	21/27		21/28
	2								
	3		4/111						
Tcrg	1♀	3·2 ± 1·2*			1/57	2/40	9/20		15/34
	1♂			3/48	4/46	4/40	19/27		19/28
	2				0/55		13/34	22/55	25/55
Hist1	1♂		6·3 ± 3·5*		1/46	2/32	16/27		17/28
Prl	1♀			2·2 ± 2·1*		1/39	9/18		14/24
	1♂					1/38	13/25		16/27
	2						12/31	19/52	24/52
Fim1	1♀				2·6 ± 1·8*		2/10		5/13
	1♂						9/16		10/18
Dhfr	1♀					42·3 ± 9·7			3/20
	1♂								2/25
	2							3/35	6/35
pe	2						8·6 ± 4·7*		3/56
Muhf								5·4 ± 3·0*	

1 = (W^{sh}/W^{sh} *bg/bg* × PWK)F_1 × C57BL/6J.*bg* backcross.
2 = (*pe/pe* × PWK)F_1 × C57BL/6J.*pe* backcross.
3 = (W^{sh}/W^{sh} *bg/bg* × PAC)F_1 × C57BL/6J.*bg* backcross.
Each fraction is the number of mice recombinant for a pair of markers/total number of mice typed, expressed × 10^{-2} ± standard error. χ^2 probabilities that the observed deviations from 1:1 segregation were due to chance fluctuation: * = $P < 0{\cdot}001$.

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males ($\chi^2 = 1{\cdot}652$). Statistically significant linkage at $P < 0{\cdot}001$ is present between *bg*, *Tcrg*, *Hist1*, *Prl*, and *Fim-1*, and between *pe*, *Dhfr*, and *Muhf*. Recombinational distances in centiMorgans are based on recombination frequency between successive loci.

4. Discussion

(i) *Comparison with prior genetic mapping studies*

The mapping results from the intra- and inter-specific backcrosses described here orient six markers relative to the anchor loci *bg* and *pe* at the proximal and distal ends of mouse chromosome 13. The map distance between *bg* and *Tcrg* in this study ($3{\cdot}2 \pm 1{\cdot}2$ cM) is consistent with previous data from an intra-specific cross utilizing C57BL/6J and C3H strains ($2{\cdot}5 \pm 1{\cdot}4$ cM, Holcombe *et al.* 1987) and a C57BL/6J × *M. spretus* inter-specific backcross ($3{\cdot}1 \pm 2{\cdot}2$ cM, Justice *et al.* 1990). The distance between *Tcrg* and *Hist1* ($6{\cdot}3 \pm 3{\cdot}5$ cM) is also consistent with previous data (Holcombe *et al.* 1987; Owen *et al.* 1986). In this study, an analysis of the segregation of alleles at the *Hist1* locus was performed in the *bg* inter-specific backcross. The map distance between *bg* and *Hist1* ($13{\cdot}3 \pm 3{\cdot}0$) is based on 128 backcross offspring, and an order of *Tcrg–Hist1–Prl* is indicated. *Prl* was previously assigned to mouse chromosome 13 by somatic cell hybrid analysis (Jackson-Grusby *et al.* 1988) and is one of a cluster of related genes including the placental lactogens and proliferin. Our linkage analysis confirms this assignment and further defines the position of *Prl* to the proximal portion of the chromosome, closely associated with the *bg* locus.

There is disagreement in the literature regarding the map distance between *Tcrg* and *Fim-1*. Sola *et al.* (1988) reported this distance at $14{\cdot}5 \pm 5{\cdot}0$ cM based on analysis of 48 animals, while Justice *et al.* (1990) mapped *Fim-1* within $3{\cdot}3 \pm 1{\cdot}3$ cM of *Tcrg* based on analysis of 181 animals. Our data places *Fim-1* $7{\cdot}5 \pm 2{\cdot}9$ cM from *Tcrg* based on analysis of 80 backcross offspring. Our study utilized a T-cell receptor γ-chain constant region probe to score allele-specific RFLPs, as did Justice *et al.* (1990). Though a C57BL/6J stock was used as the mutant strain in all three studies, the outbred parental strains (*M. spretus*, *M. musculus*) were different and it is possible that there has been a chromosomal rearrangement in one or more of these strains. The frequency of recombination between *Tcrg* and *Fim-1* observed by Justice *et al.* (1990) is significantly reduced compared with the present study and the earlier linkage data of Sola *et al.* (1988) ($\chi^2 = 5{\cdot}592$, $P < 0{\cdot}05$; $\chi^2 = 8{\cdot}997$, $P < 0{\cdot}01$ respectively). An order that combines the three maps can be described as follows: *bg–(Tcrg/Inhba/Ral/Rrm2-ps3)–Hist1–Prl–Fim-1–sa–(Lamb-1.13/D13Pas2)–(Dhfr/Hilda)–Hexb–pe–Muhf* or *Ctla-3*.

