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Marker applications in pearl millet

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Abstract

There are a multitude of potential applications of DNA marker technologies to the improvement of pearl millet (*Pennisetum glaucum* (L.) R. Br.). This presentation will delve into two major areas — the use of these molecular markers in pearl millet genetic diversity studies and the use of molecular markers for mapping quantitative trait loci (QTL) to facilitate marker-assisted (MA) breeding for economically important pearl millet traits having a large genotype × environment component to their phenotypic variation. The discussion of pearl millet genetic diversity studies builds on the general outline of this area presented earlier in this course by Dr. Bramel-Cox. The discussions of QTL mapping and MA breeding in pearl millet will be based largely on results obtained over the past nine years in a series of collaborative projects involving the International Crops Research for the Semi-Arid Tropics (ICRISAT), UK-based researchers supported by the Plant Sciences Programme (PSP) of the Department for International Development (DFID, formerly the Overseas Development Administration (ODA)) and based at the John Innes Centre for Plant Sciences Research (JIC, Norwich), the University of Wales (UW, Bangor), and the Institute of Grassland and Environmental Research (IGER, Aberystwyth), and several public-sector agencies involved in pearl millet improvement in India under the umbrella of the All-India Coordinated Pearl Millet Improvement Project (AICPMIP).

Genetic diversity studies in pearl millet

How can we extend the research that is currently being conducted on sorghum genetic resources held in ICRISAT's World Collection and transfer what we have learned to pearl millet? These two related cereals of the semi-arid tropics differ in several genetically important ways despite the many discussions in the literature of their similarities and homeologies. Pearl millet is a highly cross-pollinated species. Genetic diversity in the species is distributed both within and among cultivars. Within-cultivar diversity can be very limited in case of single-cross

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hybrids, but is substantially greater in landraces and improved open-pollinated varieties of pearl millet. Due to its highly outcrossing breeding behaviour, its apparent origin from several independent domestication events (Poncet et al., 1998), and the wide range of stressful environments in which it has traditionally been cultivated, pearl millet exhibits a tremendous amount of polymorphism at both phenotypic and genotypic levels (Liu et al., 1992, 1994a, b). The more complicated distribution of diversity in pearl millet, as well as the higher degree of marker polymorphism, will make genetic diversity studies in this species more complicated than in sorghum. Thus the breeding behaviour of pearl millet, and the structure of genetic diversity within this species, have strong implications for the use of molecular markers in its diversity assessment as we attempt to extrapolate from our previous studies in sorghum (a closely related, self-pollinated cereal in which most of the genetic diversity is distributed between rather than within cultivars, and levels of marker polymorphism are relatively low).

First, the core collection concept (Frankel and Brown, 1984) and methodology is biased towards self-pollinated and clonally propagated plant species where most of the genetic diversity is distributed between (rather than within) germplasm accessions. However, for cross-pollinated seed-propagated species, estimates of diversity need to be based upon gene frequencies within accessions and not so heavily on between-accession differences in presence/absence of particular alleles. In all crop species, phenotypic estimates of genetic diversity are biased by the environment(s) in which evaluation occurs. Further, in pearl millet and other cross-pollinated seed-propagated species, these estimates can also be heavily influenced by inbreeding depression that occurs as we impose a closed population structure during regeneration. The impact of regeneration procedures on diversity in accessions maintained in our genebank, or elsewhere, are unknown. However, these are expected to be more problematic for cross-pollinated seed-propagated species like pearl millet than for self-pollinated or clonally-propagated species. Finally, the sampling procedures used and definitions of accessions used during collection will also impact on the distribution of diversity within a germplasm collection. Thus for genetic diversity assessment in cross-pollinated species, molecular markers offer considerable advantages over methods based on phenotypic evaluation. This is especially true in pearl millet where we have a high degree of marker polymorphism.

Molecular markers developed for breeding purposes are not always directly transferable for use in genetic resources studies. There is an excellent discussion of this in the recent IPGRI publication by Karp et al. (1997). Decisions about the best markers for genetic diversity studies are largely based on requirements for cost-effectiveness and high through-put to allow analyses of a large number of samples, preferably with co-dominant markers. Further, the markers need to be neutral and unlinked in order to give unbiased genetic diversity estimates. In sorghum, these requirements lead to the conclusion that SSRs are the best marker system available.

