Identification of quantitative trait loci (QTLs) influencing early vigour, height, flowering date and seed size and their implications for breeding of narrow-leafed lupin (*Lupinus angustifolius* L.)

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Abstract. We report the first quantitative traits loci (QTL) mapped in an F_8 recombinant inbred line (RIL) population of *Lupinus angustifolius*. Traits mapped were early vigour, days to flowering, height at maturity and seed size. Twenty-two QTLs were found, located on 13 linkage groups, with alleles beneficial to the crop contributed by both parents. Early vigour was controlled by 8 QTLs on 7 linkage groups. Time to flowering was controlled by 10 QTLs and the height at maturity was found to be under the control of 4 QTLs. Seed size was linked to 2 QTLs. A region linked to the *Ku* gene that promotes early flowering by removal of the vernalisation requirement appeared to play a role in all 4 traits. The gene *mollis* controlling soft-seededness appeared to also be linked to early vigour and; *iucundis* controlling alkaloid production was linked to seed size. Five pairs of QTLs were found to be involved in epistasis, 2 of these having an effect on early vigour and another 3 influencing the time to opening of the first florets. Variation explained for each trait ranged from 28% for seed size, to 88% for days to flowering. We showed that it was possible to use this data to predict genotypes of superior progeny for these traits under Mediterranean conditions. QTL regions were compared on a second published linkage map and regions of conserved synteny with the model legume *Medicago truncatula* high-lighted.

Introduction

Narrow-leafed lupin (*Lupinus angustifolius L.*) is a relatively new crop species. In the18th and 19th centuries it was recorded as being used as a coffee substitute and as cattle fodder (Gladstones 1970), emerging in the 20th century as a food seed crop, primarily for stock feed (Edwards and van Barneveld 1998), but also for human consumption (Petterson 1998). Active domestication and breeding of this crop started soon after World War I in Germany and Poland, with an emphasis on early vigour and maturity (Barbacki 1952; Hanelt 1960) and low alkaloids (von Sengbusch 1930, 1931, 1938). Later efforts were aimed at developing soft-seeded lines (Gladstones 1958; Quinlivan 1966, 1967, 1968), early flowering and, further reductions in pod shattering (Gladstones 1967, 1977). Most of these traits were found to be under the control of one or two major genes, and could be selected for relatively easily upon incorporation into the domesticated crop. Today breeding of narrow-leaf lupins is increasingly being focused on more complex traits – especially yield, but also seed quality and resistance to pests and diseases.

In nature, many complex plant traits such as grain yield, display a large continuous range in variation approximating a Normal curve. The continuous variation in phenotypic expression of such traits frequently cannot be fitted to simple Mendelian ratios and are therefore generally considered to be the product of the interaction of a number of gene loci (Johannsen 1909; Nilson-Ehle 1909; East 1916), commonly known as quantitative trait loci (QTLs).

Early vigour, plant height and yield are complex traits of which little are known in lupins. The time to flowering too is controlled by more then one gene, although one gene (*Ku*) was found to have the greatest impact in the Western Australian environment (Rahman and Gladstones 1972). Farrington and Gladstones (1974) carried out some work on yield, primarily in assessment of the effect of recently incorporated domestication genes. There are also some reports of the effect of the *iucundis* gene (for low alkaloids) on yield, dating from the late 1930's (von Sengbusch 1938, 1942; Hackbarth and Troll 1960; Kress 1964). Recently, Dr J. Clements (pers. comm.) has carried out work on the early vigour of a range of accessions from the Australian Lupin Collection, looking at early biomass accumulation in relation to yield. But on the whole, analysis of such traits has been ignored because of a lack of suitable technology for such an assessment and, the expression of these genes is usually further complicated by environmental and gene x gene or epistatic interactions (Young 1996).

The development of molecular genetic maps now allows researchers to locate QTLs on a genetic map as first achieved by Paterson *et al*. (1988) and, make detailed analysis of their inheritance and activity. Provided that the map density and the study population are adequate, this can lead to improved prediction and selection of superior genotypes. One computer program that provides this option is QTL Network version 2.0 (Yang *et al.* 2005).

A further benefit of mapping QTLs is in exploiting synteny (conservation of linkage). It is not uncommon for related species to have near identical coding sequences for homologous genes (Moore *et al.* 1995), albeit not necessarily in identical positions on

the genome. By locating and sequencing a gene of interest it may be possible to exploit synteny to locate that gene in a related species of interest.

