Use of flanking loci to characterise variation at the *Mla* disease resistance locus in barley.

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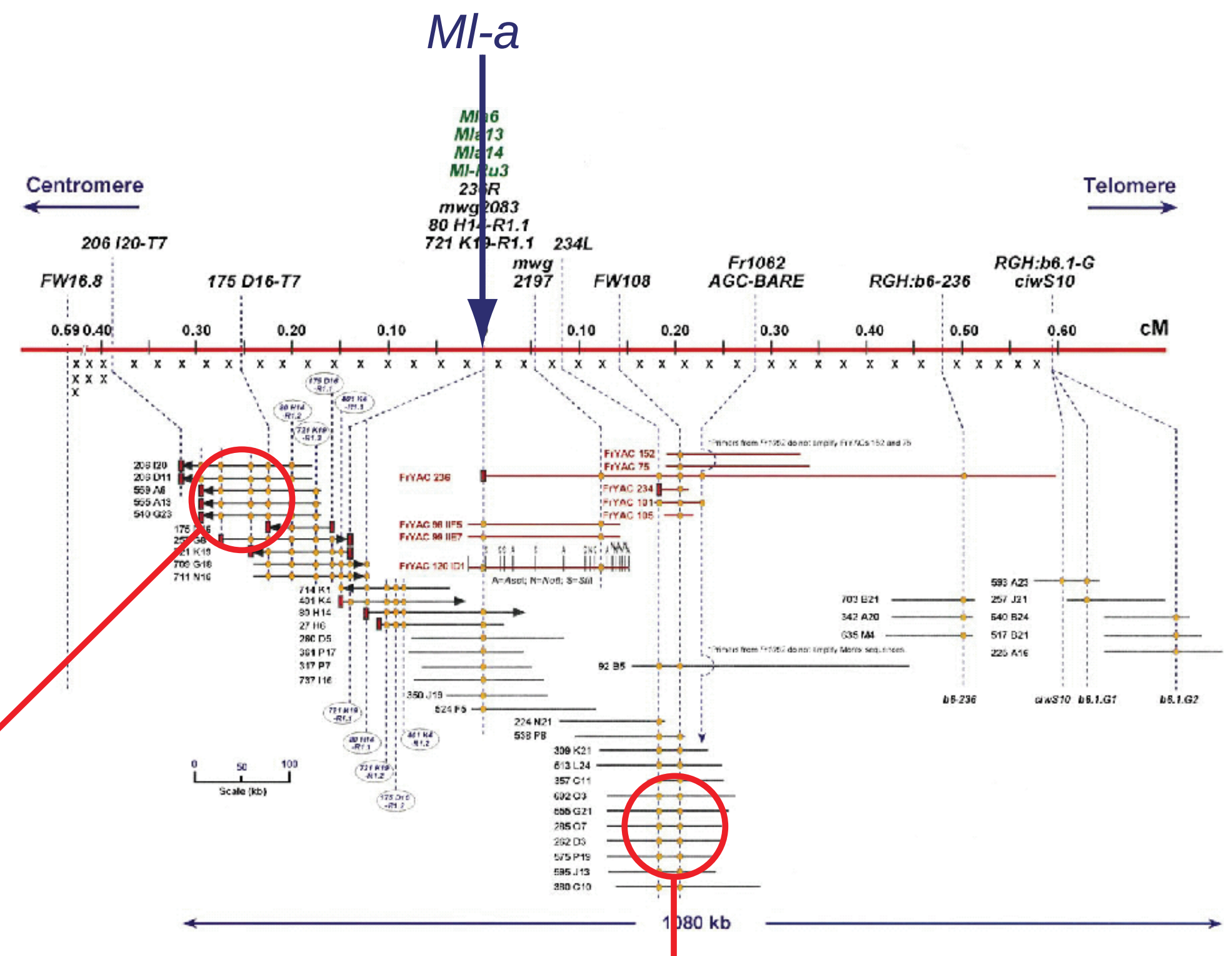
Introduction

The *Mla* locus in barley is one of several loci that confer gene-specific resistance to the powdery mildew fungus, *Blumeria graminis* f. sp. *hordei*. The locus exhibits considerable functional variation with over thirty alleles described. A 261-kb contig spanning the *Mla* gene family has been sequenced in the cultivar Morex (Wei *et al*, 2002, Plant Cell 14: 1903-1917).

