

## QTL mapping of yield, agronomic and quality traits in tetraploid potato (*Solanum tuberosum* subsp. *tuberosum*)

John E. Bradshaw · Christine A. Hackett ·  
Barnaly Pande · Robbie Waugh · Glenn J. Bryan

Received: 23 April 2007 / Accepted: 25 September 2007 / Published online: 16 October 2007  
© Springer-Verlag 2007

**Abstract** Interval mapping of quantitative trait loci (QTL) for 16 yield, agronomic and quality traits in potato was performed on a tetraploid full-sib family comprising 227 clones from a cross between processing clone 12601ab1 and table cultivar Stirling. Thirty-eight AFLP primer combinations and six SSRs provided 514 informative markers which formed a molecular marker map comprising 12 linkage groups (LGs) in 12601ab1 (nine with four homologous chromosomes) which were aligned with 12 in Stirling (11 with four homologous chromosomes), with four partial groups remaining in 12601ab1. Two LGs were identified unequivocally as chromosomes IV and V and eight others were tentatively assigned with chromosomes VII and X unidentified. All of the traits scored had moderately high heritabilities with 54–92% of the variation in clone means over 3 years and two replicates being due to genetic differences. A total of 39 QTLs were identified. A QTL for maturity was identified on chromosome V which explained 56% of the phenotypic variance, whereas the other QTLs individually explained between 5.4 and 16.5%. However, six QTLs were detected for after-cooking blackening and four for each of regularity of tuber shape, fry colour both after storage at 4 and 10°C and sprouting. Just two QTLs were found for each of yield, the two ‘overall’ scores, crop emergence, tuber size and common scab and just one QTL was detected for each of

dry matter content, keeping quality, growth cracks and internal condition. The implications for practical potato breeding and for practical linkage and QTL analysis in autotetraploids are discussed.

### Introduction

The European cultivated potato, *Solanum tuberosum* subsp. *tuberosum*, is a tetraploid ( $2n = 4x = 48$ ) species that displays tetrasomic inheritance. As a consequence, genetic analysis has proved difficult and potato breeding has remained empirical and genetically unsophisticated, particularly as most economically important traits are quantitative, displaying continuous variation. Breeders make crosses between parents with complementary traits in order to generate genetic variation. Phenotypic selection is practised over a number of vegetative generations for clones with as many desirable characteristics as possible for release as new cultivars. Statistically significant offspring on mid-parent regressions have provided evidence of heritable variation, and the slopes of the regression lines have provided measures of heritabilities. Likewise, assessment of offspring over vegetative generations has provided estimates of the genetic variation within progenies. However, in order to design more efficient breeding programmes, knowledge is required of the number of genes segregating, their chromosomal locations, and the magnitudes of their effects.

In recent years progress has been made in developing the theory of linkage analysis and quantitative trait locus (QTL) mapping in autotetraploid species for a full-sib family derived by crossing two parents (Luo et al. 2001; Hackett et al. 2001). This theory is based on the simplest

---

Communicated by M. Kearsley.

---

J. E. Bradshaw (✉) · B. Pande · R. Waugh · G. J. Bryan  
SCRI, Invergowrie, Dundee DD2 5DA, UK  
e-mail: john.bradshaw@scri.ac.uk

C. A. Hackett  
Biomathematics and Statistics Scotland,  
SCRI, Invergowrie, Dundee DD2 5DA, UK

situation that can give tetrasomic inheritance, namely random pairing of four homologous chromosomes to give two pairs of bivalents at meiosis. In practice, many departures from this simple situation could occur, in particular: multivalents and double reduction, lack of complete homology between chromosomes and hence departures from random pairing, and distorted segregation due to differential fertility and viability. The limited cytological evidence available suggests that bivalents predominate in potato, although low frequencies of quadrivalents, trivalents and univalents also occur (Swaminathan and Howard 1953). Hence when analysing real data under the assumption of crossing over restricted to bivalents, some anomalous progeny may occur and need to be eliminated from the analysis and this may bias the estimation of recombination frequencies and QTL effects. Satisfactory models of double reduction are not available because its effect on the recombination frequency depends upon whether a chain or a ring quadrivalent occurs during meiosis, and the position of the centromere when double crossovers occur.

The simple theory has been applied in potato to a cross between processing clone 12601ab1 (nematode resistance from *S. tuberosum* subsp. *andigena*) and table cultivar Stirling (late blight resistance from *S. demissum*) (Bradshaw et al. 2004; Bryan et al. 2004). QTLs of large effect were found for resistance to the white potato cyst nematode [*Globodera pallida* (Stone)] on chromosomes IV and XI (Bryan et al. 2004) and to late blight [*Phytophthora infestans* (Mont.) de Bary] on chromosome IV, and for susceptibility to late blight through early maturity on chromosome V (Bradshaw et al. 2004). This paper extends the analysis of the 12601ab1 × Stirling cross to all the yield, agronomic and quality traits routinely measured in the potato breeding programmes at SCRI. It also uses an improved algorithm to extend the partial linkage map of AFLPs constructed by Meyer et al. (1998) for a sample of 78 clones from the cross to a more extensive one based on the full set of 227 clones. The previously published maps of chromosomes IV, V and XI (Bradshaw et al. 2004; Bryan et al. 2004) were also reanalysed resulting in some map improvements. The implications for practical potato breeding and for practical linkage and QTL analysis in autotetraploids are discussed.

## Materials and methods

### Plant material

The cross 12601ab1 × Stirling was one of a diallel set of crosses made in 1992 (Bradshaw et al. 1995). The reciprocal cross was made in 1993. Seedlings were raised in a

glasshouse during the summer of 1994 and their tubers harvested in mid-September. Subsequent molecular marker analysis of 272 clones highlighted a distinct group of 45 clones from 12601ab1 × Stirling with an unusually low number of paternal bands. These clones are now thought likely to be selfs and the paternal bands to be artefacts. They were omitted from the linkage and QTL analyses, leaving 227 clones, of which 169 and 58 had 12601ab1 and Stirling, respectively, as female parent. The two parents and progeny clones were maintained in a glasshouse in 1995 and 1996, and then from 1997 to 2000 at a high-grade seed site (Blythbank Farm, West Linton, Peeblesshire) to provide tubers for yield trials.

### Yield (ware) trials

The population was grown at a ware site in 1999 (Mynefield Farm, Dundee) and again in 2000 and 2001 (Gourdie Farm, Dundee). Each year the trial had a randomised complete block design with two blocks and single-drill plots of five tubers spaced 45 cm apart. The distance between adjacent drills was 75 cm in 1999 and 90 cm in 2000 and 2001. There were enough tubers to include 217 and 162 clones in the first and second blocks in 1999, 219 clones in both blocks in 2000 and 225 clones in both blocks in 2001. Three replicates of each of the two parents were included in every block. The trials were planted on 26 April 1999, 1 May 2000 and 4 May 2001. Fertilizer, herbicide, aphicide and fungicide (for control of late blight) applications were standard for a ware crop in S.E. Scotland.

### Traits scored

Emergence (Emer) was scored on a one (none) to nine (all plants in a plot well established) scale on 26 May 1999, 1 June 2000 and 30 May 2001.

Maturity (Mat) was scored on a one (all plants in a plot dead) to nine (all plants still green) scale on 12 August 1999 and 22 August 2000. In 2001, the trial was still green at burn-down. Flower colour (blue like 12601ab1 or white like Stirling) was recorded for all clones that flowered over the 3 years of trials (201 of 225). The trials were burnt down with split applications of sulphuric acid, the first on 22 August 1999, 27 August 2000 and 19 August 2001.

Harvesting was done on 15 September 1999, 15 September 2000 and 24 September 2001 with a single-row digger. The tubers from each plot were placed in a net bag, taken into a potato store, and weighed over the period 26–29 October 1999, 23–25 October 2000 and 6–10 December 2001. The fresh weights in kg per plot (Yield) were

recorded automatically by an Avery balance connected to an Epson HX-20 portable computer. At the same time in 1999 and 2000, and on 2 October 2001, samples of three (two in 1999) and five (four in 1999) tubers were taken from each plot and stored at 4 and 10°C, respectively, until 28 February 2000, 29 January 2001 and 28 January 2002 (i.e. a minimum of 3 months).

Dry matter content (DM) was determined in 1999 by measuring the specific gravity of a 3.63 kg sample of tubers with a hydrometer at the same time as the plots were weighed. In contrast, in 2000 and 2001 the specific gravity (SG) of the five tubers which had been stored at 10°C was determined on 29 and 30 January 2001 and from 1 to 18 February 2002 by weighing them in air ( $x$ ) and then under water ( $y$ ) [ $SG = x/(x-y)$ ]. Dry matter content was then estimated from the relationship with specific gravity found by von Scheele et al. (1937).

Visual assessments of the tubers from each plot were made on 10 and 11 November 1999, from 23 to 25 October 2000 and from 6 to 10 December 2001. Tuber size (Size), regularity of tuber shape (Shape), and resistance to growth cracks (Grow) were scored on a one (low) to nine (high) scale. Tubers with deep eyes would get a low score for Shape, but so would ones that showed the more common occurrence of departures from a regular round, oval or long oval shape. In 2001 resistance to common scab (Scab) [*Streptomyces scabies* (Thaxt.) Waksman and Henrici] was scored on a one (susceptible) to nine (resistant) scale. Three large tubers from each plot were cut open to check for internal defects (incipient hollow heart, hollow heart, internal necrosis and flecking) and the sample given an internal condition (IC) score on a one (all three tubers with severe defect such as hollow heart) to nine (no defects) scale. The plots were then given two overall scores on a one to nine scale of increasing preference, one (OverY) which included yield but not internal condition and the other (Over) which included internal condition but not yield.

Sprouting (Sprt), keeping quality (Keep) and after-cooking blackening (ACB) were determined between 6 and 10 March 2000, on 29 and 30 January 2001 (but not keeping quality) and from 1 to 18 February 2002 on the samples stored at 10°C. Sprouting was scored on a one (extensive) to nine (none) scale and keeping quality was also scored on a one (tubers very soft) to nine (tubers still hard) scale. Two tubers from each plot were steamed for 45 min and then cut open. ACB was scored 4–5 h later on a one (completely black) to nine (none) scale.

Fry colour of the 4°C (Fry4) samples was determined from 28 February to 2 March 2000, on 12 and 13 February 2001, and from 28 to 31 January 2002. The 10°C (Fry10) samples were done from 6 to 10 March 2000, on 14 and 15 February 2001, and from 1 to 18 February 2002. Each tuber (two in 1999 and three in 2000 and 2001) was cut in half

and a 1–1.5 mm-thick slice was taken and fried in vegetable oil at 175°C in a thermostatically controlled chip fryer until all of the water had boiled off (bubbling ceased). Colour was assessed on a 1–9 scale by comparison with a standardized chart (1 = extremely dark to 9 = extremely pale) where 5 and above is an acceptably light colour (Mackay and Dale 1990).