Recently two molecular maps have been published for narrow-leafed *lupin* (*Lupinus angustifolius* L), the latter one also determining regions of synteny with the model legume species, *Medicago truncatula.* Genome coverage is estimated to be 80% at a moderate to high density (Boersma *et al.* 2005; Nelson *et al.* 2006). These maps allow additional genes including QTLs to be mapped with a high degree of accuracy, opening up possibilities for marker-assisted selection in the breeding program and the future exploitation of any synteny discovered.

In this study, we examined the genetic basis of quantitative variation in early plant vigour, plant height at maturity, days to flowering and seed weight. QTLs found were positioned on the published maps and regions of micro-synteny with *M. truncatula* identified.

Materials and Methods

Plant materials

The marker population used in this study was an F_8 recombinant inbred line (RIL) of a domestic x wild type (DxW) cross of *L. angustifolius* previously developed by the Department of Agriculture and Food Western Australia (DAFWA), using as parents lines 83A:476 (domesticated) and P27255 (wild). The same 89 RILs that had been used in the mapping study by Boersma *et al.* (2005) were grown over four years.

Genetic markers

Several new markers were added to the map by Boersma *et al.* (2005). The protocols and naming details of all but one are as described in that paper. One marker, mtmt_GEN_00024_04_1 (referred to as mtmtGEN00024041 from here on) is of a type not previously described by us. Details are as follows:

The primers for marker mtmtGEN00024041 were designed using *M. truncatula* databases. Primer sequences are: (mtmtGEN00024041_L: TTGGTGATGGATGCTGTTGT; mtmtGEN00024041_R: CATCGTCATCTGTGTGACCC). PCR volumes were 20µl, including 25ng template DNA, 0.25µM of each primer, 1.25 U *Taq* DNA polymerase, 1.5mM $MgCl_2$, 2µl 1x PCR buffer (Invitrogen), 0.25 µM dNTPs (Fermentas), and 2 µg BSA (Sigma). The marker was amplified by PCR on an MJ Research PTC-200 thermocycler over 34 cycles after an initial denaturation at 95^{o}C for 3 min, with each cycle consisting of: 94^{o}C for 20 s, 53.6^{o}C for 20 s, 72^{o}C for 2 min. The final extension was 5 min at 72^{o}C. PCR products were separated in a 2% agarose gel (19.8 x 25 cm) at 120V over 4 h alongside a 100bp ladder (Fermentas No. SM0623). They were visualised by staining with Ethidium Bromide and exposure to a U.V. light source.

Segregation of all new markers were observed in the same RIL population as described in the above reference and the markers placed into the map of Boersma *et al.* (2005) using MapManager QTX (Manly *et al.* 2001).

Plant measurements

Data was collected for two consecutive years - 2005, 2006 (winter sown) in a screen-house and the adjacent field respectively as randomised plots, each with three replicates. To ensure rapid germination, all hard-seeded lines were scarified prior to sowing. In 2005, plots were arranged into three continuous rows with each plot consisting of 3 plants in a 10cm interval. In 2006, plots were grown as individual rows 1.5m long consisting of ~30 plants, with rows spaced at 50cm intervals. The sowing date in 2006 was 2 calendar days later then in 2005. Plots were irrigated as required.

Plant heights were measured to the nearest 0.5cm at 10 weeks after sowing (early vigour) and, to the nearest 1cm at maturity just prior to senescence. Three representative plants were measured for each plot.

The date on which the first floret was fully opened was taken as the date of flowering for that particular plot.

Seed produced in 2005 and 2006 were not harvested. Instead, seed weights for each RIL were determined as 100 seed weights (g) of seed produced in screen-house plots during 2002 and 2003 under similar growing conditions.

Data analysis

Initial data analysis carried out included a simple regression analysis giving means and standard deviations for each trait and year. Further analysis was carried out using the program QTL Network 2.0 (Yang *et al.* 2005) as detailed below.

The map by Boersma *et al.* (2005) was used to place the QTLs, the average marker interval on this map being 3.2cM. Marker intervals were also identified on the map by Nelson *et al.* (2006) in instances where synteny had been identified with *M. truncatula*. In comparing the maps we also made use of a composite map created by merging raw data from both original maps (Nelson *et al.* un-published)

Map data from Boersma *et al.* (2005) was imported into the program QTL Network 2.0 from MapManager. Input of trait data was as 2 environments (years) and 3 replicates (except seed weights). Seed weights based on 100 seeds per RIL were analysed separately for 2 environments, but without replication. The significance threshold chosen for declaring a putative QTL was at $P = 0.05$. Data was analysed at 1cM intervals ('walk speed') with 1000 permutations. Putative QTLs were separated by a minimum of 10cM ('filtration window') before a decision was made that more then 1 QTL was present in any one linkage group.