At least seven alleles at this locus (*Ml-a1, Ml-a3, Ml-a6, Ml-a7, Ml-a9, Ml-a12 & Ml-a13*) have been used in past breeding programmes in Northwest Europe in attempts to introduce resistance to powdery mildew.. These resistance gene introgressions suffered from classic boom and bust cycles as the fungus developed virulence to the specific resistance alleles introduced.

Although the gene is no longer a major target of selection in modern breeding programmes the introduction of the various alleles has had a profound impact on the variation present in this genomic region in modern elite barley varieties.. This study was initiated to study the effect of the introgression of the *Ml-a* alleles into elite germplasm and we present preliminary results here.



Schematic diagram of genomic region surrounding *Ml-a*
Wei et al, 1999. Genetics 153: 1929-1948

Haplotype surrounding *Ml-a* locus

Although the gene is cloned and the surrounding BAC sequence known, the application of this genomic sequence to the study of variation at the locus is complicated by duplication within the *Ml-a* locus itself and duplication between the region and homologous loci elsewhere in the genome.

In order to circumvent the problems inherent in studying the variation at the *Ml-a* locus we have characterised the variation within the genomic region around *Ml-a* through sample sequencing at a number of single copy loci flanking the locus. The results from two single copy genes on BAC clones flanking the locus (~500 kb, ~0.35 cM apart) are presented in the table opposite.

The derived primers were used on DNA from stock accessions previously described as type designations of particular *Ml-a* alleles (Jørgensen, 1993). The resulting derived sequences of the two flanking loci indicate that several *Ml-a* alleles reside within identical haplotypes (*Ml-a1, Ml-a5, Ml-a7, Ml-a10, Ml-a11* and *Ml-a30*) that possibly indicates a recent diversification of these alleles from a common ancestor.

Other flanking haplotypes (such as that for *ml-a*) indicate possible recombination between the two flanking loci. This recombination may relate to the known derivation of the null allele *ml-a* from an unequal crossing over event within the *Ml-a* locus (Halterman, and Wise, 2004, Wei et al., 2002). The instability of the locus in heterozygotes and loss of resistance has previously been described (Wise and Ellingboe, 1985).