If appropriate marker systems are available, as they are to some extent in pearl millet (RFLP, STS, and AFLP markers are now available, with SSR markers currently under development), then we have at least three research areas where they could be used. First, they can be used in descriptions of baseline diversity for collection purposes. An assessment of pearl millet genetic diversity distribution within traditional cropping systems in Rajasthan, at field, village, and regional levels, is currently underway that will relate this information to farmers' seed management practices. The information generated will have direct implications to on-farm conservation schemes and to the potential efficacy of participatory breeding schemes in this highly cross-pollinated crop. Marker information can also be used to assess regeneration procedures, and identify effective methods that will be satisfactory both for the ICRISAT germplasm bank and for NARS programs. For example, we have examined the effect of 12 pollination cycles on the frequency distribution of flowering time, plant height, panicle length, and panicle thickness among individuals in several pearl millet accessions. Both qualitative and quantitative shifts were noted. It has been important to separate the effect of inbreeding on accession means as a result of imposing a closed population from loss of alleles (the really important issue). All four regeneration methods resulted in a general reduction in the frequency of some classes for quantitative traits, so that later regeneration cycles were generally earlier and shorter with thinner, shorter, well-exserted panicles. Plants had fewer leaves, thinner stems, and lighter grains. All of these changes could be due to both inbreeding depression and loss of alleles. However, when qualitative traits were examined, the shifts were not as evident. In general, sibbing looks better than cluster-bagging or selfing. Neutral markers would have been a better, more efficient method to evaluate these regeneration procedures than the phenotypic methods used in this study. Finally, Lamy et al. (1994) are using pearl millet molecular markers to follow the introgression of genomic segments from the wild progenitors of this crop into several populations based on crosses of wild and cultivated accessions from various parts of western and central Africa.

Future opportunities for use of markers in pearl millet diversity studies include marker development in conjunction with mapping to identify a core set of markers appropriate for diversity assessment. They also include use of linked markers to screen for traits in order to better understand the allelic and map distribution of diversity for specific traits among sources and hence better target mapping and transfer of new QTLs for specific traits. We see development of appropriate markers (SSRs) as a critical need, and this will be a very important research focus for ICRISAT and its research partners (Cornell University and the John Innes Centre for Plant Science Research) in the short term.

To recapitulate, molecular markers provide an extremely valuable tool for the study of genetic diversity in pearl millet. However, the sample numbers needed to estimate diversity in cross-

pollinated crops is very large because of the need to determine both within and between accession allelic distribution. Thus cost and ease of measurement are very important marker attributes. One complication is that marker choice will be affected by the high degree of polymorphism in this species. This is not usually a problem in self-pollinated species, but could make use of SSR markers for diversity studies problematic in pearl millet. This situation can best be addressed by studies comparing SSR markers with RFLP and/or RAPD markers for this purpose in this species.

QTL mapping and marker-assisted selection in pearl millet

Molecular marker-based genetic linkage map

Initial work to develop a molecular marker-based genetic linkage map of pearl millet was reported by Liu et al. (1992, 1994a, 1994b). This began with a focus on the use of restriction fragment length polymorphism (RFLP) markers detected using homologous pearl millet probes, with ^{32}P-mediated autoradiography to visualise banding differences. A few heterologous probes from rice, wheat and barley — along with several isozymes and known-function probes — were also included in this base map. The result was a map of over 180 loci that covered approximately 350 cM and contained the expected seven linkage groups, one extra couplet, and a floating point. This map probably corresponds to the centromeric regions of the seven pearl millet chromosome pairs. It was used to map QTLs for resistance to pearl millet downy mildew (caused by the pseudo-fungus *Sclerospora graminicola* (Sacc.) J. Schr¬t.) by Jones et al. (1994, 1995). Markers linked with resistance QTLs, and additional low copy probes having high levels of polymorphism in initial variablilty studies, were converted to sequence tagged site (STS) markers (Money et al., 1994), but these have proven to be disappointing because much of the polymorphism of the RFLP markers on which they were based can no longer be detected without the use of multiple restriction enzymes. In many cases, this additional enzyme cost essentially negated any advantages of moving from co-dominant RFLP markers to the polymerase chain reaction (PCR)-compatible STS marker format.