QTL Network 2.0 analyses the data as multi-factorial matrices using a Mixed Model Composite Interval Mapping (MCIM) approach (Yang & Zhu 2005) to give the following outputs for a RIL population:

(i) Population means and variances including Genetic (G), Environmental (E) and GxE interaction variances.

(ii) QTL positions including range, Standard Error(s) (SE), Additive gene effect (A), Additive x Environmental (AxE) effects and the Probability (P)-value of each.

(iii) Epistatic gene positions and intervals including nomination of genes acted upon.

(iv) Heritability estimates (narrow-sense)

(v) Genotype of a predicted superior offspring.

To confirm the veracity of detected epistasis' and the predicted superior genotypes, we conducted a further analysis on the early vigour of plants carrying the 4 possible gene combinations of the epistatic gene pair located on LGs 17 (CQTL 3) and 23 using the 'fit-model' function of the JMP statistic software (SAS Institute).

Results

Marker mtmtGEN00024041

The amplified Marker mtmtGEN00024041 product was found to segregate in a dominant fashion, with RILs carrying the maternal allele showing a band with a length of approximately 950bp.

Genetic Maps

QTL data was mapped to the previously published map by Boersma *et al.* (2005) and compared to the one by Nelson *et al.* (2006). Eleven new markers including mtmtGEN00024041 were added to the map by Boersma *et al.* as two linkage groups (22, 23) with a combined length of 62.7cM (Figure 1) increasing the total map length to 1605.7cM. These two groups were found to carry QTLs for early vigour and time to flowering (Table 2). (Insert Figure 1 near here)

Several QTL regions were found to have both an additive and an epistatic effect, or were active in more then one trait. We have designated such QTLs as a 'Common

QTL' (CQTL) and numbered these from 1 – 5. In three instances it was unclear whether the same QTL was involved or one that was closely linked to another on the same linkage group. These were marked with an asterisk (Table 2).

Phenotypic variation of traits

Table 1 shows parental, mean, minimum, maximum and standard deviation of trait values over 2 years for early vigour, height at maturity, days to flowering and seed weight. The traits measured in the present study mostly exhibit continuous variation (Figure 2), indicative that they are under the influence of multiple genes, with only days to flowering being clearly divisible into two major groups clustered around the parental values. All traits showed transgressive segregation, indicating that alleles with positive effects were distributed among the parents. There was no obvious increase in early vigour over the domesticated parent, although there were some RILs exhibiting a reduction in early vigour when compared to the wild parent. Seed weight data were not as complete and more variable, with data for the wild parent only available for year 1 (2002). (Insert Table 1, Figure 2 near here)

Detection of QTLs

Twenty-two QTLs were found for the four traits on 13 linkage groups including the 2 additional linkage groups (Table 2, Figure 1). (Insert Table 2 in this section)

Early vigour: Eight QTLs were found to influence the early vigour of lupins (Table 2, Figure 3a) including two epistatic loci. All but one of the gene alleles contributing to early vigour were from the domesticated parent. Linkage groups involved were 8, 10

(2), 13, 14, 17 (2), 18 and 23. Most of these showed no environmental interaction, the exception being the locus on LG 8 (± 0.8cm). One locus on LG14 (CQTL 1) was also found to have an epistatic effect on a locus identified as active on LG17 (CQTL 2) and, a second locus on LG17 (CQTL 3) was found to be epistatic on one of the loci on LG23. The range of the locus on LG8 includes *Mollis*, the gene associated with soft-seededness. The range of CQTL 2 on LG17 is closely linked to, but does not include the *Ku* gene that removes the vernalisation requirement for flowering. The two loci having the greatest effects were CQTLs 1, 2 on LGs 14, 17, together accounting for approximately 43% of the observed phenotypic variation. The 8 QTLs in total accounted for 61% of the variation, with 58.3% being due to additive effects and a further 2.7% due to epistatic effects (additive x additive). (Insert Figure 3a near here)