#### Genomic DNA isolation and molecular marker assays

Details of the genomic DNA isolation from leaves of the parents and progeny clones, and of the AFLP and SSR assays, can be found in Bradshaw et al. (2004). Parents of an ultra high density (UHD) mapping population, SH and RH (Van Os et al. <http://www.genetics.org/cgi/content/abstract/173/2/1075>), were also included in the marker assays to enable identification of AFLP alleles which co-migrated with previously mapped markers and hence to help with chromosomal identification of linkage groups (LGs), as done by Rouppe van der Voort et al. (1997). AFLP markers were derived from 33 *PstI/MseI* combinations and 5 *EcoRI/MseI* combinations. AFLP fragment nomenclature is PABMDEF\_XYZ and EABCMDEF\_XYZ where AB, ABC and DEF are the selective base extensions on the *PstI*, *EcoRI* and *MseI* primers, respectively, and XYZ is the size of the AFLP fragment in basepairs. Autoradiograms were scored manually, each band being scored as a locus with a dominant (present) versus recessive (absent) allele. Intensity differences were observed in the segregating bands, but visual interpretation was not considered reliable enough to assign allelic dosage to a given individual in the population. Summary information on the six SSRs used to identify LGs is given in Table 1.

#### Statistical analyses

GENSTAT 5 Release 3 (GENSTAT 5 Committee 1993) was used to analyse the data by the method of residual maximum likelihood (REML) with years, clones and blocks as random effects.

The heritability ( $h^2$ ) of clone means was estimated as

$$h^2 = a\sigma_c^2 / (a\sigma_c^2 + b\sigma_{cy}^2 + \sigma^2),$$

where  $\sigma_c^2$ ,  $\sigma_{cy}^2$  and  $\sigma^2$  are the variance components for clones, clones  $\times$  years interaction and residual plot to plot variation;  $a \leq 6$  (number of years  $\times$  number of replicates) and  $b \leq 2$  (number of replicates).

Clone means over years were based on the best linear unbiased prediction (BLUP) of clone effects from the REML analysis.

**Table 1** Summary information on the six SSRs used to identify LGs IV, V and XI including length in base pairs and optimum annealing temperature

SSR	Repeat	LG	Sequence	Length (bp)	Annealing temperature
STM 5140 (est p104b1)	(AAT) <sub>7</sub>	IV	F: GCTATTGTTGCAGATAATACG R: GCCATGCACTAATCTTTGA	180–190	45°C
STM 3160	(AG) <sub>25</sub>	IV	F: ACTCAGAACACCGAATGG R: CTCCACTACTGGTCAAATCC	105–127	54°C
STM 3179	(AT) <sub>10</sub>	V	F: ACCATAACCATCATCGTCTAC R: GATAGTGATTGGTGGTGATTC	168–185	54°C
STM 5148	(GAA) <sub>17</sub>	V	F: TCTTCTTGATGACAGCTTCG R: ACCTCAGATAGTTGCCATGTCA	380–440	55°C
STM 5109 (estp2OB8)	(TA) <sub>9</sub>	XI	F: ACAGCTTTACTCTGCTCAACAA R: TGACGGCGTTATTACAAAC	208–212	57°C
STM 5130 (estp21B15)	(CGG) <sub>5</sub>	XI	F: AAAGTACAGCGAAGATGACGAC R: TTACCTTTGCAACCTTGCC	240–250	57°C

### Construction of linkage map of markers

The reader unfamiliar with autotetraploid linkage analysis may find it useful to refer to Fig. 1 where the four homologous chromosomes present in each parent are shown for LG VI. The most likely dosage for each marker, conditional on the observed parent and offspring phenotypes, was identified using the approach of Luo et al. (2000). A preliminary cluster analysis was done on each parent for the markers identified as simplex, using the simple matching coefficient, which is equivalent to the recombination frequency for simplex coupling linkages. This identified markers on the same chromosome. All simplex, duplex and multiallelic markers were then analysed by group average cluster analysis to partition them into LGs (Luo et al. 2001), analysing markers from the two parents separately. For each LG, recombination frequencies and lod scores between every pair of markers were calculated for all possible phases using the EM algorithm, as described by Luo et al. (2001). The recombination frequencies and lod scores from the phase with the highest likelihood were used to order the markers. A simulated annealing algorithm (Hackett et al. 2003) was used to identify the order with the minimum value of the weighted least squares criterion (Stam 1993) and to calculate map distances between the markers. Once the LGs were established for each parent, significant coupling linkages between simplex and double-simplex markers (simplex markers present in both parents) were identified as a means of associating LGs from the two parents. Double-simplex markers are not shown in Fig. 1, but simplex markers linked to double-simplex ones are in bold and arrows indicate linkage to a common double-simplex marker. Repulsion linkages between simplex and double-simplex markers were excluded, as were linkages involving

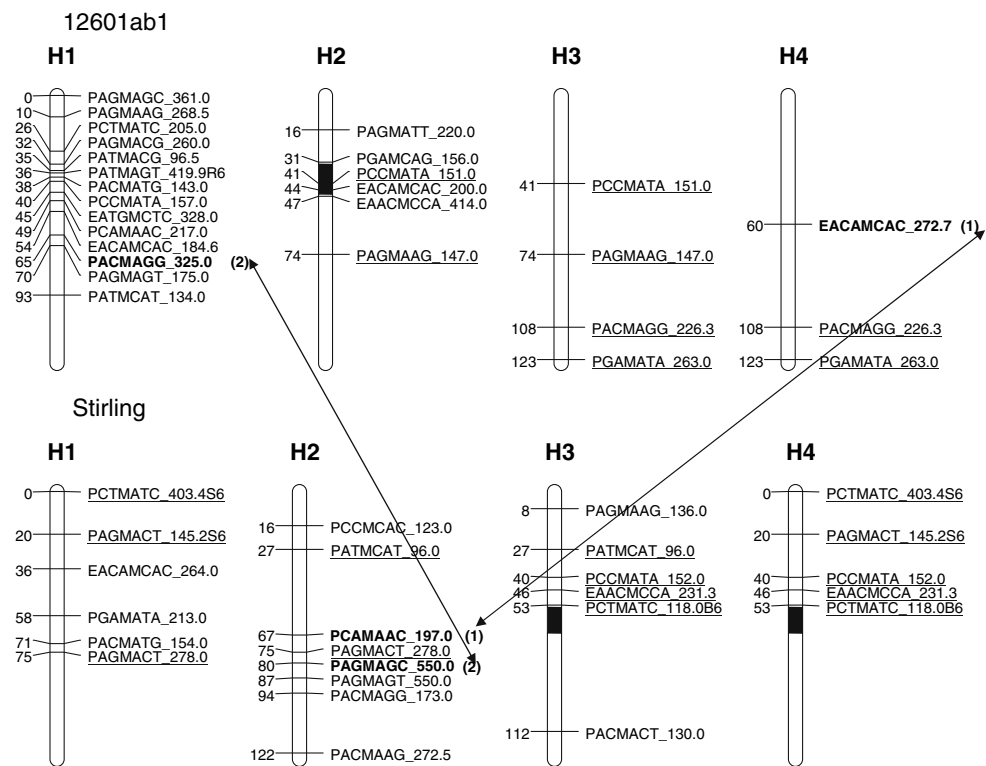
dominant markers present in both parents where the dosage was higher than double-simplex, because they are very uninformative about recombination. A permutation test (Churchill and Doerge 1994) was used to establish a 99% threshold for declaring a simplex to double-simplex linkage significant for this set of marker information; the results reported here have a chi-squared statistic >16 from a test of independent segregation with 1 degree of freedom. This corresponds to a significance level of 0.00006 in an individual test. All of these analyses were done using the TetraploidMap software programme of Hackett and Luo (2003) which is available from the authors.

Two chromosomal identifications of LGs were done by the presence of mapped SSR markers, one was done by the presence of locus *P* for blue flower colour (Van Eck et al. 1993) and others were done on the basis of two or more AFLP markers co-migrating with ones in the SH × RH UHD reference population (<http://potatodbase.dpw.wau.nl/UHDdata.html> and <http://www.dpw.wageningen-ur.nl/uhd/top50.html>). Such markers are coded R (present in RH), S (present in SH) and B (present in both). If required, their bin numbers can be found in the reference population database just given.

### Analysis of trait data for QTLs

An interval mapping approach was used as described by Hackett et al. (2001). QTL models were fitted for the two parents separately, as there were insufficient bridging markers to align the two maps reliably. A normal mixture model with separate means for each of the six possible QTL genotypes (gametes Q<sub>1</sub>Q<sub>2</sub>, Q<sub>1</sub>Q<sub>3</sub>, Q<sub>1</sub>Q<sub>4</sub>, Q<sub>2</sub>Q<sub>3</sub>, Q<sub>2</sub>Q<sub>4</sub> and Q<sub>3</sub>Q<sub>4</sub>) derived from the parent (Q<sub>1</sub>Q<sub>2</sub>Q<sub>3</sub>Q<sub>4</sub>) was fitted at a 2 cM grid of locations along the chromosome. Based

**Fig. 1** Linkage Group VI showing the four homologous chromosomes (H1 to H4) in each parent and QTL alleles for ACB (*shaded*). Duplex markers are underlined and simplex markers linked to double-simplex ones in coupling are shown in *bold*, but the double-simplex ones themselves are not shown. *Arrows* show that two simplex markers are linked to the same double-simplex marker and hence align the two parents. *Markers* co-migrating with ones in the UHD reference population are *marked R, S and B*. H2 in 12601ab1 has a QTL allele for increased blackening and H3 and H4 in Stirling have a dominant QTL allele for decreased blackening



on a small number of permutation tests (100 permutations for several trait and chromosome combinations), a QTL was declared to be significant ( $P < 0.05$ ) for a lod  $> 4.0$ , and non-significant for a lod  $< 3.0$ . For lod scores between 3.0 and 4.0, a further 100 permutations for that trait and chromosome combination were performed to obtain an improved estimate of the significance threshold.

If a QTL was found to be significant, alternative models were fitted at the same location and compared using a likelihood ratio test. Let the QTL genotype for an offspring inheriting homologous chromosomes  $i$  and  $j$  from the parent being analysed be denoted by  $Q_{ij}$ . Ten possible alternative models were explored, comparing the full model of six QTL genotypes to either (a) a simplex allele (Q) on each homologous chromosome in turn (e.g.  $Q_{12} + Q_{13} + Q_{14}$  versus  $Q_{23} + Q_{24} + Q_{34}$  to test for a simplex allele on homologue 1, reported as +H1 versus -H1), making four models, or (b) a dominant duplex allele (QQ) on pairs of homologous chromosomes in turn (e.g.  $Q_{12} + Q_{13} + Q_{14} + Q_{23} + Q_{24}$  versus  $Q_{34}$  to test for a dominant allele on homologues 1 and 2, absent from homologues 3 and 4, and reported as H1/2 versus H34), making a further six models. H1/2 means that offspring have inherited homologues 1 and/or 2 and H34 means that they have inherited homologues 3 and 4. This is illustrated in Fig. 1 with QTLs for ACB. In 12601ab1 there is a simplex allele on H2 for more blackening (lower score)

which is reported in Table 5 as +H2 4.8, -H2 5.2. In Stirling there is a dominant duplex allele on H3 and H4 for less blackening (higher score) which is reported in Table 5 as H3/4 5.1, H12 4.6. If one of the alternative models was not significantly different from the full model, the interval mapping was repeated using the simpler model. Permutation tests for the alternative model were performed by fitting each of the ten alternative models to each permuted set of trait values and selecting the maximum lod score over all the models. Finally, if all of the alternative models were a significantly worse fit than the full model, and the genotypes associated with the largest and smallest trait means involved all four homologues, an additive model QQ, Qq, qq was investigated with the mean trait value for Qq at the mid-point of the trait values for QQ and qq, for example H12 = QQ, H34 = qq, H13 = H14 = H23 = H24 = Qq, where H12 means homologues 1 and 2, etc.