This initial map was transferred to several additional crosses (Busso et al., 1995; Liu et al., 1996) in studies of sex-specific recombination rates in cultivated $\times$ cultivated and cultivated $\times$ wild crosses, and a pearl millet world reference mapping population was developed based on the cross 81B $\times$ ICMP 451 (Hash and Witcombe, 1994). This map has been used for high saturation marker genotyping using amplified fragment length polymorphism (AFLP) markers, additional homologous probes from pearl millet, and heterologous probes from other grasses. This latter group of markers has allowed us to improve our understanding of the complex relationships between the pearl millet genome and those of other cultivated graminaceous

species (Devos and Gale, 1997; Devos et al., 1999). This work has extended the total pearl millet genetic linkage map length to approximately 600 cM. Unfortunately, attempts to cap the linkage groups with telomeres have not proven successful as all polymorphic telomeric sequences detected to date in pearl millet segregate independently from the seven previously detected linkage groups. Currently, work is underway at JIC to develop sets of simple sequence repeat (SSR)-based markers for pearl millet that are PCR-compatible, co-dominantly inherited, and can be multiplexed for simultaneous marker genotype characterization at a number of loci within or across linkage groups.

QTL mapping already underway in pearl millet

Downy mildew resistance

Jones (1994) and Jones et al. (1994, 1995), have described QTLs for pearl millet downy mildew resistances effective against pathogen populations from western and central Africa as well as populations from south Asia from “resistant” parents P 7-3, and ICMP 85410, and “susceptible” parent LGD-1-B-10. Independent inheritance of resistance to pathogen populations from India, Senegal, Niger, and Nigeria was shown. QTLs with large effects contributed a large portion of the detected variation in disease reaction to each pathogen population, and were consistently detected in repeated screens. QTLs of smaller effects and variable effects were also detected. No single QTL was effective against all four of the pathogen populations studied, indicating that pathotype-specific resistance is a major mechanism of downy mildew resistance. For most, but not all QTLs, resistance was inherited from the “resistant” parent. Similarly, resistance at most QTLs was inherited in a dominant or over-dominant manner. Similar results have been found from screens against multiple pathogen populations of Asian and African origin of several subsequently developed pearl millet mapping populations based on parental lines ICMP 451, H 77/833-2, PRLT 2/89-33, P 310-17, W 504-1-1, P 1449-2 and PT 732B:

- we have identified many pathogen-population-specific downy mildew resistance QTLs, but to date none confers complete resistance to all tested field populations of the pathogen,
- most of these resistance QTLs are dominant or over-dominant in their inheritance, and
- overall susceptibility of a parental line does not preclude it from contributing alleles for improved resistance at some QTLs.

As a result of these findings, we now recommend routinely screening parents of all pearl millet mapping populations against specific downy mildew populations (e.g., Thakur et al., 1998) from regions where this disease is an important, or potentially important, constraint to pearl millet production. Where differential reactions are observed for pearl millet mapping population parent pairs, then progenies from that mapping population can be screened and the phenotypic scoring data thus obtained can be used to map downy mildew resistance QTLs

currently effective against that particular pathogen population. Similar approaches are probably relevant for other pearl millet diseases, as well as any other trait of sufficient economic importance to pearl millet producers and consumers.

As part of on-going research at ICRISAT-Patancheru, we are attempting marker-assisted backcross transfer of mapped downy mildew resistance QTLs from sources P 7-3, ICMP 85410, and LGD-1-B-10 to the genetic background of an economically important hybrid seed parent maintainer line, 843B (Stegmeier et al., 1998), and from source ICMP 451 (Hash et al., 1995) to another economically important hybrid pollinator line, H 77/833-2. The first homozygous products of these marker-assisted breeding programs have been developed and are currently being evaluated. A large number of additional substitution lines, near-isogenic for resistance donor chromosome segments associated with the target resistance QTLs (and their flanking markers), are nearing completion in the background of 843B. We have also initiated such marker-assisted backcrossing programs to enhance downy mildew resistance of other economically important hybrid parental lines such as 81B, 841B, 842B, ICMB 88006, and PT 732B.

Thesis research being conducted at JIC during the past three years with support from the European Union (EU) has attempted to use near-isogenic lines from the marker-assisted backcrossing program described above, combined with a recently developed pearl millet bacterial artificial chromosome (BAC) library, to identify markers very tightly linked to the major resistance QTL on linkage group 1 of resistance source P 7-3. The aim of this research is two-fold—to facilitate resistance gene isolation (to support another EU-supported project that will use transgenic approaches to improve pearl millet downy mildew resistance in hybrid seed parents for Africa) and to provide more tightly linked markers to improve the efficiency of marker-assisted selection for this resistance QTL.