Height at maturity: Four QTLs on Linkage groups 1, 4, 8 and 17 were found to influence the height at maturity (Figure 3b), three with additive effects only and a fourth that had both an additive and GxE effect. The first three loci, including one on LG17 were contributed by the wild parent. The fourth QTL also exhibiting GxE was contributed by the domesticated parent. The QTL on LG17 (range 11.9 – 15.0 cM) having the greatest impact (25.7% of phenotypic variation) overlapped the corresponding one for early vigour (CQTL 3), with the *Ku* gene just 2cM outside of that range. The QTL on LG8 (range 51.9 – 67.9) was distant from the QTL for early vigour on the same LG (range 15.2 – 19.6). The total amount of height variation that could be explained by these QTLs was almost 40%, with 3 of the 4 QTLs conferring increased height coming from the wild parent. (Insert Figure 3b near here)

Days to Flowering: Ten QTLs on 8 LGs were found to influence the time interval from sowing to opening of the first floret (Figure 3c). Six loci had additive gene effects only. The QTL on LG17 (CQTL 2) having the largest impact (A = (-)10.56 days, explaining 81% of the observed variation) also had a small but significant genotype x environment (GxE) interaction. This locus (range 18.8 – 20.8) was positioned adjacent to the mapped position of the gene *Ku* (17.0cM). Two of the additive QTLs (LG13, 22) were also involved in epistatic interactions and a further two pairs of QTLs were involved in separate epistasis'. The locus on LG13 is immediately adjacent to *Lentus*, one of the two reduced-pod-shatter genes incorporated into domesticated lupin at 35.5cM. In total, 88.6% of the variation in flowering could be accounted for. (Insert Figure 3c near here)

Seed Weight: Two QTLs were found having an influence on seed weight. One QTL was positioned on the gene *Ku* (LG17) and also had a substantial GxE interaction component (Figure 3d). The positioning of this QTL is immediately adjacent to and overlapping CQTL 2 and, 1cM downstream of CQTL 3. The second QTL on LG9 was positioned on the gene *Iucundis* associated with plant alkaloid levels (von Sengbusch 1930; Hackbarth and von Sengbusch 1934). Increased seed size was positively correlated with the dominant allele associated with high alkaloids carried by the wild parent. Together the 2 loci accounted for between 28 and 34% of all variation in this trait with the QTL on LG17 having approximately twice as great an impact as the QTL on LG9. (insert Figure 3d near here)

One QTL on LG17 (CQTL 2) was found to influence both early vigour and time to opening of the first floret. A short segment of LG17 (CQTLs 2, 3; range10.6 – 19.8cM) was associated with all four traits

Comparison to Nelson et al. (2006) and Synteny with M. truncatula: Several regions of synteny with *M. truncatula* could be identified when QTL regions were super-imposed on the map by Nelson *et al.* (2006).

(i) The region on LG8 adjacent to *Mollis* and associated with early vigour, could be matched to LG03 of the linkage map by Nelson *et al.* (2006) (Map 2), being placed between *Mollis* and marker Lup111a. No syntenic region for these markers was found in *M. truncatula*. The second region on LG8 (map 1) associated with height at maturity, was outside of the locus found to have some synteny.

(ii) One of the two QTLs on LG13 corresponded to a region on LG05 of Map 2 between the *Le* gene and 212Len, while the second could be placed near marker LSSR05. Neither of these 2 markers was associated with synteny in the *M. truncatula* genome.

(iii) Common QTL 1 on LG14, associated with both early vigour and the opening of the first floret, could be located on LG12 of Map 2 as corresponding to the region of Lup251 and UWA064. Lup251 had also been mapped to a position on pseudo-chromosome 5 of *M. truncatula*.

(iv) The two regions on LG17adjacent to the *Ku* gene could be transposed onto LG01 of Map 2 although the marker order differed with that of map 1. CQTL 2 (associated with three out of the four traits) appears to be aligned with markers

UWA232, Lup054 and UWA214 and includes the *Ku* gene. CQTL 3, associated with both early vigour and height at maturity, corresponded to the region of markers Lup158 and VBP1 on the far side of the *Ku* gene. Both of these regions have some synteny on *Medicago truncatula* pseudo-chromosome 7, albeit on three widely separated loci.