The use of *pe* in our analysis provides the means for defining the linkage of those loci that map to the distal portion of chromosome 13 that appear to be unlinked to the proximal marker *bg*. *Dhfr* and *Muhf* have been previously mapped to mouse chromosome 13 (Killary *et al.* 1986; Sola *et al.* 1988), consistent with our inter-specific backcross map location. The empirical order of genes as determined in this study is also consistent with the report by Justice *et al.* (1990). We have shown that the two molecular loci flank the *pe* locus, with *Dhfr* $8{\cdot}6 \pm 4{\cdot}7$ cM proximal to *pe*, and *Muhf* $5{\cdot}4 \pm 3{\cdot}0$ cM distal to *pe*. The calculated map distance between *Dhfr* and *Muhf*, based on analysis of 80 mice from the *pe* and *bg* inter-specific backcrosses is $13{\cdot}8 \pm 3{\cdot}9$ cM.

(ii) *Comparative mapping between mouse and human genomes*

The *Tcrg* locus has been mapped previously to human chromosome 7p15 (Murre *et al.* 1985). Though *Tcrg* is only 3 cM distal to *bg* in the mouse genome, this linkage is not maintained in humans, based on analysis of RFLP patterns in five families with Chediak–Higashi syndrome (CHS) (Holcombe *et al.* 1987). The human H1 complex appears to map to multiple chromosomes, specifically chromosomes 1, 6 and 12 (Tripputi *et al.* 1986), though Chandler *et al.* (1979) has localized genes encoding histone proteins at 7q22. Murine *Prl* is one of a family of genes including the placental lactogens and proliferin clustered in a small region of chromosome 13 (Jackson-Grusby *et al.* 1988). *Prl* is located on human chromosome 6 (Owerbach *et al.* 1981), while the placental lactogens have been mapped to human chromosome 17 (Owerbach *et al.* 1980; George *et al.* 1981; Harper *et al.* 1982). If the linkage between *Prl* and *Hist1* demonstrated in the mouse genome by inter-specific backcross analysis is conserved, it is possible that the murine *Hist1* complex is equivalent to the H1 complex that maps to human chromosome 6. Alternatively, if the linkage between *Tcrg* and *Hist1* is conserved, the human homologue of *Hist1* may be located at 7q22. This latter scenario is less likely given the large distance between 7p15 (the location of *Tcrg*) and 7q22. The *Fim-1* locus maps to human chromosome 6p23 (Van Cong *et al.* 1989). A potential region of homology between mouse and human therefore, would include the genes for *Hist1*, *Prl*, and *Fim-1*, all of which map within approximately 5 cM on mouse chromosome 13 by the analysis presented here, and all of which exhibit homologous human loci on human chromosome 6.

The region adjacent to *bg* appears to have undergone extensive rearrangement during evolution (Fig. 2). The location of the gene responsible for Chediak–Higashi syndrome (Barak & Nir, 1987) is unknown. The human *Tcrg* locus is not linked to CHS and maps to human chromosome 7, and a cluster of genes distal to *Tcrg* on mouse chromosome 13 define a potential region of conserved linkage with human chromosome 6. Eventual identification of markers centromeric to