Seedling heat tolerance

To date, our efforts to identify QTLs for the ability of seedlings of some pearl millet genotypes to withstand temperatures in excess of 50C (Howarth et al., 1994) have been largely unsuccessful. We believe that this lack of success is largely due to inadequacies of our phenotyping procedures, which were initially based on comparisons of partially inbred F_4 self bulks derived from individual skeleton-mapped F_2 plants from crosses ICMP 451 $\times$ H 77/833-2 and H 77/833-2 $\times$ PRLT 2/89-33. Vigor differences within and between the segregating partially inbred bulks were sufficient to mask any other genetic effects on seedling thermo-tolerance. By moving to F_3 testcross progenies as test units, we should be able to overcome this problem—at least under controlled environment conditions at IGER where we can ensure uniform moisture availability to the progenies being evaluated. If we succeed in identifying QTLs associated with large components of the higher seedling thermotolerance of parental line

H 77/833-2, we should then be able to use flanking markers associated with these QTLs to transfer them by backcrossing to the genetic backgrounds of otherwise elite, thermosensitive, pearl millets such the Iniadi landrace (Andrews and Kumar, 1996) and its derivatives.

Terminal drought tolerance of Iniadi-based inbred lines

In what began as a spin-off from the thermotolerance mapping studies described above, we have succeeded in mapping QTLs for terminal drought tolerance from two Iniadi-based inbred lines, PRLT 2/89-33 and 863B (Yadav et al., 1999a, b). F3-testcross hybrids produced using elite inbred lines as testers and F3 progenies derived from individual skeleton-mapped F2 plants were grown in paired environments in which one was fully irrigated and the other was drought stressed by withholding irrigation from the time the trial reached flowering. Regression was used to remove any statistically significant effects of genetic potential under fully irrigated conditions, and flowering time in the drought-stressed environment, on individual grain and stover yield component traits measured in the stressed environment. The entry-wise residuals from this regression, or the entry-wise ratio of performance in the stress environment over that in the irrigated control, were then treated as the quantitative trait of interest (i.e., drought tolerance for an individual yield component) and mapped. In both PRLT 2/89-33 and 863B, a substantial portion of the variation in drought tolerance mapped to linkage group 2, while in case of 863B a major QTL on linkage group 6 was also detected (Yadav et al., 1999a, b). We have recently initiated several different marker-assisted backcrossing schemes to transfer one or more of these putative drought tolerance QTLs to the genetic backgrounds of elite drought-sensitive hybrid parental lines 841B and H 77/833-2 (Hash et al., 1999). These will be used as an example in a later presentation in this seminar on concepts for application of marker techniques in Africa.

Grain and stover yield components (and maturity*)*

Since all of our drought stress tolerance screening studies have included a fully irrigated control environment, along with the paired stress environment(s), it has been possible for us to use the testcross data sets from these experiments to examine the potential of mapping QTLs for components of grain and stover yield potential. The marker genotyping has already been done, the phenotypic data collection has already been done. What is now required is simply to conduct further data analyses with phenotype data from a restricted set of environments—those where yields were not constrained by moisture availability. While these studies are still underway, preliminary analysis clearly shows the expected strong relationship between QTLs for maturity (flowering time) and stover yield (Table 1). Essentially, 30-50% of phenotypic variation for stover dry matter yield observed among testcrosses on a given tester was controlled by only two or three QTLs. These stover yield QTLs were usually associated with a QTL for flowering time such that delayed flowering time and increased stover yield co-segregate. Only one of the four QTLs for increased stover yield was not associated with

delayed flowering. One of the three QTLs for increased stover yield associated with delayed flowering was found in testcrosses of both the early flowering, high tillering, thin stemmed inbred tester (H 77/833-2) and the late flowering, low tillering, thick stemmed inbred tester (PPMI 301). The remaining two QTLs for increased stover yield associated with delayed flowering were detected only with tester PPMI 301. Interestingly, all increases in stover yield associated with delayed flowering came from the thinner stemmed mapping population parental line 841B, which is the maintainer for the seed parent of a released hybrid (Pusa 322) that has inbred tester PPMI 301 as its male parent (Govila et al., 1996). However, the most interesting stover yield QTL detected thus far, from an applied breeding point of view, is that from 863B in linkage group 3, which segregated independently from flowering time QTLs. It may be possible to improve the stover yield potential of (some) hybrids of male-sterile line 841A, without delaying their maturity, by marker-assisted backcross transfer of part of linkage group 3 from 863B into the genetic background of 841B. The male-sterile line counterpart of this new version of 841B could then be used to produce higher stover yield versions of hybrids currently produced on 841A. Work to produce this new version of 841B has recently been initiated at ICRISAT-Patancheru (Hash et al., 1999). Regressing out flowering time effects on testcross stover yield, and then QTL-mapping the residuals may allow us to detect additional stover yield QTLs not associated with maturity.