Superior Genotype: By assuming that the ideal genotype is at either one end of the phenotypic spectrum or the other, we predicted a "superior" genotype (Table 2). For maximum early vigour, only one epistatic allele was required from the wild parent. For height at maturity to be maximised, three out of four alleles were required from the wild parent. The earliest flowering offspring had two additional dominant alleles from the wild parent. Seed weight was maximised by one allele for early flowering from the domesticated parent and the other for high alkaloids from the wild parent.

An analysis of the 4 possible genotypes for early vigour of the epistatic QTL pair CQTL 3 on LG17, and LG23 (Table 2), revealed (Figure 4) that the 10 week height (indicative of early vigour) ranged from a mean low of 20.5cm for plants carrying both wild type genes (BB) to a mean maximum height of 31.9cm for plants carrying one domesticated and one wild type gene (AB). Plants having the parental genotype (AA) were relatively shorter, having a mean height of 28.8cm and those with the BA genotype were intermediate (although variable) to the two parental genotypes at 24.8cm. (Insert Figure 4 near here)

Discussion

Lupinus angustifolius is still a relatively new crop and knowledge about relationships between various traits and crop production is limited. It is generally accepted that in Western Australia early flowering to avoid heat induced abortion during flowering and drought stress at grain filling are desirable to maximise grain yield and size. The present study investigated in two trials, some of traits thought to be of importance in grain production and harvesting in a Mediterranean environment.

A number of observations were made, relating seed size as well as early vigour to regions near the *Ku* gene on LG17. We have observed that there is linkage between *Ku* and early vigour (CQTL 2). However, the role of this QTL in promoting early vigour is not so pronounced. This could be explained by the presence of several further QTLs with effects of similar magnitude (LGs 8, 10, 13, 14, 18) and, the negative effect on early vigour of a second, epistatic locus on LG17 (CQTL 3) not far away from CQTL 2. The linkage to a region adjacent to *Mollis* (LG 8) was surprising in that seed of all hard-seeded RILs had been scarified to ensure rapid imbibition and germination upon sowing. Consequently, no specific measurements had been taken of emergence dates and casual observations at the time did not reveal any obvious differences.

The first QTL for early vigour on LG17 (CQTL 2) was also found to have a large, significant effect on days to flowering (DTF). From previous work (Gladstones 1970, Gladstones and Hill 1969) we know that *Ku* is the only gene to have a very large effect (2 – 5 weeks) on the reduction in DTF of narrow-leaf lupins. It therefore appears appropriate that it should have been included in this locus as occurs on Map 2 (Nelson *et al.* 2006) and may indicate that some revision of the map order in this region (Map 1

353 – Boersma *et al*. 2005) can be expected at a future date as more suitable markers are
354 generated.
355
356 Other QTL genes influencing DTF cumulatively have almost as great an effect (A,
357 AxA) on flowering date as *Ku* in this experiment (Table 2), although the heritability of
358 4 of the 8 is low. In this particular population, DTF in two RIL lines was advanced by
359 just 3 days in 2006 from 73 days (domesticated parent) after sowing (DAS) to 70 days
360 by inclusion of two QTLs from the wild parent. Current Australian cultivars have DTF
361 (measured at 50% of first florets open) similar to this. For example, Mandelup the
362 earliest flowering cultivar has an approximate DTF of 68 days (Dr B. Buirchell pers.
363 comm.).
364
365 Four QTLs having additive effects on mature plant heights were found. Lack of rainfall
366 can drastically reduce the plant height at maturity in a crop, while high rainfall
367 situations can lead to excessive vegetative growth and lodging. Both extreme situations
368 can make harvest difficult or even impossible. The gene combination of the
369 domesticated parent tending to reduce plant height (height QTL on LG1) would
370 therefore be suited to a relatively high rainfall area (e.g. 400mm p.a.), with a further
371 reduction in height potentially available under very high rainfall (> 450mm p.a.)
372 conditions. In a low rainfall situation there is the opportunity to breed for increased
373 height using the QTLs identified on LG's 4 and 8. The QTL on LG17 (CQTL 3) having
374 by far the greatest (reducing) effect on height is in the vicinity of the *Ku* gene and may
375 be involved in promoting both early vigour and early flowering. It will be necessary to