## Results

### Phenotypic data

The heritabilities were all moderately-high to high with between 54 and 92% of the variation in clone means over years and replicates due to genetic differences between clones (Table 2). The lowest heritabilities were for



common scab, which was severe enough to be assessed in only one of the 3 years, and internal condition, keeping quality and ACB where clones  $\times$  years interaction as well as plot-to-plot variation contributed to the lower values. In contrast, maturity, which could not be assessed in 1 year with a late season, nevertheless had the highest heritability for the 2 years when it could be assessed.

The two parents had similar scores for emergence, maturity, growth cracks, internal condition and ACB (Table 3). The population means were also similar to the scores of the two parents, but the release of genetic variation can be seen in the large population ranges. There were however few growth cracks and few internal defects with 214 and 135 of 225 clones, respectively, scoring 8.5 or more for growth cracks and internal condition. The other three traits displayed continuous, unimodal, approximately normal distributions and the distributions for maturity and ACB are shown in Fig. 2 as QTLs of interest were found.

The two parents differed for the other traits (Table 3), Stirling having higher Yield, lower DM, larger Size, better Shape, better resistance to common scab, higher OverY and Over scores, but more sprouting, worse keeping quality and worse Fry4 and Fry10. The population mean was either intermediate between the two parents (DM, common scab, sprouting, keeping quality and Fry4 and Fry10) or close to the worst parent (Yield, Size, Shape, OverY and Over). The distributions were continuous, unimodal and approximately normal. Those for Yield, Shape, sprouting and Fry4 are shown in Fig. 2 as QTLs of interest were found.

**Table 2** Components of variance  $\sigma_c^2$ ,  $\sigma_{cy}^2$  and  $\sigma^2$  for clones, clones  $\times$  years interaction and residual variation and heritability  $h^2$

Trait	$\sigma_c^2$	$\sigma_{cy}^2$	$\sigma^2$	$h^2$
Emergence	2.119	0.498	1.126	0.849
Maturity	2.476	0.160	0.507	0.916
Yield	4.440	0.627	1.640	0.896
DM	1.677	0.560	1.068	0.807
Size	0.6862	0.0962	0.3648	0.873
Shape	0.4417	0.0530	0.3454	0.845
Growth cracks	0.1450	0.0872	0.0776	0.766
Common scab	0.957	–	0.982	0.660
Internal condition	0.502	0.534	1.333	0.544
OverY	0.7700	0.1298	0.4048	0.867
Over	0.6604	0.0863	0.3881	0.868
Sprouting	1.796	0.683	0.926	0.816
Keeping quality	0.8026	0.4660	0.6833	0.653
ACB	0.4563	0.4108	0.9154	0.599
Fry4	0.5558	0.1585	0.5149	0.790
Fry10	0.4443	0.1541	0.4001	0.778

## Correlations between traits

The correlations between traits are shown in Table 4. With over 200 degrees of freedom, a correlation coefficient as low as  $r = 0.138$  would be statistically significant at  $P < 0.05$ . However, only correlations with  $r > 0.40$  are shown, as lower values are not considered of sufficient biological interest. The two visual preference scores (OverY and Over) were highly correlated and both were correlated with Yield, despite the fact that Over was not meant to include yield. In other words, scorers found it almost impossible to ignore yield. Hence the three traits are all assessments of yield. Tuber size is a component of yield and hence not surprisingly Size was correlated with Yield and the preference scores (OverY and Over). Fry colour after tuber storage at 4°C (Fry4) was correlated with fry colour after storage at 10°C (Fry10). Keeping quality was correlated with lack of sprouting. Finally fast emergence was correlated with high Yield (and OverY) and early sprouting in store (i.e. lack of dormancy).

## Linkage map

For the 12601ab1 parent, 326 AFLP markers were scored. Of these, 208 segregated in a 1:1 ratio ( $P < 0.001$ ) and 80 segregated in a 5:1 ratio ( $P < 0.01$ ). Preliminary analysis showed that the mapping process is less robust to distorted segregation ratios in duplex markers, and hence the more stringent threshold for these (i.e. more are rejected as significant departures by setting  $P < 0.01$  rather than  $P < 0.001$  because null hypothesis is no distortion). Together with SSR markers, a total of 293 markers were analysed for the map of 12601ab1. At a recombination frequency of 0.3, the simplex markers formed 85 LGs composed of markers in coupling phase (coupling LGs), with 46 isolated markers and 39 groups of two or more markers. Inspection of the dendrogram from group average cluster analysis indicated a preliminary division into 13 homology groups, with five to 51 markers, although three groups were subsequently divided following more detailed assessments of recombination frequencies in these groups. Any marker that was a poor fit in the ordering was moved to another group or omitted.

For Stirling, 260 AFLP markers were scored. Of these, 171 segregated in a 1:1 ratio ( $P < 0.001$ ) and 41 segregated in a 5:1 ratio with  $P < 0.01$ . Including the SSRs, 221 markers were analysed for the Stirling map. At a recombination frequency of 0.3, the simplex markers formed 74 coupling LGs, with 39 isolated markers and 35 groups of two or more. Cluster analysis separated the markers into 11 homology groups, with 10–32 markers, although one group was subsequently divided.

**Table 3** Parents and F1 population mean and extremes

Trait	12601ab1 mean	Stirling mean	Population mean	Population extremes
Emergence	6.014	5.653	5.630	1.582–8.359
Maturity	4.604	4.958	4.767	1.654–7.506
Yield	7.250	12.01	7.747	1.425–12.818
DM	25.40	20.87	22.19	19.01–26.12
Size	3.792	6.416	4.222	1.636–6.245
Shape	4.528	5.222	4.270	2.414–6.030
Growth cracks	9.000	8.778	8.890	4.628–9.000
Common scab	4.500	6.583	5.278	2.616–6.746
Internal condition	8.472	8.111	8.350	4.444–8.840
OverY	3.014	6.153	3.217	1.148–5.440
Over	3.180	6.014	3.242	1.459–5.535
Sprouting	6.611	2.028	3.660	1.172–8.062
Keeping quality	6.667	4.417	5.117	2.473–6.959
ACB	5.361	5.444	5.003	3.012–6.791
Fry4	6.050	2.683	4.091	2.371–6.544
Fry10	7.417	5.333	6.351	4.554–8.025

#### Chromosomes identified by SSRs

**Chromosome IV** SSR markers STM3160 and STM5140 identify and align the 12601ab1 and Stirling maps of chromosome IV (Fig. 3a). Both maps also contain a marker (S4) present on chromosome IV of the UHD map, and seven double-simplex markers are linked to both maps, one of which is also on chromosome IV of the UHD map. Markers are located on all four homologous chromosomes for both parents.

**Chromosome V** SSR markers STM3179 and STM5148 identify and align the 12601ab1 and Stirling maps of chromosome V (Fig. 3b). The four STM3179 alleles (a, b, c and d) were mapped as separate alleles and not as a single locus. Hence the map distances between a and c, and b and d are not zero due to sampling effects. The 12601ab1 map also has two closely linked markers (S5) which are also closely linked on chromosome V of the UHD map, and the Stirling map has one marker in common (R5). However, it should be noted that both parents have a marker from another chromosome on the UHD map (R3 and S12). Three double-simplex markers are linked to both maps. Again markers are located on all four homologous chromosomes for both parents, although one chromosome of Stirling has only two markers.

#### Chromosome identified by flower colour locus *P*

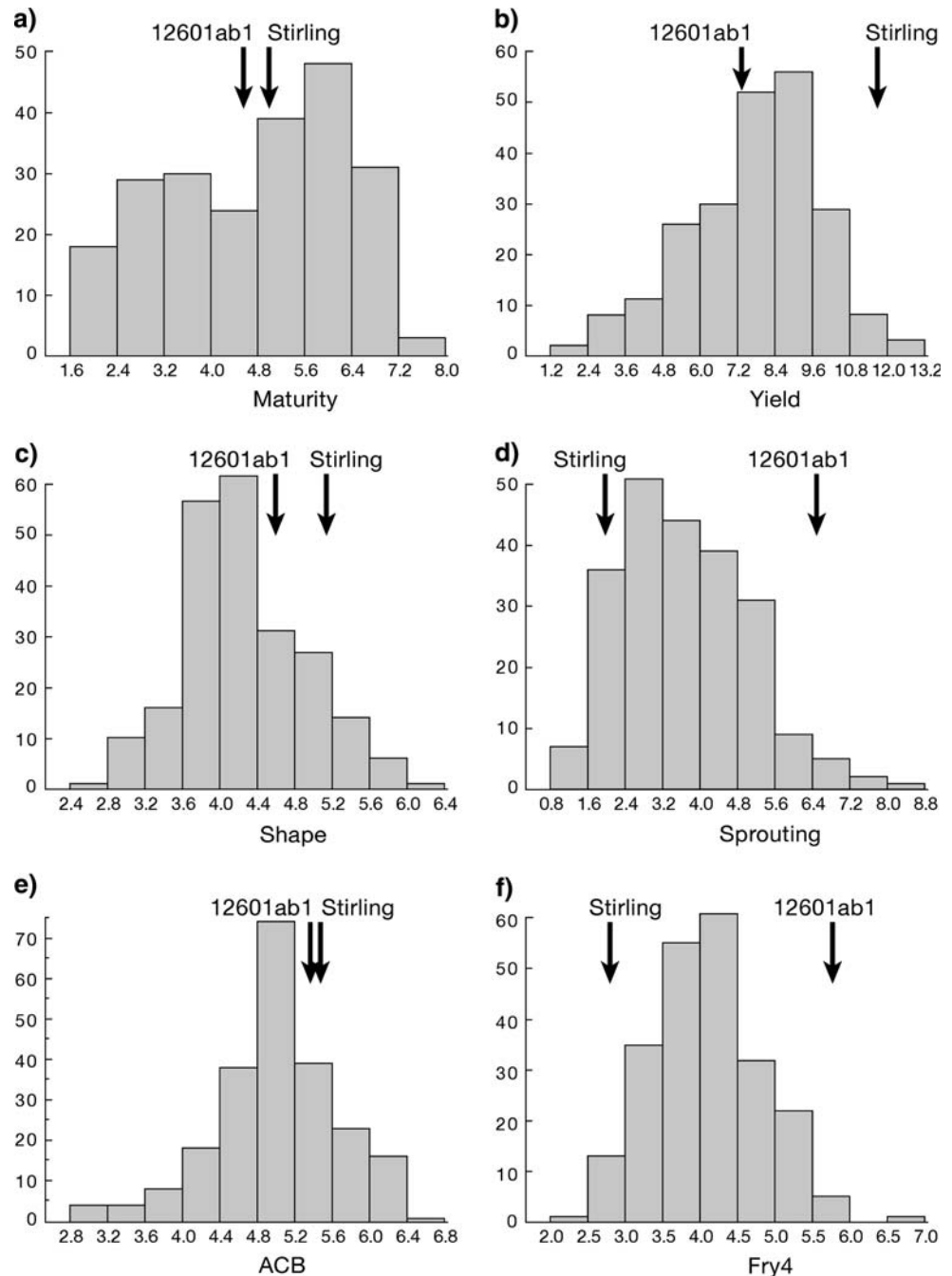
**Chromosome XI** The flower colour locus *fc* (Fig. 3c), assumed to be *P*, maps to a LG from 12601ab1 which has

four homologous chromosomes, although two of these have only one marker. There were no markers from the UHD chromosome XI present in 12601ab1 only. Two double-simplex markers link this to a group of four homologous chromosomes from Stirling, one of which has only one marker. This is shown in Fig. 3c as XIa. No markers are in common with the UHD map. However, one of the double-simplex markers also shows a weaker linkage ( $\chi^2_1 = 13.3$ ) to a second LG from Stirling with four homologous chromosomes and five markers from the UHD chromosome XI (one R11, three S11 and one B11). S11 markers PACMATA\_345.5 and PGAMATC\_207.0 are closely linked whereas PACMATG\_248.9 is 26 cM away. In SH, the first two markers are in the same bin and the third one is in an adjacent bin. This LG also had a marker (B12) from UHD chromosome XII. These maps are shown in Fig. 3c as Stirling XIb. A final complication is a duplex SSR allele (STM5109b) for a locus on chromosome XI that is linked to another group of mainly duplex markers from 12601ab1, on two homologous chromosomes. However, this SSR allele shows no significant linkages with any other markers. This group is shown in Fig. 3c as 12601ab1 XIc. In summary, LGs from 12601ab1 and Stirling have been identified as chromosome XI, but these cannot be combined for either parent, and these difficulties prevent the presentation of final linkage maps for this chromosome.