Ml-a allele	Cultivar	Stock	abc15612																				538p8																																																	
			131	133+	350	523	546	616	641	783	835	936	959	1037	1104	1108	1115	1207	1246	1269	1356	1451	29	30	35	52	70	77	87	124	127	130+	161	169	185	198	202	206	209	211	212	240	246	265	283	291	295	298	302	303	310	323	324	327	332	337	338	340	342	343	361	380	381	382	407	409	411	427+	448	457	463	506
Ml-a1	Algerian	CIho 1179	A	dl	C	C	A	T	G	T	T	G	A	G	C	A	A	G	C	G	-	A	T	T	T	T	T	G	C	T	A	t8	C	T	C	A	T	C	A	A	A	T	G	G	T	A	T	A	A	G	A	C	A	A	G	C	C	T	T	T	G	C	G	A	G	G	G	dl	C	T	T	G
Ml-a2	Black Russian	Ciho 2202	G	in	C	C	A	T	G	T	T	G	A	A	C	A	A	A	C	G	-	A	T	T	T	T	T	G	C	T	A	t8	C	C	C	A	A	C	A	A	A	C	G	G	T	A	C	A	A	G	G	C	A	A	G	C	C	T	T	T	G	C	G	A	A	G	G	dl	C	C	T	A
Ml-a3	Ricardo	CIho 6306	A	dl	C	T	A	T	A	T	C	C	A	A	C	A	A	G	C	G	-	A	A	T	T	T	C	G	C	T	A	t7	C	C	C	A	A	C	A	A	A	C	G	G	T	A	C	A	T	T	G	C	A	A	T	C	C	A	T	T	G	C	G	A	G	G	G	dl	C	C	A	A
Ml-a5	Gopal	CIho 1091	A	dl	C	C	A	T	G	T	T	G	A	G	C	A	A	G	C	G	-	A	T	T	T	T	T	G	C	T	A	t8	C	T	C	A	T	C	A	A	A	T	G	G	T	A	T	A	A	G	A	C	A	A	G	C	C	T	T	T	G	C	G	A	G	G	G	dl	C	T	T	G
Ml-a6,Ml-a14	Franger	CIho 16151	A	dl	C	T	G	T	A	T	T	C	A	A	T	A	A	G	T	G	-	A	A	T	T	T	T	G	C	T	A	t8	C	C	C	A	A	C	A	A	A	C	G	G	T	A	C	A	A	G	G	C	A	A	G	C	C	T	T	T	G	C	G	A	A	G	G	dl	C	C	T	A
Ml-a7	Multan	CIho 3401	A	dl	C	C	A	T	G	T	T	G	A	G	C	A	A	G	C	G	-	A	T	T	T	T	T	G	C	T	A	t8	C	T	C	A	T	C	A	A	A	T	G	G	T	A	T	A	A	G	A	C	A	A	G	C	C	T	T	T	G	C	G	A	G	G	G	dl	C	T	T	G
Ml-a8	Heil Hanna No3	CIho 682	G	in	C	C	A	T	G	T	T	G	A	A	C	A	G	A	C	G	-	A	A	T	T	T	T	G	C	T	A	t8	C	C	T	A	A	C	A	A	A	C	G	G	T	A	C	A	A	G	G	C	A	A	G	C	T	T	T	T	G	C	G	A	A	G	G	dl	C	C	T	A
Ml-a9	Monte Cristo	CIho 1017	G	in	T	C	A	C	G	C	T	C	C	A	C	G	A	G	C	A	T	G	T	T	A	T	T	T	C	T	T	t7	C	C	C	A	A	C	C	A	A	T	G	G	T	G	C	A	A	G	G	C	A	T	G	G	C	T	T	T	G	T	G	A	G	C	A	dl	C	C	T	A
Ml-a10	Durani	CIho 16149	A	dl	C	C	A	T	G	T	T	G	A	G	C	A	A	G	C	G	-	A	T	T	T	T	T	G	C	T	A	t8	C	T	C	A	T	C	A	A	A	T	G	G	T	A	T	A	A	G	A	C	A	A	G	C	C	T	T	T	G	C	G	A	G	G	G	dl	C	T	T	G