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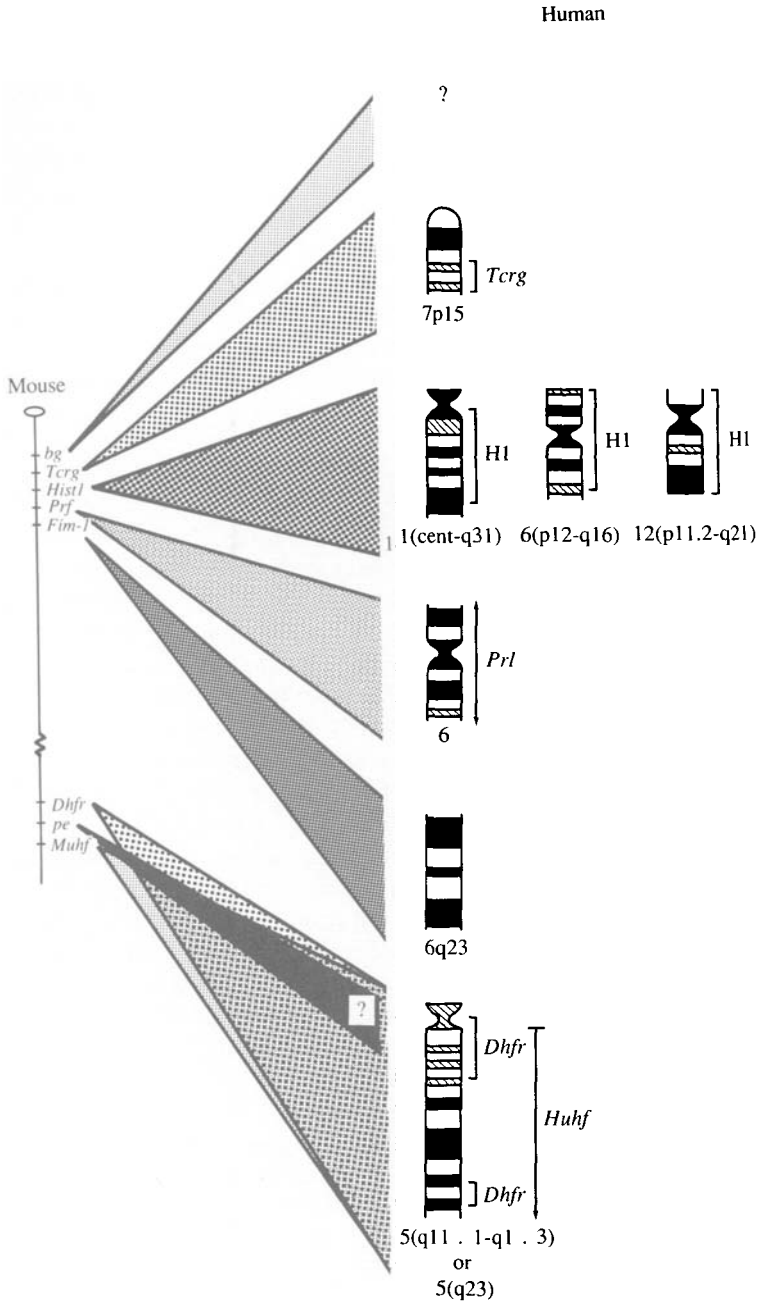

Fig. 2. Comparison of loci on murine chromosome 13 ans various homologous loci in humans. Chromosome 13 drawn with centromere to the top. Human chromosome number and specific bands indicated to the right.

bg should further elucidate the pattern of distribution of genes and gene segments over time, and may allow the chromosomal localization of CHS in man.

The regions immediately proximal and distal to the *pe* locus at the distal end of mouse chromosome 13 appear to have been conserved through evolution (Fig. 2). The human equivalents to these genes have both been mapped to human chromosome 5; *Dhfr* to either q11.1-q13.3 or q23 (Anagnou *et al.* 1984, 1988; Mauer *et al.* 1984, 1985), and *Huhf* (CTLA-3) to 5q (Gerschenfeld *et al.* 1988). It is likely therefore, that these genes have moved through evolution as a conserved segment of DNA. The pearl mutation, along with several other pigment mutations, has been proposed as a murine homologue of Hermansky–Pudlak syndrome (HPS) (Novak *et al.* 1984; Witkop *et al.* 1988), a disease characterized by partial albinism and a platelet storage pool defect (Novak *et al.* 1985; DePinho & Kaplan, 1985). The identification of two flanking markers which suggest an extended region of homologous synteny between mouse and human may be significant for the localization of the gene for HPS. If *pe* is homologous to HPS, it is likely that the gene for HPS is located on human chromosome 5q. Documentation of this through RFLP analysis of a family affected with HPS will be a first step toward the

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eventual cloning of the HPS gene, and the understanding of platelet storage pool defects at a molecular level.

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