#### Chromosomes identified by markers from UHD map

**Chromosome I** A large group of 46 markers from 12601ab1, with seven markers from chromosome I of the UHD map, were merged by the cluster analysis. However, no single marker order was a good fit to this group, and a further cluster analysis showed that these markers separated into three clusters, with two, 20 and 24 markers. The cluster of two markers showed no significant associations to any other markers, but the larger clusters could both be ordered without difficulties. The resulting maps, designated chromosome Ia and Ib, are shown in Fig. 3d, and have four (1R1, 2S1 and 1B) and three (3S1) markers from the UHD chromosome I, respectively (and one R7). Chromosome Ia contains mainly duplex markers and has only two homologous chromosomes, while chromosome Ib has markers on all four homologues. There are also two LGs in Stirling with markers from the UHD chromosome I, but there is no evidence from this population to associate these. They will be referred to as Stirling chromosomes Ia and Ib, and are shown in Fig. 3d. Chromosome Ia has markers on all four homologous chromosomes, with five markers (2R1, 2S1 and 1B1) from the UHD chromosome I. Chromosome Ib has three markers (2S1 and 1B1) from the UHD chromosome I, and several markers on two homologous

**Fig. 2** Histograms **a** Maturity, **b** Yield, **c** Shape, **d** Sprouting, **e** ACB and **f** Fry4



chromosomes, but only a single marker on the other two. Three double-simplex markers, one also from the UHD chromosome I, associate the 12601ab1 and Stirling pairs Ia-Ia, Ib-Ia and Ib-Ib. Where comparisons can be made, map distances between R, S and B markers are greater than found on the UHD map.

**Chromosome II** A group of markers from 12601ab1, with four markers (3R2 and 1S2) from the UHD

chromosome II (and one S7), formed a LG with four homologous chromosomes, although one of these had only a single marker. The four markers in common with the UHD map were in the same order, but further apart than on the UHD map. A single double-simplex marker associates the 12601ab1 LG with one from Stirling, which has two markers (R2 and S2) from the UHD chromosome II. This map has markers on all four homologous chromosomes. These maps are shown in Fig. 3e.



**Table 4** Correlation coefficients between traits for those with  $|r| > 0.4$ 

Traits	$r$
OverY and Over	0.941
OverY and Yield	0.857
Keeping quality and Sprouting resistance	0.768
Over and Yield	0.718
Fry4 and Fry10	0.701
Size and OverY	0.619
Size and Yield	0.609
Size and Over	0.606
Emergence and Yield	0.579
Emergence and Sprouting resistance	-0.460
Emergence and OverY	0.451

**Chromosome III** Markers from 12601ab1, with three markers (1S3 and 2B3) from the UHD chromosome III, formed a LG with four homologous chromosomes. The two B3 markers were in the same bin but 14 cM apart in 12601ab1. This LG is associated by two double-simplex markers to a group of four homologous chromosomes from Stirling. There were no markers from the UHD chromosome III present in Stirling only. These maps are shown in Fig. 3f. A double-simplex marker from UHD chromosome III was significantly linked to the Stirling group. Its most significant association to 12601ab1 was to this group, but this was not significant by the permutation test, although  $P < 0.001$ .

**Chromosome VI** A LG from 12601ab1, with one marker (R6) from the UHD chromosome VI, is linked by two double-simplex markers to a LG from Stirling with three markers (2S6 and 1B6) from the UHD chromosome VI. The two S6 markers are in the same bin, but 20 cM apart in Stirling, and B6 is much closer to them in the UHD map. Both LGs have four homologous chromosomes. The maps are shown in Figs. 1 and 3g.

**Chromosome VIII** A LG from 12601ab1 has two markers (S8 and B8) from the UHD chromosome VIII. This group has four homologous chromosomes. It is linked by two double-simplex markers to a group from Stirling, consisting of a single homologous chromosome and one further marker linked in repulsion with these. Only one marker from the UHD chromosome VIII was present in Stirling only, and this shows no linkage to this group. The maps are shown in Fig. 3h. The designation of these as chromosome VIII is regarded as tentative until further marker data are obtained.

### Other groups

**A, possibly chromosome IX** Only one marker (R9) from the UHD chromosome IX was found, a duplex marker present in the 12601 parent. This was linked in repulsion to a single homologous chromosome. Two double-simplex markers linked this in turn to a group from Stirling, which had four homologous chromosomes, although one had only a single marker. These maps are shown in Fig. 3i.

**B, possibly chromosome XII** A LG from 12601ab1 has one marker (S12) from the UHD chromosome XII. This group has four homologous chromosomes. It is linked by three double-simplex markers to a group from Stirling, consisting of four homologous chromosomes, but the one marker from the UHD map was an S8 (chromosome VIII). The maps are shown in Fig. 3j.

**C** A LG from 12601ab1 has one marker (R12) from the UHD chromosome XII but a previous analysis (Bryan et al. 2004) provided some evidence for this LG being linked to STM5109b on chromosome XI (see chromosome XI above). However, in this reanalysis, marker STM5109 caused problems which could not be resolved. The group has four homologous chromosomes. It has two double-simplex markers, but neither of these shows a significant linkage to Stirling. The map is shown in Fig. 3k.

**D** A LG from 12601ab1 with two homologous chromosomes was found with one marker (S1) from the UHD chromosome I. There are no double-simplex markers linked to this group, which is shown in Fig. 3l.

**E** A LG from 12601ab1, with five duplex markers linked in repulsion to a simplex marker, is shown in Fig. 3m. Again one marker (R1) is from the UHD chromosome I.

**F** A single homologous chromosome from 12601ab1 is shown in Fig. 3n.

### QTL mapping

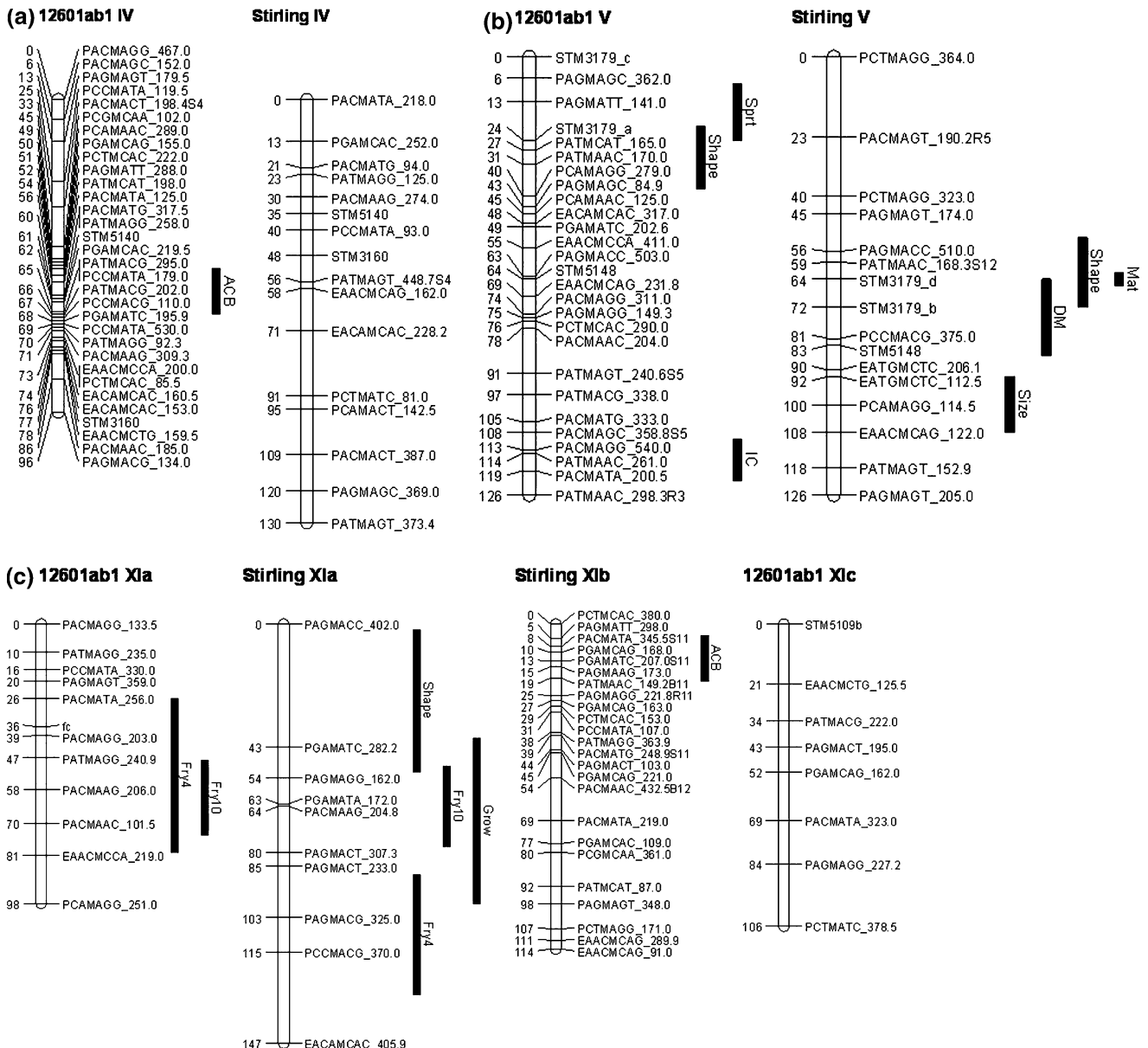
Figure 3 shows the locations of the QTLs, with one-lod support intervals. Table 5 shows the positions, lod scores and percentage phenotypic variance explained for the full model of six QTL genotypes, and for a simpler model if there is one that fits the data. Where a simpler model is

adequate, it can be seen that there is little change in the position of the QTL but a number of examples of lower lod scores. The genotypes with the highest and lowest means are also shown together with their standard errors (SE). Where a simplex QTL allele was found, the closest simplex marker in coupling linkage is also given.