Ml-a11	A222	CIho 11555	A	dl	C	C	A	T	G	T	T	G	A	G	C	A	A	G	C	G	-	A	T	T	T	T	T	G	C	T	A	t8	C	T	C	A	T	C	A	A	A	T	G	G	T	A	T	A	A	G	A	C	A	A	G	C	C	T	T	T	G	C	G	A	G	G	G	dl	C	T	T	G
Ml-a12	Emir	CIho 11790	A	dl	C	T	A	T	A	T	T	C	A	A	C	A	A	G	C	G	-	A	A	A	T	T	T	G	C	T	A	dl	C	C	C	A	A	C	A	A	A	C	G	G	T	A	C	A	A	G	G	C	A	A	G	C	C	T	T	T	G	C	G	A	A	G	G	dl	C	C	T	A
Ml-a13	Rupee	CIho 16155	A	dl	C	C	A	T	G	T	T	G	A	G	C	A	A	G	C	G	-	A	A	T	T	T	T	G	C	T	A	t7	C	C	C	A	A	C	C	A	A	C	G	G	T	A	C	A	A	G	G	C	A	A	G	G	C	T	T	T	G	C	A	A	G	G	G	dl	C	C	T	A
Ml-a22	Hor1657	CIho 13134	G	in	C	C	A	T	G	T	T	G	A	A	C	A	A	A	C	G	-	A	A	T	T	T	T	G	C	T	A	t8	C	C	T	A	A	C	A	A	A	C	G	G	T	A	C	A	A	G	G	C	A	A	G	C	C	T	T	T	G	C	G	A	A	G	G	dl	C	C	T	A
Ml-a23	Hor1402	PI 328650	A	dl	C	C	A	T	G	T	T	G	A	G	C	A	A	G	C	G	-	A	A	T	T	T	T	G	A	T	A	t7	C	T	C	A	A	C	A	A	A	C	A	G	T	A	C	A	A	G	G	T	-	A	G	C	C	T	T	T	G	C	G	A	G	G	G	dl	C	C	T	G
Ml-a24	Engledow India	CIho 7555	A	dl	C	C	A	T	G	T	T	G	A	G	C	A	A	G	C	G	-	A	A	T	T	T	T	G	C	T	A	t7	C	C	C	A	A	C	C	A	A	C	G	G	T	A	C	A	A	G	G	C	A	A	G	G	C	T	T	T	G	C	A	A	G	G	G	dl	C	C	T	A
Ml-a30	Nigrate	CIho 2444	A	dl	C	C	A	T	G	T	T	G	A	G	C	A	A	G	C	G	-	A	T	T	T	T	T	G	C	T	A	t8	C	T	C	A	T	C	A	A	A	T	G	G	T	A	T	A	A	G	A	C	A	A	G	C	C	T	T	T	G	C	G	A	G	G	G	dl	C	T	T	G
Ml-a31	Turkey 290	CIho13646	G	in	C	C	A	T	G	T	T	G	A	A	C	A	G	A	C	G	-	A	A	T	T	T	T	G	C	T	A	t8	C	C	C	A	A	A	A	G	A	C	G	G	T	A	C	A	A	G	G	C	A	A	G	C	C	T	T	T	G	C	G	T	G	G	G	in	C	C	T	A
ml-a	Manchuria		A	dl	C	C	A	T	G	T	T	G	A	G	C	A	A	G	C	G	-	A	A	T	T	T	T	G	C	T	A	t7	T	C	C	G	A	C	A	A	A	C	G	T	T	A	C	A	A	G	G	C	A	A	G	C	C	T	T	T	A	C	G	A	G	G	G	dl	C	C	T	A
Ml-a X	Golden_Promise		G	in	C	T	A	T	G	T	T	G	A	A	C	A	G	A	C	G	-	A	A	T	T	-	T	G	C	T	A	t9	C	C	C	A	A	C	A	A	-	C	G	G	T	A	C	A	A	G	G	C	A	A	G	C	C	T	G	A	G	C	G	A	G	G	G	dl	C	C	T	A
Ml-a Z	Igri		A	dl	C	C	A	T	G	T	T	G	A	G	C	A	A	G	C	G	-	A	A	T	T	T	T	G	C	G	A	t7	C	C	C	A	A	C	A	A	A	C	T	G	C	A	C	-	A	G	G	C	A	A	G	C	C	T	T	T	G	C	G	A	G	G	G	dl	C	C	T	A