Ruminant nutritional quality of crop residues

Another trait that we have targeted for QTL mapping and marker-assisted selection in the (841B $\times$ 863B)-derived pearl millet mapping population is improved ruminant nutritional quality of stover fractions (leaf blades, leaf sheaths, and stem internodes). Ruminant nutritional quality is a very complex trait—dependent on maturity of the stover, the degree of nodal tillering, nitrogen content, soluble carbohydrate content, and fiber chemistry. We are conducting this research, based initially on stover samples produced in the irrigated control treatments of the drought tolerance mapping testcross trials described above, in collaboration with a livestock nutritionist from ILRI and a group of forage biochemists in Australia and the UK. Very preliminary results (Table 1) indicate the presence of a QTL for significant differences in in vitro rumen gas production among hybrids produced with the early, thin stemmed, high tillering inbred tester (H 77/833-2), at least for the leaf blade fractions of their stover. We are also examining testcross hybrids produced on four diverse male-sterile lines with the parental lines of all existent pearl millet mapping populations of ICRISAT origin, evaluating them for grain and stover yield components, as well as stover fraction ruminant nutritional quality. Our intention here, as described above for downy mildew resistance, is to identify additional pairs of parental lines that differ significantly for one or more traits of interest so as to further exploit the investments made in F2-skeleton mapping of existing or future pearl millet mapping population progeny sets.

Future target traits for QTL mapping and MAS in pearl millet

There are a number of economically important constraints to pearl millet production and/or utilization that appear to warrant the use of QTL mapping in the near future. High priority target traits include superior ability to access soil phosphate (e.g., Wissuwa et al., 1998) including that from poorly available sources such as rock phosphate, insect resistance (if resistance sources and phenotyping methods have been developed sufficiently), and grain quality traits such as improved meal keeping quality (Chavan and Hash, 1998) and reduced goitrogen levels in the bran fraction.

Summary and conclusions

In summary, there has been a relatively large amount of effort put into development of molecular markers as tools for pearl millet improvement over the past decade. These tools are now available for application in selected areas of pearl millet genetic diversity and applied breeding. However, much remains to be done. The most important area for short-term improvement in pearl millet marker technology would be design and development of appropriate sets of SSR markers to facilitate rapid, low cost, efficient high through-put genotyping within and across linkage groups using automated, multiplexed marker genotyping systems.

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Table 1. Eleven statistically significant (in bold) quantitative trait loci (QTLs) identified from MapMaker/QTL following QTL analysis of 36 phenotypic traits, using the additive genetic model, for F_3 testcross progenies from the (841B × 863B)-derived pearl millet mapping population evaluated in the 1998 summer drought nursery (fully-irrigated control treatment) in field RP 11 at ICRISAT, Patancheru 502 324, A.P., India.