**Maturity**

The largest lod score by far was for Mat on Stirling homology group (=LG) V, where the presence/absence of a

simplex allele on homologue one (H1) explained 54% of the phenotypic variance, and the simplex allele was associated with earlier maturity. This homologue (+H1) was also associated with small effects for better Shape and higher DM, despite Stirling being the lower DM parent. These small effects explain the small but significant correlations between Mat and Shape ( $r = -0.362$ ) and Mat and DM ( $r = -0.236$ ). Once the maturity effects were removed by regression of these two traits on maturity, analysis of the residuals showed no further associations with this region. When the residuals from the regression of Shape on Mat were analysed for 12601ab1 homology group V, the same



**Fig. 3** Linkage Groups and QTLs (shaded). The two parents are shown separately but the four homologous chromosomes are merged into a single map. a LGIV, b LGV, c LGXI, d LGI, e LGII, f LGIII, g LGVI, h LGVIII, i LGA, j LGB, k–n 12601ab1 LGC-F

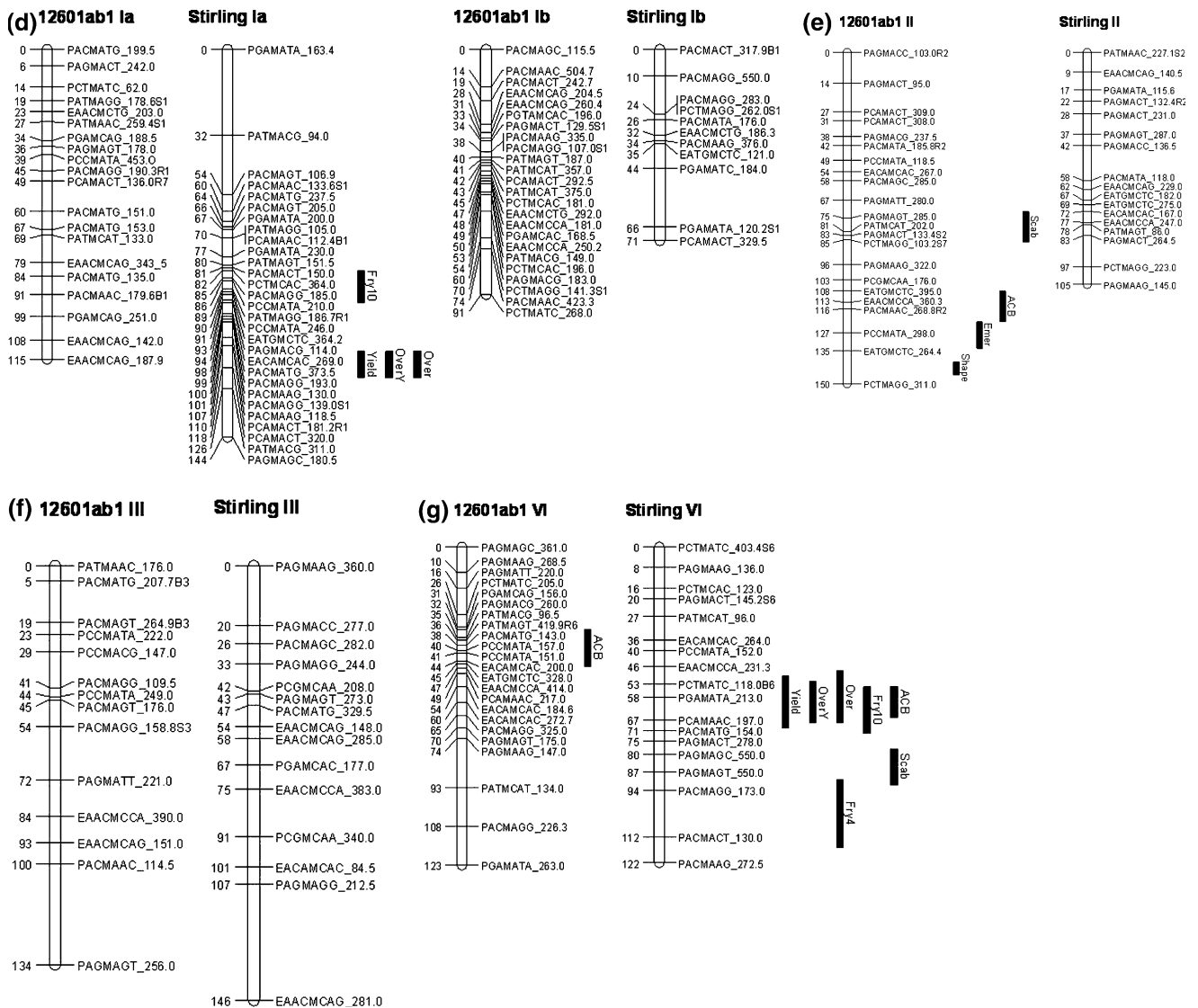


Fig. 3 continued

QTL was detected as in the analysis of Shape, but the lod score increased (see below). A QTL for Size was also mapped to Stirling homology group V, but this showed no relationship with Mat (see below).

*Fresh-weight yield, overall scores, tuber size and emergence*

Quantitative trait loci for Yield, OverY and Over map to the same locations on Stirling homology group VI and Ia. The models are similar in each case, with the highest yields and overall scores for genotypes with homologous chromosomes two and three from homology group VI, and homologous chromosomes one and four from homology group Ia. It can be concluded that OverY and

Over are assessments of Yield and that at the locus on Ia there is dominance for low yield. This is consistent with the population mean being close to the lower yielding parent. QTLs for Fry10 and ACB were mapped to the same region of Stirling homology group VI but different combinations of homologous chromosomes gave the extreme phenotypes. QTLs for Fry4 and Scab were mapped to the same homology group but again different combinations of homologous chromosomes gave the extreme phenotypes.

Size is a component of Yield and the two traits are correlated. However, the two QTLs which were detected for Size are in different homology groups to Yield, OverY and Over. Yield and OverY were also correlated with Emer, but again, the QTLs which were detected for Emer are in different homology groups.

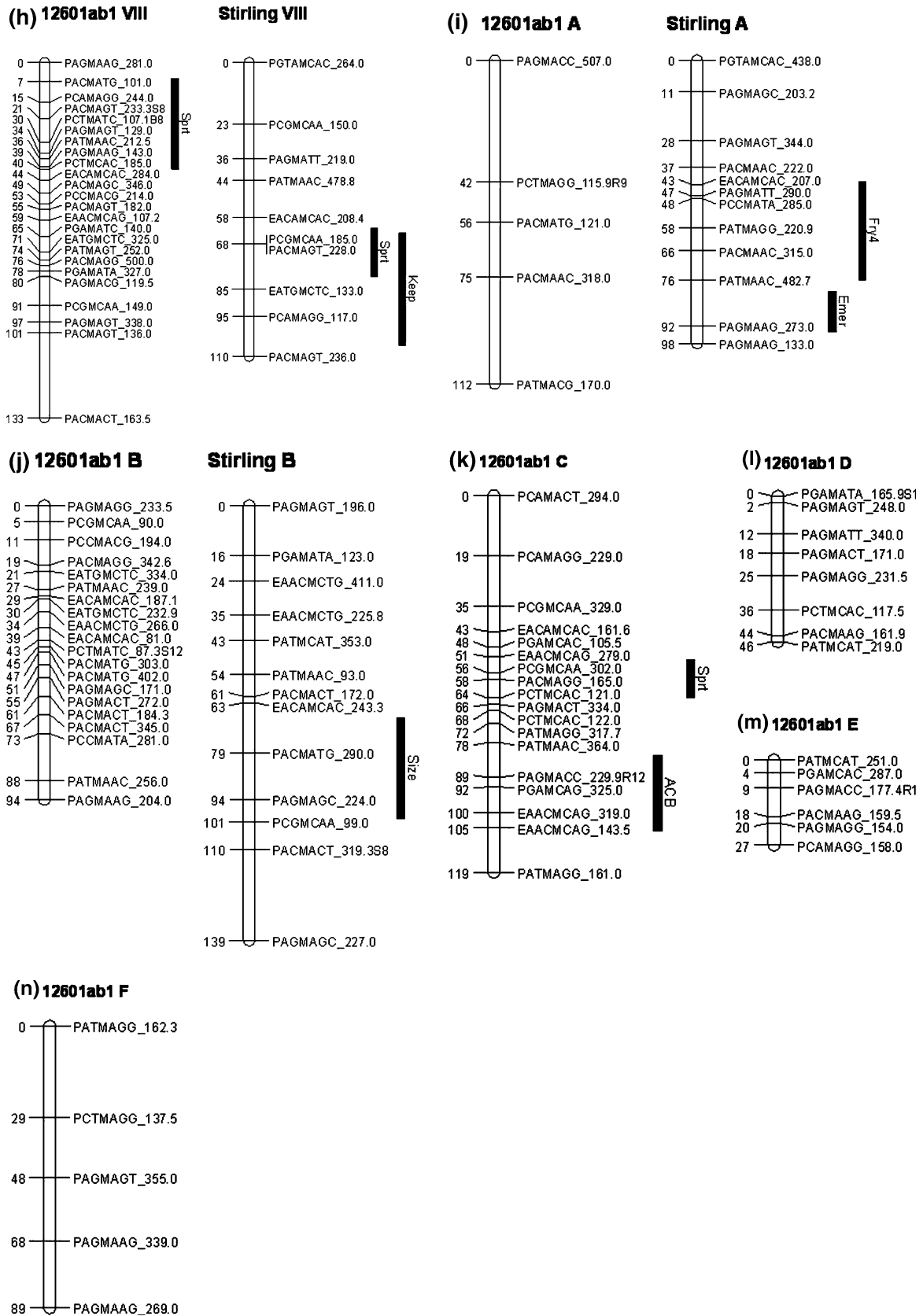


Fig. 3 continued

**Table 5** Estimated parameters from QTL analysis where *HG* homology group and  $R^2 = \%$  phenotypic variance explained—if a simpler model is not significantly different from the full model, its parameters are shown on the next line, where +H1 is presence of homologous chromosome 1 (Q) and simplex marker is closest in

coupling, –H1 is absence of homologue 1 (q), H1/2 is presence of H1 and/or H2 (Q–) and H3/4 is presence of H3 and H4 (qq). Otherwise H1/2 is presence of H1 and H2 and means genotype  $Q_{12}$ . QQ, qq are used to indicate an additive model