Table of the SNP haplotypes at two loci flanking *Ml-a* locus for barley accessions with 17 known *Ml-a* alleles

		abc15612												538p8																															
		523	546	616	641	783	835	936	959	1037	1104	1108	1115	130+	161	169	180	185	198	202	209	207	240	246	265	283	291	295	298	302	303	310	327	332	337	338	340	342	343	361	380	381	407	409	411
Ml-a1	**Algerian**	C	A	T	G	T	T	G	A	G	C	A	A	t8	C	T	C	C	A	T	A	A	T	G	G	T	A	T	A	A	G	A	A	G	C	C	T	T	T	G	C	G	G	G	G
Ml-a7	**Multan**	C	A	T	G	T	T	G	A	G	C	A	A	t8	C	T	C	C	A	T	A	A	T	G	G	T	A	T	A	A	G	A	A	G	C	C	T	T	T	G	C	G	G	G	G
	Amazone	C	A	T	G	T	T	G	A	G	C	A	A	t8	C	T	C	C	A	T	A	A	T	G	G	T	A	T	A	A	G	A	A	G	C	C	T	T	T	G	C	G	G	G	G
	Annabell	C	A	T	G	T	T	G	A	G	C	A	A	t8	C	T	C	C	A	T	A	A	T	G	G	T	A	T	A	A	G	A	A	G	C	C	T	T	T	G	C	G	G	G	G
	Canut	C	A	T	G	T	T	G	A	G	C	A	A	t8	C	T	C	C	A	T	A	A	T	G	G	T	A	T	A	A	G	A	A	G	C	C	T	T	T	G	C	G	G	G	G
	Cellar	C	A	T	G	T	T	G	A	G	C	A	A	t8	C	T	C	C	A	T	A	A	T	G	G	T	A	T	A	A	G	A	A	G	C	C	T	T	T	G	C	G	G	G	G
	Claret	C	A	T	G	T	T	G	A	G	C	A	A	t8	C	T	C	C	A	T	A	A	T	G	G	T	A	T	A	A	G	A	A	G	C	C	T	T	T	G	C	G	G	G	G
	Cooper	C	A	T	G	T	T	G	A	G	C	A	A	t8	C	T	C	C	A	T	A	A	T	G	G	T	A	T	A	A	G	A	A	G	C	C	T	T	T	G	C	G	G	G	G
	Dram	C	A	T	G	T	T	G	A	G	C	A	A	t8	C	T	C	C	A	T	A	A	T	G	G	T	A	T	A	A	G	A	A	G	C	C	T	T	T	G	C	G	G	G	G
	Graphic	C	A	T	G	T	T	G	A	G	C	A	A	t8	C	T	C	C	A	T	A	A	T	G	G	T	A	T	A	A	G	A	A	G	C	C	T	T	T	G	C	G	G	G	G
	Hood	C	A	T	G	T	T	G	A	G	C	A	A	t8	C	T	C	C	A	T	A	A	T	G	G	T	A	T	A	A	G	A	A	G	C	C	T	T	T	G	C	G	G	G	G
	Pipkin	C	A	T	G	T	T	G	A	G	C	A	A	t8	C	T	C	C	A	T	A	A	T	G	G	T	A	T	A	A	G	A	A	G	C	C	T	T	T	G	C	G	G	G	G
	Prestige	C	A	T	G	T	T	G	A	G	C	A	A	t8	C	T	C	C	A	T	A	A	T	G	G	T	A	T	A	A	G	A	A	G	C	C	T	T	T	G	C	G	G	G	G
Ml-a3	**Ricardo**	T	A	T	A	T	C	C	A	A	C	A	A	t7	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	T	T	G	A	T	C	C	A	T	T	G	C	G	G	G	G
	Colada	T	A	T	A	T	C	C	A	A	C	A	A	t7	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	T	T	G	A	T	C	C	A	T	T	G	C	G	G	G	G
Ml-a6	**Franger**	T	G	T	A	T	T	C	A	A	T	A	A	t8	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Agneta	T	G	T	A	T	T	C	A	A	T	A	A	t8	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Carnival	T	G	T	A	T	T	C	A	A	T	A	A	t8	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Goldmaker	T	G	T	A	T	T	C	A	A	T	A	A	t8	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Torrent	T	G	T	A	T	T	C	A	A	T	A	A	t8	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
Ml-a9	**Monte Cristo**	C	A	C	G	C	T	C	C	A	C	G	A	t7	C	C	C	C	A	A	C	A	T	G	G	T	G	C	A	A	G	G	T	G	G	C	T	T	T	G	T	G	G	C	A
	Force	C	A	C	G	C	T	C	C	A	C	G	A	t7	C	C	C	C	A	A	C	A	T	G	G	T	G	C	A	A	G	G	T	G	G	C	T	T	T	G	T	G	G	C	A
	Kym	C	A	C	G	C	T	C	C	A	C	G	A	t7	C	C	C	C	A	A	C	A	T	G	G	T	G	C	A	A	G	G	T	G	G	C	T	T	T	G	T	G	G	C	A
Ml-a12	**Emir**	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Aramir	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Blenheim	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Chad	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Chalice	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Chariot	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Corniche	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Decanter	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Egmont	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Extract	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Fergie	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Hart	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Henni	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Landlord	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Maris Mink	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Nudinka	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Optic	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