Trait[1] (Mean, variance, & skewness)	Quantitative trait loci detected							
	Linkage group	Position	Genetics	Weight	Dominance	% Variation explained	LOD	Proposed QTL names
HBloomM (+43.04, +1.53, --0.03)	LG5	M345+02.4	Additive	--1.226	+0.000	28.7	5.02	
	LG7	M269+09.0	Additive	--1.084	+0.000	21.0	2.65	
	LG5	**M345+03.6**	**Additive**	**--1.150**	**+0.000**	\		***QDtf.icp-LG5.1***
	LG7	**M269+09.8**	**Additive**	**--1.005**	**+0.000**	/ **45.8**	**7.88**	***QDtf.icp-LG7.1***
HStDWM (+376.71, +30.75, +0.27)	LG3	M325+00.0	Additive	+14.432	+0.000	10.7	1.76	
	LG5	M345+00.5	Additive	--19.896	+0.000	18.8	3.04	
	LG5	M735+11.3	Additive	--17.307	+0.000	15.6	2.54	
	LG3	**M325+00.1**	**Additive**	**+13.663**	**+0.000**	\		***QSdw.icp-LG3.1***
	LG5	**M345+00.3**	**Additive**	**--19.198**	**+0.000**	/ **28.2**	**4.98**	***QSdw.icp-LG5.1***
HBGas36M (+25.34, +5.33, +0.33)	**LG7**	**M718+04.0**	**Additive**	**+3.912**	**+0.000**	**22.8**	**2.70**	***QBg3.ilr-LG7.1***
PBloomM (+43.09, +1.28, --0.75)	LG5	M345+04.4	Additive	--0.703	+0.000	13.4	2.05	
	LG6	M588+09.0	Additive	--0.911	+0.000	18.1	2.78	
	LG6	M870+15.8	Additive	--0.863	+0.437	17.2	2.23	
	LG7	M269+13.9	Additive	--0.887	+0.000	22.6	3.66	
	LG5	**M345+07.0**	**Additive**	**--0.722**	**+0.000**	\		***QDtf.icp-LG5.1***
	LG6	**M588+12.8**	**Additive**	**--0.710**	**+0.000**	|- **46.6**	**8.88**	***QDtf.icp-LG6.1***
	LG7	**M618+14.1**	**Additive**	**--0.801**	**+0.000**	/		***QDtf.icp-LG7.2***
PStDWM (+324.53, +27.58, +0.18)	LG4	M345+03.2	Additive	--15.744	+0.000	14.7	2.22	
	LG6	M514+01.0	Additive	--24.542	+0.000	27.4	4.51	
	LG6	M870+17.8	Additive	--20.870	+0.000	22.1	2.88	
	LG7	M618+04.5	Additive	--18.613	+0.000	19.5	2.66	
	LG5	**M345+05.1**	**Additive**	**--14.690**	**+0.000**	\		***QSdw.icp-LG5.1***
	LG6	**M588+09.2**	**Additive**	**--19.330**	**+0.000**	|- **49.3**	**8.53**	***QSdw.icp-LG6.1***
	LG7	**M618+07.6**	**Additive**	**--13.951**	**+0.000**	/		***QSdw.icp-LG7.1***

[1] Traits:

HBloomM	Mean 50% bloom date (days after sowing) of H 77/833-2 testcrosses
HStDWM	Mean stover dry weight per square meter (g) of H 77/833-2 testcrosses
HBGas36M	Mean gas production (mL) from leaf blade samples of H 77/833-2 testcrosses incubated for 36 h
PBloomM	Mean 50% bloom date (days after sowing) of PPMI 301 testcrosses
PStDWM	Mean stover dry weight per square meter (g) of PPMI 301 testcrosses

Table 2. Marker-based genetic linkage skeleton map (Haldane cM, and recombination percentage) of 32 markers across seven linkage groups (LG) for a pearl millet mapping population based on the cross 841B × 863B.

Linkage Group	Map distance		Markers
LG1			
1-2	16.5 cM	14.1%	M567
2-5	9.7 cM	8.8%	M761
5-9	6.3 cM	5.9%	M891
9-10	32.5 cM	23.9%	M52
	====		M196
	65.0 cM		
LG2			
11-12	26.7 cM	20.7%	M708.1
12-16	11.7 cM	10.5%	M322
16-20	5.0 cM	4.8%	M380
20-21	30.0 cM	22.6%	M443
	====		M708.2
	72.4 cM		
LG3			
22-25	6.5 cM	6.1%	M174
25-29	2.7 cM	2.7%	M325
	====		M525
	9.2 cM		
LG4			
30-33	4.8 cM	4.6%	M265
33-34	17.6 cM	14.9%	M527
34-35	26.8 cM	20.7%	M837
35-36	11.0 cM	9.9%	M1003D
36-38	6.3 cM	5.9%	M716
	====		M84
	66.5 cM		
LG5			
39-42	12.8 cM	11.3%	M345
42-45	12.2 cM	10.8%	M735
	====		MP2001
	25.0 cM		
LG6			
46-48	4.7 cM	4.5%	M202
48-50	14.1 cM	12.3%	M588
50-51	2.5 cM	2.5%	M514
51-52	23.5 cM	18.8%	M737
52-54	20.9 cM	17.1%	M870
	====		WG110
	65.7 cM		
LG7			
55-56	16.6 cM	14.2%	M718
56-57	16.2 cM	13.9%	M269
57-59	14.1 cM	12.3%	M618
	====		M526
	46.9 cM		