Trait	Parent	HG (=LG)	Position cM	Lod	$R^2$	Simplex marker Highest mean (SE)	Simplex marker Lowest mean (SE)
Mat	Stirling	V	62	33.8	55.5		STM3179_d
			62	32.4	54.4	–H1 5.9 (0.10)	+H1 3.6 (0.10)
DM	Stirling	V	76	4.8	9.7	STM3179_d	
			74	3.9	9.1	+H1 22.6 (0.12)	–H1 21.8 (0.12)
Yield	Stirling	VI	56	5.2	13.3	H23 8.9 (0.28)	H24 6.6 (0.29)
Yield	Stirling	Ia	118	3.1	5.4		
			118	3.0	7.1	H14 8.9 (0.32)	H2/3 7.5 (0.16)
OverY	Stirling	VI	56	6.0	16.5	H23 3.8 (0.11)	H24 2.8 (0.12)
OverY	Stirling	Ia	118	4.4	8.6		
			118	3.9	9.7	H14 3.7 (0.13)	H2/3 3.1 (0.06)
Over	Stirling	VI	56	5.1	13.7		
			56	3.9	11.2	H23 3.8 (0.11)	H1/4 3.1 (0.06)
Over	Stirling	Ia	116	4.8	10.3		
			118	4.0	9.9	H14 3.7 (0.12)	H2/3 3.1 (0.06)
Size	Stirling	B	82	3.8	7.9	H24 4.6 (0.12)	H14 3.8 (0.13)
Size	Stirling	V	102	3.5	7.4		
			102	3.1	8.3	H24 4.7 (0.12)	H1/3 4.1 (0.06)
Emer	12601	II	126	4.6	8.0		
			126	3.4	7.0	H2/4 5.8 (0.10)	H13 4.7 (0.26)
Emer Shape	Stirling 12601	A V	94	3.8	10.2	H12 6.1 (0.19)	H13 4.5 (0.27)
			30	7.2	15.5		PATMCAT_165.0
Shape	Stirling	V	30	5.4	13.2	–H4 4.5 (0.06)	+H4 4.1 (0.06)
			62	6.4	11.6	STM3179_d	
Shape	Stirling	XIa	62	6.0	12.1	+H1 4.5 (0.06)	–H1 4.1 (0.06)
			34	4.4	9.5		
Shape	12601	II	30	3.8	10.1	H14 4.8 (0.10)	H2/3 4.2 (0.04)
			146	4.7	8.8		
Sprt	12601	V	146	3.4	7.6	H1/4 4.3 (0.05)	H23 3.7 (0.12)
			14	4.5	9.6	PATMCAT_165.0	
Sprt	12601	VIII	14	3.3	8.1	+H4 4.0 (0.13)	–H4 3.3 (0.12)
			28	3.5	7.2	PAGMAGT_129.0	
Sprt	Stirling	VIII <sup>a</sup>	28	2.8	7.1	+H4 4.0 (0.13)	–H4 3.3 (0.11)
			68	3.1	5.8	–H1 4.0 (0.13)	PCGMCAA_185.0 +H1 3.4 (0.11)
Sprt	12601	C	58	3.3	4.8		PACMAGG_165.0
			58	2.9	5.7	–H1 3.9 (0.12)	+H1 3.3 (0.13)
Keep	Stirling	VIII <sup>a</sup>	58	2.9	5.7	–H1 3.9 (0.12)	PCGMCAA_185.0
			76	2.3	5.4	–H1 5.4 (0.09)	+H1 4.9 (0.08)
ACB	Stirling	VI	58	6.4	13.5		
			58	4.6	9.6	H3/4 5.1 (0.05)	H12 4.6 (0.10)
ACB	Stirling	XIb	14	4.2	8.0	H12 5.2 (0.11)	H14 4.7 (0.11)
ACB	12601	VI	40	3.8	7.2		EACAMCAC_200.0
			40	2.8	6.4	–H2 5.2 (0.06)	+H2 4.8 (0.06)
ACB	12601	C	90	4.1	7.0	H23 5.2 (0.10)	H12 4.7 (0.10)



**Table 5** continued

Trait	Parent	HG (=LG)	Position cM	Lod	R <sup>2</sup>	Simplex marker Highest mean (SE)	Simplex marker Lowest mean (SE)
ACB	12601	IV	62	3.7	6.9	PCCMATA_179.0	
			56	3.2	8.3	+H2 5.2 (0.06)	–H2 4.8 (0.06)
ACB	12601	II	114	4.0	6.6		PACMAAC_268.8R2
			114	3.8	8.0	–H3 5.2 (0.06)	+H3 4.8 (0.06)
Fry4 <sup>b</sup>	Stirling	XIa	100	4.4	8.8	H14 4.5 (0.12)	H23 3.7 (0.15)
Fry4 <sup>b</sup>	Stirling	A	100	3.4	7.6	QQ	qq
			54	4.1	7.5	H13 4.5 (0.11)	H24 3.9 (0.11)
Fry4	Stirling	VI	48	3.3	6.5	QQ	qq
			94	4.0	6.9		
Fry4	12601	XIa	96	3.6	8.1	H12 4.5 (0.11)	H3/4 4.0 (0.05)
			64	3.4	6.5	H12 4.3 (0.11)	H24 3.8 (0.11)
Fry10	Stirling	VI	66	4.7	8.1	PCAMAAC_197.0	
			66	3.9	7.8	+H2 6.5 (0.06)	–H2 6.1 (0.07)
Fry10	12601	XIa	60	3.7	7.9	H12 6.6 (0.10)	H34 6.1 (0.11)
Fry10 <sup>b</sup>	Stirling	Ia	88	3.9	7.3	H23 6.7 (0.11)	H14 6.1 (0.11)
			86	3.0	7.8	QQ	qq
Fry10	Stirling	XIa	64	3.8	5.9	H12 6.6 (0.09)	H23 6.0 (0.14)
Grow	Stirling	XIa	62	3.4	5.0		
			62	3.2	6.3	H1/4 8.9 (0.03)	H23 8.6 (0.09)
Scab	12601	II	80	4.3	8.1		
			80	3.1	7.1	H3/4 5.4 (0.06)	H12 4.8 (0.12)
Scab	Stirling	VI	86	3.6	6.9	H34 5.6 (0.12)	H12 4.9 (0.13)
IC	12601	V	116	4.5	8.6		PACMAGC_358.8S5
			116	3.7	8.5	–H1 8.5 (0.06)	+H1 8.2 (0.06)

<sup>a</sup> In this LG, only possible model is +H1 versus –H1

<sup>b</sup> Indicates traits where an additive model was an alternative to the full model

### Regularity of tuber shape

Four QTLs were detected for Shape, but none could be attributed to segregation at known major gene loci for tuber shape (round/long contrast) (Van Eck et al. 1994) and eye depth (Li et al. 2005). Parent 12601ab1 has two copies of a dominant allele for good Shape on homology group II and a single copy of an allele for poor Shape on homology group V. Stirling likewise has one copy of an allele for good Shape on homology group V, linked to early Mat, which may be the same locus as in 12601ab1 as both are close to STM3179. Stirling also has two copies of a dominant allele for poor Shape on homology group XIa. Thus, the favourable alleles are dispersed between the parents and there is ambidirectional dominance. These results are consistent with the observed transgressive segregation (population range 3.62) from similar parents (difference 0.69). Although segregation at each QTL was of small effect, the sum of their individual phenotypic variances is 43% (45% for full models), but this would only be the total variance explained if they acted independently

because any correlations will reduce the variance explained. Likewise the differences between the highest and lowest means sum to a total of 2.00 compared with the population range of 3.62. It was not possible to compare this predicted total of 2.00 with actual marker combinations. Whilst the simplex alleles in 12601ab1 and Stirling on homology group V mapped close to simplex markers in coupling, the two copies of the dominant alleles in 12601ab1 homology group II and in Stirling group XIa were not closely linked to markers in coupling.

### Sprouting and keeping quality

Four QTLs were detected for Sprt. Segregation at each QTL was of small and similar effect. Three have single alleles on chromosomes in homology groups V, VIII and C of 12601ab1. The first two give resistance to sprouting and the third one susceptibility. The first one is linked to a QTL allele for poor Shape. The fourth QTL has a single allele on a chromosome in homology group VIII of Stirling and

confers susceptibility to sprouting. It is linked to a QTL allele for poor keeping quality (Keep) and could in fact be the same allele as the two traits are associated. Likewise 12601ab1 homology group VIII had a possible QTL for keeping quality which was almost significant with  $0.1 > P > 0.05$  by the permutation test (lod 3.4). It is not possible to tell if the same locus has been detected in the two parents for group VIII. The results are consistent with 12601ab1 having better sprouting resistance than Stirling, the population mean being intermediate between the parents and the population displaying some transgressive segregation (i.e. population extremes higher and lower than parents). The four simplex QTLs were closely linked in coupling to four simplex markers [PATMCAT\_165.0 (13 cM away), PAGMAGT\_129.0 (6 cM), PACMAGG\_165.0 (0 cM) and PCGMCAA\_185.0 (0 cM)]. When these four markers were included as the independent variables in a multiple regression analysis of sprouting, each marker, but none of the interactions between markers, was statistically significant ( $P < 0.05$ ). Together they explained 16.3% of the phenotypic variation, compared with 26.7% from adding the individual  $R^2$ s. The estimated extreme phenotypes were 2.7 (SE 0.17) for individuals with markers PACMAGG\_165.0 and PCGMCAA\_185.0 and 4.7 (SE 0.18) for individuals with markers PATMCAT\_165.0 and PAGMAGT\_129.0, a difference of 2.0 compared with 2.6 from adding the individual estimates. Hence there is evidence for four loci acting independently and additively to give the observed phenotypes.

#### *After-cooking blackening*

Six QTLs were detected for ACB. Segregation at each QTL was of small and similar effect but collectively their individual phenotypic variances sum to 47% (49% for full models) and the differences between the highest and lowest means sum to a total of 2.70 compared with a population range of 3.78. This represents transgressive segregation as the parents and population mean were all similar. Three QTLs have single alleles on chromosomes in homology groups II, IV and VI of 12601ab1. The ones on II and VI are for increased blackening and the one on IV is for less blackening. There is also a QTL on homology group C of 12601ab1 but no simple model to explain the variation. The other two QTLs are in homology groups VI and XIb of Stirling. In group VI there are two copies of a dominant allele for decreased blackening. The two alleles for increased blackening are linked to ones for good Fry4 but susceptibility to Scab. There is no simple model for the QTL in group XIb. There are insufficient bridging markers to determine whether the QTLs in homology group VI are the same locus in the two parents.

#### *Fry colour*

Four QTLs were detected for each of Fry4 and Fry10. Although these traits are correlated, there were differences in the QTLs. Stirling, the parent with poor Fry4 and Fry10, has two copies of a dominant allele for poor Fry4 in homology group VI and QTLs in groups XIa and A, where the difference in fry colour is associated with two different pairs of homologous chromosomes. An additive model (QQ versus Qq versus qq) is a good fit to their Fry 4 data. Stirling also has QTLs for Fry10 in groups VI and XIa, but at apparently different locations and with different alleles. Thus, in group VI there is a single allele for good Fry10 but no simple model for the QTL in group XIa where genotype Q<sub>23</sub> (H23) is associated with poor Fry10 and more growth cracks. There is also a QTL in group Ia where the difference in fry colour is associated with two different pairs of chromosomes and an additive model fits the Fry10 data. Clone 12601ab1, the parent with good Fry4 and Fry10, has a QTL for both Fry4 and Fry10 in group XIa at similar locations with genotype Q<sub>12</sub> (H12) giving the better fry colour. However, the model is simpler for Fry10 as the other two homologous chromosomes give the poorer fry colour (an approximation to an additive model for two alleles), whereas for Fry4 the extremes are Q<sub>12</sub> (H12) and Q<sub>24</sub> (H24). It is not possible to tell if the same locus in group XIa has been detected in Stirling and 12601ab1. The allele for blue flower colour from the parent 12601ab1 is also on H2 in homology group XIa.