	Prisma	T	A	T	A	T	T	C	A	A	C	A	A	dl	C	C	C	C	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	A	G	G
Ml-a13	**Rupee**	C	A	T	G	T	T	G	A	G	C	A	A	t7	C	C	C	C	A	A	C	A	C	G	G	T	A	C	A	A	G	G	A	G	G	C	T	T	T	G	C	A	G	G	G
	Brise	C	A	T	G	T	T	G	A	G	C	A	A	t7	C	C	C	C	A	A	C	A	C	G	G	T	A	C	A	A	G	G	A	G	G	C	T	T	T	G	C	A	G	G	G
	Cocktail	C	A	T	G	T	T	G	A	G	C	A	A	t7	C	C	C	C	A	A	C	A	C	G	G	T	A	C	A	A	G	G	A	G	G	C	T	T	T	G	C	A	G	G	G
	Colston	C	A	T	G	T	T	G	A	G	C	A	A	t7	C	C	C	C	A	A	C	A	C	G	G	T	A	C	A	A	G	G	A	G	G	C	T	T	T	G	C	A	G	G	G
	Pewter	C	A	T	G	T	T	G	A	G	C	A	A	t7	C	C	C	C	A	A	C	A	C	G	G	T	A	C	A	A	G	G	A	G	G	C	T	T	T	G	C	A	G	G	G
Ml-a8	**Heil Hanna No.3**	C	A	T	G	T	T	G	A	G	C	A	A	t8	C	C	C	T	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	T	T	T	T	G	C	G	A	G	G
	Diamant	C	A	T	G	T	T	G	A	G	C	A	A	t8	C	C	C	T	A	A	A	A	C	G	G	T	A	C	A	A	G	G	A	G	C	T	T	T	T	G	C	G	A	G	G
Ml-a X	**Golden Promise**	C	A	T	G	T	T	G	A	A	C	A	G	t9	C	C	C	C	A	A	A	:	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	G	A	G	C	G	G	G	G
	Armelle	C	A	T	G	T	T	G	A	A	C	A	G	t9	C	C	C	C	A	A	A	:	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	G	A	G	C	G	G	G	G
	Carlsberg II	C	A	T	G	T	T	G	A	A	C	A	G	t9	C	C	C	C	A	A	A	:	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	G	A	G	C	G	G	G	G
	Gull	C	A	T	G	T	T	G	A	A	C	A	G	t9	C	C	C	C	A	A	A	:	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	G	A	G	C	G	G	G	G
	Ingrid	C	A	T	G	T	T	G	A	A	C	A	G	t9	C	C	C	C	A	A	A	:	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	G	A	G	C	G	G	G	G
	Kenia	C	A	T	G	T	T	G	A	A	C	A	G	t9	C	C	C	C	A	A	A	:	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	G	A	G	C	G	G	G	G
	Maja	C	A	T	G	T	T	G	A	A	C	A	G	t9	C	C	C	C	A	A	A	:	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	G	A	G	C	G	G	G	G
	Maythorpe	C	A	T	G	T	T	G	A	A	C	A	G	t9	C	C	C	C	A	A	A	:	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	G	A	G	C	G	G	G	G
	Proctor	C	A	T	G	T	T	G	A	A	C	A	G	t9	C	C	C	C	A	A	A	:	C	G	G	T	A	C	A	A	G	G	A	G	C	C	T	G	A	G	C	G	G	G	G
Ml-a Y	**Igri**	C	A	T	G	T	T	G	A	G	C	A	A	t7	C	C	C	C	A	A	A	A	C	T	G	C	A	C	:	A	G	G	A	G	C	C	T	T	T	G	C	G	G	G	G
	Clarine	C	A	T	G	T	T	G	A	G	C	A	A	t7	C	C	C	C	A	A	A	A	C	T	G	C	A	C	:	A	G	G	A	G	C	C	T	T	T	G	C	G	G	G	G
	Dura	C	A	T	G	T	T	G	A	G	C	A	A	t7	C	C	C	C	A	A	A	A	C	T	G	C	A	C	:	A	G	G	A	G	C	C	T	T	T	G	C	G	G	G	G
	Intro	C	A	T	G	T	T	G	A	G	C	A	A	t7	C	C	C	C	A	A	A	A	C	T	G	C	A	C	:	A	G	G	A	G	C	C	T	T	T	G	C	G	G	G	G
	Melusine	C	A	T	G	T	T	G	A	G	C	A	A	t7	C	C	C	C	A	A	A	A	C	T	G	C	A	C	:	A	G	G	A	G	C	C	T	T	T	G	C	G	G	G	G
	Sonja	C	A	T	G	T	T	G	A	G	C	A	A	t7	C	C	C	C	A	A	A	A	C	T	G	C	A	C	:	A	G	G	A	G	C	C	T	T	T	G	C	G	G	G	G
	Violet	C	A	T	G	T	T	G	A	G	C	A	A	t7	C	C	C	C	A	A	A	A	C	T	G	C	A	C	:	A	G	G	A	G	C	C	T	T	T	G	C	G	G	G	G
Ml-a Z	**Atem**	C	A	T	G	T	T	G	A	A	C	A	A	t8	T	T	T	C	A	A	A	T	C	A	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	G	G	G
	Cambrinus	C	A	T	G	T	T	G	A	A	C	A	A	t8	T	T	T	C	A	A	A	T	C	A	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	G	G	G
	Dandy	C	A	T	G	T	T	G	A	A	C	A	A	t8	T	T	T	C	A	A	A	T	C	A	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	G	G	G
	Franken II	C	A	T	G	T	T	G	A	A	C	A	A	t8	T	T	T	C	A	A	A	T	C	A	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	G	G	G
	Julia	C	A	T	G	T	T	G	A	A	C	A	A	t8	T	T	T	C	A	A	A	T	C	A	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	G	G	G
	Riviera	C	A	T	G	T	T	G	A	A	C	A	A	t8	T	T	T	C	A	A	A	T	C	A	G	T	A	C	A	A	G	G	A	G	C	C	T	T	T	G	C	G	G	G	G
ml-a	**Manchuria**	C	A	T	G	T	T	G	A	G	C	A	A	t7	T	C	C	C	G	A	A	A	C	G	A	T	A	C	A	A	G	G	A	G	C	C	T	T	T	A	C	G	G	G	G
	Morex	C	A	T	G	T	T	G	A	G	C	A	A	t8	T	C	C	C	G	A	A	A	C	G	A	T	A	C	A	A	G	G	A	G	C	C	T	T	T	A	C	G	G	G	G
	Steptoe	C	A	T	G	T	T	G	A	G	C	A	A	t9	T	C	C	C	G	A	A	A	C	G	A	T	A	C	A	A	G	G	A	G	C	C	T	T	T	A	C	G	G	G	G

Table of the SNP haplotypes at two loci flanking *Ml-a* locus for past and current Northwest European barley cultivars showing range o *Ml-a* alleles

Genotyping Barley varieties for *Ml-a* alleles

The integration of the sequence information has enabled the genotyping of barley lines for *Ml-a* alleles by proxy through the genotyping of closely physically linked loci. When used on a range of current and past northwest European barley varieties the flanking sequences correctly predicted the *Ml-a* allele in previously characterised cultivars (Jørgensen and Jensen , 1983) and gave an indication of which allele had been inherited in current uncharacterised material for which pedigree information was known.

In addition the characterisation of *Ml-a* alleles within recent European barley material indicted not only the presence of the seven introgressed alleles but also four other haplotypes which presumably were present in ancestral Northwest European barley landraces. These four European haplotypes include the functionally characterised weak allele *Ml-a8* (Jørgensen, 1993). Interestingly other barley cultivars that have been characterised as *Ml-a8* such as the variety Golden Promise have a different haplotype (denoted *Ml-a X*). This could be due to the genotype of the Heil Hanna No.3 plant used for this study not carrying *Ml-a8* or alternatively that the Japanese isolate used to functionally characterise *Ml-a8* could possibly have carried avirulence to two old European *Ml-a* resistance alleles.

Given the maintenance of linkage disequilibrium (LD) over ~0.35 cM around the *Ml-a* locus in elite material that has allowed us to genotype for *Ml-a* by proxy, we are currently investigating how far this LD extends. Initial studies with primers derived from AF085164 the receptor-like kinase *LRK10* gene (Feuillet and Keller , 1999) ~3cM distal of *Ml-a* indicate a near complete maintenance of the donor haplotype in current barley varieties. This corresponds to past findings of correlations of allelic states at *Ml-a* and the flanking hordein seed storage proteins (Shewry et al 1979) We are continuing this with other genes on 1HS to determine whether there may be selection acting on alleles at other loci brought in via linkage drag with the introgressions of the *Ml-a* alleles.

Summary

The use of flanking loci has enabled an investigation of the relationships between Ml-a alleles and highlighted a possible role of recombination in their evolution

These loci have also allowed us to follow complex breeding history of Northwest European barleys that includes repeated introgression of resistance alleles.

The characterisation of older European barley lines has also indicated that the functionally characterised Ml-a8 allele may encompass two separate sequence haplotypes.

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