#### *Other traits*

Two copies of a dominant allele for resistance to growth cracking (Grow) were detected in Stirling homology group XIa, with the two recessive alleles associated with poor fry colour. Two QTLs were detected for Scab resistance, one in 12601ab1 with two copies of a dominant allele for resistance in homology group II, the other in Stirling group VI with genotype Q<sub>34</sub> (H34) more resistant than Q<sub>12</sub> (H12). Genotype Q<sub>12</sub> was also associated with poor ACB but good Fry4. Finally, a QTL was detected for internal condition (IC) with a single copy of an allele for increased defects in 12601ab1 homology group V.

## **Discussion**

### Phenotypic data

The parental data were as expected for cultivar Stirling (a low-dry matter, maincrop table variety) and clone 12601ab1 (a high-dry matter, maincrop clone for

processing). The heritabilities were all moderately high to high with between 54 and 92% of the phenotypic variation in clone means over years and replicates due to genetic differences between clones. This means that the SCRI breeding practice of reducing the number of clones from 1,000 to 60 on the basis of 3 years of Dundee ware trials with two replicates and small plots was basically sound (Mackay 2005). The traits with the lower heritabilities were as expected from previous breeding experience. Common scab was sufficiently severe to be scored in only 1 year, and hence there is a good case for establishing a special disease nursery. The clones  $\times$  years interactions for internal condition and ACB are consistent with differences between clones being more obvious in some years than others. Likewise, it is easier to score differences in sprouting than differences in keeping quality based on tuber firmness.

The correlations between traits all have simple explanations. The overall preference scores were highly correlated with each other and yield because there were few internal defects and scorers unconsciously included yield in Over as well as consciously in OverY. Tuber size is a component of yield and hence was correlated with yield and the overall preference scores. The correlation of fry colours after storage at two temperatures presumably reflected differences between clones in reducing sugar content with the darker colours for Fry4 than Fry10 due to higher contents (Brown et al. 1990). The correlations of poor keeping quality and faster emergence after planting with early sprouting in store can both be interpreted in terms of lack of dormancy. Finally, fast emergence and high yield can be interpreted in terms of rate of canopy establishment. There were no strong correlations between maturity and other traits, probably a reflection of both parents having main crop maturity and the trials being burnt down with sulphuric acid once most clones were up to size (standard practice), rather than waiting for natural senescence.

As all of the traits displayed continuous variation, it is not possible from their phenotypic distributions to infer the number of genes segregating, their chromosomal locations and the magnitudes of their effects. However, it was possible to detect QTLs through their linkages to molecular markers.

#### Molecular marker map

As the potato is a tetraploid ( $2n = 4x = 48$ ) which displays tetrasomic inheritance, a complete linkage map would comprise 12 sets of four homologous chromosomes (homology or LGs), correctly aligned and orientated with previously published diploid maps of potato including the

SH  $\times$  RH UHD reference population (Van Os et al. <http://www.genetics.org/cgi/content/abstract/173/2/1075>). Furthermore, the 12 LGs would be numbered from I to XII to agree with those now adopted for the 12 unique chromosomes of potato which have been aligned with those of tomato (Dong et al. 2000; Celebi-Toprak et al. 2005). This ideal was not achieved, but ten groups of four homologous chromosomes were identified for clone 12601ab1, with six groups of less than four remaining, and 11 groups of four were established for cultivar Stirling with one group of two remaining. The 12 Stirling groups (total map length 1,234 cM) were aligned with those from 12601ab1 (total map length 1,202 cM), with four of the latter remaining unaligned. However, there were insufficient bridging markers for this to be done accurately and QTL models were therefore fitted to the two parents separately. There were also insufficient markers in common with the UHD map for an accurate alignment by this method. The map was an improvement on the previously published one for these parents which was based on a similar number of AFLP primer combinations (39 compared with 38 here) but a smaller population size (78 compared with 227 here) (Meyer et al. 1998). The total map lengths for the sets of homologous chromosomes are the right order of magnitude for potato (Celebi-Toprak et al. 2005), but it is difficult to be certain about whether they are over or under estimates. Likewise it is difficult to assess bias in the recombination frequencies as a result of double reduction and other failures of the assumptions in the simple model for tetrasomic inheritance. However, where comparisons could be made, marker order was similar to that found in the UHD map, but the markers were further apart in the tetraploid map.

The linkage maps for chromosomes IV and V were the most satisfactory because each was identified by two well characterized chromosome specific SSR markers; there were AFLP markers on all four homologous chromosomes of each LG in each parent; double-simplex markers (seven and three for chromosomes IV and V, respectively) linked the maps of the two parents; and there was a total of five AFLP markers co-migrating with ones from chromosomes IV and V of the parents of the UHD reference map. However, there were two AFLP markers on chromosome V which appeared to co-migrate with ones from different UHD chromosomes, but co-migration can be difficult to assess in marker dense regions if bands are not sharp. Overall, 5 out of 52 (~10%) co-migrating markers were apparently not homologous, a figure close to the 11% originally reported by Rouppe van der Voort et al. (1997). They recommended as a rule of thumb that at least three putatively homologous markers per LG should be used for chromosome identification. Hence the chromosomal identification of the other LGs is tentative, with varying degrees of uncertainty, until they can be associated with well

characterized SSRs, a greater number of co-migrating AFLP markers, or other chromosome specific markers. Despite recent advances in the selection of highly informative and user-friendly SSRs in potatoes (Ghislain et al. 2004), there is still a need to develop and utilize more SSRs to ensure adequate coverage of both arms of all 12-potato chromosomes for mapping purposes. Multiallelic SSRs will also result in a more accurate alignment of LGs from the two parents than can be done through estimates of map distance from coupling linkages between simplex and double-simplex markers. The double-simplex dominant markers, which segregated 3: 1, were in fact omitted from the linkage maps of the parents because the inevitable repulsion linkages between simplex and double-simplex markers are very uninformative about recombination. The same applied to linkages involving dominant markers present in both parents where the dosage was higher than double-simplex. More SSRs are also required to resolve the problems with LGs I and XI.

Mapping studies in potato still rely very heavily on AFLP markers. Hence for QTL analysis it is desirable to have sufficient simplex markers on each of the 48 chromosomes in each parent in order to adequately track the products of meiosis in their offspring. This is necessary because one has to assume that there could be four different QTL alleles in each parent. As potato chromosomes are ~100 cM long, five evenly spaced markers would occur every 20 cM and ten would occur every 10 cM, making totals of 480 and 960 markers, respectively, for all 96 chromosomes. The 38 primer combinations resulted in 208 simplex markers in 12601ab1 and 171 in Stirling, or an average of ten per primer combination, but the distribution was not even. In 12601ab1 there were 46 isolated markers and 39 groups of two or more, giving an average of just over four per group. In Stirling there were 39 isolated markers and 35 groups of two or more, giving an average of just under four per group. Hence in future mapping the number of primer combinations used should be at least 48 and ideally around 96. Duplex markers are required for identifying homologous chromosomes. As recombination frequencies from simplex/duplex markers in coupling and repulsion are estimated with equal accuracy, in theory, a single duplex marker per LG would be sufficient. Hackett et al. (1998) used 14 simplex and 4 duplex markers per set of four homologous chromosomes in their simulation study and concluded that a population size of at least 150, and preferably 250, should be used to identify homologous chromosomes. In other words, 48 duplex markers per parent should have been adequate in this study. In 12601ab1 there were 80 duplex markers and in Stirling there were 41, but the distribution was not even. Hence the target number should be increased and a sensible one would be all six potential combinations of duplex markers

for each set of four homologous chromosomes (1 and 2, 1 and 3, 1 and 4, 2 and 3, 2 and 4 and 3 and 4), making a total of 72 duplex markers for each parent. The suggested increase in the number of primer combinations should provide more than enough duplex markers present in one parent and absent in the other. It remains to be seen if in future SNPs will provide the required density of markers more cheaply and easily, but the same problems will be faced over linkage phase.

#### QTLs and implications for potato breeding

A total of 39 QTLs were identified, although the number of separate loci may be less, possibly 27. Those for Mat (one), ACB (six) and Shape (four) accounted for 56, 49 and 45% of the phenotypic variance, while four QTLs for each of Fry4, Fry10 and Sprt accounted for 30, 29 and 27%, on the simplifying assumption that the variances for QTLs are additive. Hence these figures must be taken as upper bounds because any correlations between QTLs in their action and distribution will reduce the percentage of the phenotypic variance explained. The same applies to the two QTLs for each of Yield, OverY and Over which explained 19, 25 and 24% of the variation and the two for each of Emer, Size and Scab which explained 18, 15 and 15%. Just one QTL explaining less than 10% of the variation was detected for each of DM, Keep, Grow and IC. Some QTLs of large effect may have been missed through inadequate marker coverage on some chromosomes, but it is more likely that many QTLs of small effect have gone undetected. QTLs with small to intermediate effects, rather than ones of large effect, have been reported by other researchers for some of the traits just mentioned. Bonierbale et al. (1993) identified 26 loci from seven of the potato LGs where restriction fragment length polymorphisms (RFLPs) were associated with measured yield characteristics in diverse tetraploid germplasm. Schäfer-Pregl et al. (1998) identified eight putative QTLs for tuber yield on eight LGs using RFLP markers on two different crosses among dihaploid breeding lines. Van den Berg et al. (1996a, b) detected QTLs affecting tuberization and dormancy in reciprocal backcrosses between *S. tuberosum* and *S. berthaultii*. Eleven distinct loci on seven LGs were associated with variation in tuberization. Most of the loci had small effects but a QTL explaining 27% of the variance was found on LG V (tuberization in long days could be interpreted as early maturity). QTLs were detected on nine LGs that affected tuber dormancy at 13°C in the dark, either alone or through epistatic interactions. Freyre et al. (1994) identified six QTLs (one on each of six LGs) for dormancy in a population derived from diploid (*S. tuberosum* × *S. chacoense*) × *S. phureja*. In the same

population a total of ten putative QTLs for specific gravity (DM) were located on six LGs (Freyre and Douches 1994). Menendez et al. (2002) mapped QTLs for cold sweetening (Fry4 in this paper) in diploid potato. QTLs for sugar content were located on all potato chromosomes with ones explaining >10% of the variability for reducing sugars located on six LGs. They also provided evidence that allelic variants of enzymes operating in carbohydrate metabolism contribute to the genetic variation in cold sweetening.

As the linkage maps of 12601ab1 and Stirling were not aligned with certainty, QTL effects are based on comparisons of pairs of alleles segregating from one parent ( $Q_1Q_2$ ,  $Q_1Q_3$ ,  $Q_1Q_4$ ,  $Q_2Q_3$ ,  $Q_2Q_4$ ,  $Q_3Q_4$ ), averaged over the alleles from the other parent ( $Q_5Q_6$ ,  $Q_5Q_7$ ,  $Q_5Q_8$ ,  $Q_6Q_7$ ,  $Q_6Q_8$ ,  $Q_7Q_8$ ). Thus  $Qq \times Qq$  would be interpreted as a difference between  $Qq$  and  $qq$  segregating from each parent and not as a comparison of  $QQ$ ,  $Qq$ ,  $qq$  where statements about dominance could be made. Likewise, with three alleles, in the cross  $Q_1Q_2Q_2 \times Q_3Q_3Q_3$ , it is  $Q_1Q_2Q_3$  that is compared with  $Q_2Q_2Q_3$  where  $Q_3$  may be the superior, intermediate or inferior allele. Bearing these limitations in mind, the 39 segregating QTLs can be classified as 14 simplex ( $Qqq$ ), 12 duplex with complete dominance ( $QQq$  where  $QQ$  and  $Qq$  have same phenotype), three duplex additive ( $QQq$  where  $Qq$  intermediate between  $QQ$  and  $qq$ ) and ten more complicated. Hence for 29 of the 39 QTLs a very simple gene model explains the variation. Any QTL genotypes that could arise only by double reduction would have been misclassified as different genotypes, and might have caused some bias in the estimation of QTL effects.

The results confirm the models of classical quantitative genetics that explain the observed phenotypic variation in terms of Mendelian genetics and underpin the theory and practice of conventional potato breeding. In these models the effects of genes are measured in terms of their quantitative effects on the traits of interest, and not in terms of their effects at the levels of the transcriptome, proteome and metabolome. Examples can be found of different loci whose effects on a trait are additive (four simplex alleles for *Sprt*); of dispersion between the parents of alleles with positive and negative effects on a trait (*Shape*, *Sprt* and *ACB*); and of loci displaying ambidirectional dominance for a trait (*Shape*). Examples can also be found of chromosomes associated with effects on more than one trait. The association between the QTL allele for early maturity on chromosome V of *Stirling* and susceptibility to late blight has been analysed and discussed previously (Bradshaw et al. 2004). The chromosome for early maturity was also associated with better *Shape* and higher DM. Other examples were resistance to sprouting and poor *Shape* on the same chromosome, and increased

*ACB*, good *Fry4* and susceptibility to *Scab* on the same two chromosomes. These associations may be due to the pleiotropic effects of a single gene or linkage of different genes on the same chromosome. Linkages can be broken through recombination, but in the short-term both pleiotropy and linkage will result in correlated responses to selection and breeders need to be aware of them. In contrast, the associations between yield and overall scores and between sprouting resistance and keeping quality probably simply reflect different ways of assessing the same trait.

The conclusion from this and the other studies mentioned above is that the genetic variation in yield, agronomic and quality traits results from the segregation of alleles at many quantitative trait loci (QTLs). These allelic differences usually result in small to intermediate differences in the traits of interest, but with the occasional larger difference such as the one found for maturity. More genes of large effect may be found as complex traits are dissected and candidate genes are sought. This has already happened for quantitative resistance to cyst nematodes (Bryan et al. 2002, 2004) and late blight (Bradshaw et al. 2004). For these genes the breeder will want to use molecular breeding methods (gene cloning and marker-assisted selection) to ensure that they are introduced into new cultivars as quickly as possible. However, efficient exploitation of most of the genetic variation in potato breeding programmes will continue to rely on knowledge from classical quantitative genetics. The most important implications for practical potato breeding come from the number of segregating genetic loci. The 39 (or 27) found is clearly an underestimate. Again, naively adding up all of the individual QTL variances and dividing by the sum of the heritabilities (expressed as percentages) suggests that an upper bound of 30% of the total genotypic variation has been explained and that the total number of loci segregating may be as many as 128. There is no way in which desirable alleles from that number of loci can be combined into a new cultivar through one round of crossing and selection in typical potato breeding programmes with seedling population sizes from 20,000 to 200,000. This can be seen by considering the simplest additive QTL model with a single copy of a desirable allele at all loci ( $Qqq \times qqq$ ). As the number of alleles segregating increases from 14 to 18, the frequency of the genotype with all of the desired alleles decreases from 1 in 16,384 to 1 in 262,144 and passes the one in a million mark with segregation at as few as 20 loci. Hence efficient multitrait genotypic recurrent selection is essential for continued steady progress in the genetic improvement of the potato (Bradshaw et al. 2003). Otherwise, breeders will simply be shuffling genes with each generation of crosses without making real progress.



**Acknowledgements** Thanks are due to Margaret McInroy, Karen McLean, Drummond Todd and Ralph Wilson for technical support, Jim McNicol for statistical advice, and to the British Potato Council (Ph.D. studentship to B.P.), the Scottish Executive Environment and Rural Affairs Department and the United Kingdom Biotechnology and Biological Sciences Research Council (GAIT Project) for funding. Dr. Zewei Luo, now at the University of Birmingham, developed the theory and software for linkage analysis whilst on the GAIT project.

## References

- Bonierbale MW, Plaisted RL, Tanksley SD (1993) A test of the maximum heterozygosity hypothesis using molecular markers in tetraploid potatoes. *Theor Appl Genet* 86:481–491
- Bradshaw JE, Stewart HE, Wastie RL, Dale MFB, Phillips MS (1995) Use of seedling progeny tests for genetical studies as part of a potato (*Solanum tuberosum* subsp. *tuberosum*) breeding programme. *Theor Appl Genet* 90:899–905
- Bradshaw JE, Dale MFB, Mackay GR (2003) Use of mid-parent values and progeny tests to increase the efficiency of potato breeding for combined processing quality and disease and pest resistance. *Theor Appl Genet* 107:36–42
- Bradshaw JE, Pande B, Bryan GJ, Hackett CA, McLean K, Stewart HE, Waugh R (2004) Interval mapping of quantitative trait loci for resistance to late blight [*Phytophthora infestans* (Mont.) de Bary], height and maturity in a tetraploid population of potato (*Solanum tuberosum* subsp. *tuberosum*). *Genetics* 168:983–995
- Brown J, Mackay GR, Bain H, Griffith DW, Allison MJ (1990) The processing potential of tubers of the cultivated potato, *Solanum tuberosum* L., after storage at low temperatures. 2. Sugar concentration. *Pot Res* 33:219–227
- Bryan GJ, McLean K, Bradshaw JE, De Jong WS, Phillips M, Castelli L, Waugh R (2002) Mapping QTLs for resistance to the cyst nematode *Globodera pallida* derived from the wild potato species *Solanum vernei*. *Theor Appl Genet* 105:68–77
- Bryan GJ, McLean K, Pande B, Purvis A, Hackett CA, Bradshaw JE, Waugh R (2004) Genetical dissection of H3-mediated polygenic PCN resistance in a heterozygous autotetraploid potato population. *Mol Breed* 14:105–116
- Celebi-Toprak F, Watanabe JA, Watanabe KN (2005) Molecular markers in identification of genotypic variation. In: Razdan MK, Mattoo AK (eds) Genetic improvement of Solanaceous crops: potato, vol 1. Science Publishers, Enfield, pp 115–141
- Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. *Genetics* 138:963–971
- Dong F, Song S, Naess SK, Helgeson JP, Gebhardt C, Jiang J (2000) Development and applications of a set of chromosome-specific cytogenetic DNA markers in potato. *Theor Appl Genet* 101:1001–1007
- Freyre R, Douches DS (1994) Development of a model for marker-assisted selection of specific gravity in diploid potato across environments. *Crop Sci* 34:1361–1368
- Freyre R, Warnke S, Sosinski B, Douches DS (1994) Quantitative trait locus analysis of tuber dormancy in diploid potato (*Solanum* spp.). *Theor Appl Genet* 89:474–480
- GENSTAT 5 Committee (1993) GENSTAT 5 release 3 reference manual. Clarendon Press, Oxford
- Ghislain M, Spooner DM, Rodriguez F, Villamon F, Nunez J, Vasquez C, Waugh R, Bonierbale M (2004) Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. *Theor Appl Genet* 108:881–890
- Hackett CA, Bradshaw JE, Meyer RC, McNicol JW, Milbourne D, Waugh R (1998) Linkage analysis in tetraploid species: a simulation study. *Genet Res Camb* 71:143–154
- Hackett CA, Bradshaw JE, McNicol JW (2001) Interval mapping of QTLs in autotetraploid species. *Genetics* 159:1819–1832
- Hackett CA, Pande B, Bryan GJ (2003) Constructing linkage maps in autotetraploid species using simulated annealing. *Theor Appl Genet* 106:1107–1115
- Hackett CA, Luo ZW (2003) TetraploidMap: construction of a linkage map in autotetraploid species. *J Hered* 94:358–359
- Li XQ, De Jong H, De Jong DM, De Jong WS (2005) Inheritance and genetic mapping of tuber eye depth in cultivated diploid potatoes. *Theor Appl Genet* 110:1068–1073
- Luo ZW, Hackett CA, Bradshaw JE, McNicol JW, Milbourne DM (2000) Predicting parental genotypes and gene segregation for tetrasomic inheritance. *Theor Appl Genet* 100:1067–1073
- Luo ZW, Hackett CA, Bradshaw JE, McNicol JW, Milbourne DM (2001) Construction of a genetic linkage map in tetraploid species using molecular markers. *Genetics* 157:1369–1385
- Mackay GR (2005) Propagation by traditional breeding methods. In: Razdan MK, Mattoo AK (eds) Genetic improvement of Solanaceous crops: potato, vol 1. Science Publishers, Enfield, pp 65–81
- Mackay GR, Dale MFB (1990) Quality in potatoes. SCRI Annual Report 1990, pp 9–12
- Menendez CM, Ritter E, Schäfer-Pregl R, Walkemeier B, Kalde A, Salamini F, Gebhardt C (2002) Cold sweetening in diploid potato: mapping quantitative trait loci and candidate genes. *Genetics* 162:1423–1434
- Meyer RC, Milbourne DM, Hackett CA, Bradshaw JE, McNicol JW, Waugh R (1998) Linkage analysis in tetraploid potato and association of markers with quantitative resistance to late blight (*Phytophthora infestans*). *Mol Gen Genet* 259:150–160
- Roupe van der Voort JNAM, van Zandvoort P, van Eck HJ, Folkertsma RT, Hutten RCB, Draaistra J, Gommers FJ, Jacobsen E, Helder J, Bakker J (1997) Use of allele specificity of comigrating AFLP markers to align genetic maps from different potato genotypes. *Mol Gen Genet* 255:438–447
- Schäfer-Pregl R, Ritter E, Concilio L, Hesselbach J, Lovatti L, Walkemeier B, Thelen H, Salamini F, Gebhardt C (1998) Analysis of quantitative trait loci (QTLs) and quantitative trait alleles (QTAs) for potato tuber yield and starch content. *Theor Appl Genet* 97:834–846
- Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: Joinmap. *Plant J* 3:739–744
- Swaminathan MS, Howard HW (1953) The cytology and genetics of the potato (*Solanum tuberosum*) and related species. *Bibliogr Genet* 16:1–192
- Van den Berg JH, Ewing EE, Plaisted RL, McMurry S, Bonierbale MW (1996a) QTL analysis of potato tuberization. *Theor Appl Genet* 93:307–316
- Van den Berg JH, Ewing EE, Plaisted RL, McMurry S, Bonierbale MW (1996b) QTL analysis of potato tuber dormancy. *Theor Appl Genet* 93:317–324
- Van Eck HJ, Jacobs JME, van Dijk J, Stiekema WJ, Jacobsen E (1993) Identification and mapping of three flower colour loci of potato (*S. tuberosum* L.) by RFLP analysis. *Theor Appl Genet* 86:295–300
- Van Eck HJ, Jacobs JME, Stam P, Ton J, Stiekema WJ, Jacobsen E (1994) Multiple alleles for tuber shape in diploid potato detected by qualitative and quantitative genetic analysis using RFLPs. *Genetics* 137:303–309
- Von Scheele C, Svensson G, Rasmussen J (1937) Die bestimmung des starkegehalts und der trockensubstanz der kartoffel mit hilfe des spezifischen gewichts. *Landw Vers Sta* 127:67–96