376 further clarify the positions of *Ku* and both QTLs before their usefulness in the
377 manipulation of plant heights can be determined.
378
379 Early flowering and pod-set allows for a longer period of seed production – both in the
380 extension of the flowering period and seed growth and may result in a significant
381 increase in seed size as found by Farrington and Gladstones (1974). It is therefore to be
382 expected that a QTL with close linkage to *Ku* was found to have a positive influence on
383 seed size. The second QTL associated with development of larger seeds centred on the
384 gene *Iucundis*. The recessive form of this gene, one of several known to limit
385 development of alkaloids in *L. angustifolius* (Hackbarth and von Sengbusch 1934;
386 Gladstones 1970), has been incorporated into all modern Australian cultivars and is here
387 associated with a smaller seed size. This analysis lends weight to the suggestion by
388 some researchers that this gene causes a reduction in yield (von Sengbusch 1938, 1942;
389 Hackbarth and Troll 1960; Kress 1964; Oram 1983). However, these two QTLs only
390 explain 30% of the observed variation in seed size, probably indicative of insufficient
391 replication in this particular trial and, of further QTLs having a significant effect. For
392 example, in cereals, a large number of QTL have been reported to be involved in yield
393 components such as ears per plant, grain weight and number per ear (Börner *et al.* 2002;
394 Huang *et al.* 2003; Quarrie *et al.* 2005). There is some evidence that seeds per pod may
395 also be an important component of yield in lupins (B. Wolko *et al.* pers. comm.) and, it
396 seems likely that this would have a (negative) correlation with seed size.
397
398 The number of QTLs found here for each trait are in all probability only a subset of the
399 total number of controlling genes as not all environments will reveal their presence

(Tanksley 1993; Young 1996). It is also possible that in this particular cross both parents carry the same set of alleles for a particular QTL. For instance, lines have been found exhibiting far greater early vigour then demonstrated in this population (J. Clements pers. comm.) and, in the Australian Lupin Collection are many lines (of *L. angustifolius*) that produce very small seeds. There are also a small number of lines tending to produce seed larger then those produced by this particular population. To locate all of the genes involved in these traits may require substantially more and larger populations to be generated and grown over a number of sites and years.

We found that several lupin QTL regions corresponded to regions of synteny on the *M. truncatula* pseudo-chromosome. Of particular interest are the QTLs associated with early vigour and days to flowering. These two traits are important to many crops including pasture legumes in dry Mediterranean environments. The identification of syntenic regions on the *M. truncatula* genome may thus lead to improved selection of superior lines by developing the appropriate molecular markers into forms useful across one or more related species.

The prediction of a superior genotype is an important part of plant breeding and the selection of parents has in the past frequently been on the basis of observations of superior performance in the field without fully understanding its genetic basis. For example, it had long been known that in China the F_1 hybrid rice 'Shanyou 63' was superior to both parents. A careful QTL analysis of that particular cross using the program QTL Network 1.0, revealed the basis for the superiority of the F_1 and, that there was still the potential to further increase yield gains from the same parental

424 combination (Yang and Zhu 2005). The outcome of our study confirms that in this
425 particular parental combination it is possible to select individuals having a higher early
426 vigour, demonstrating the potential to improve narrow-leaf lupins by using a similar
427 approach. Consequently, in Table 2 we inserted a column titled 'Superior genotype'
428 under which we have predicted plants genotypes that are expected to lead to superior
429 trait expressions as based on this experiment. However as noted earlier, the optimum
430 for a particular trait such as height at maturity may in fact be somewhere between the
431 two extremes in which case the predicted superior genotypes ought to be re-assessed.
432
433 Low gene heritability is a common problem in breeding. In this study, some of the least
434 heritable QTLs were involved in either a GxE interaction or epistasis (Table 2, Figure
435 4). By developing molecular markers closely linked to these QTLs it may be possible
436 to more efficiently select plants having the desirable alleles, leading to enhanced rates of
437 genetic improvement. The need for close linkage is high-lighted in Figure 4 where
438 especially the data for plants putatively of the BA genotype showed a large range in
439 height well beyond that found within the other gene combinations, suggesting that
440 cross-overs may have occurred between the QTL gene and the nearest associated
441 marker. The traits (for increased) early vigour, height at maturity and especially (a
442 reduction in the) days to flowering would benefit from this approach as all three have
443 several QTLs with substantial impact but of very low heritability.
444
445 In conclusion, this work has shown that the currently available genetic maps of narrow-
446 leaf lupin have opened the door of opportunity to a careful analysis of yield component
447 (and other) traits with a view to improved selection of parental genotypes and

448 accelerated breeding gains. However, the current marker population needs to be
449 expanded to allow for fine mapping of certain traits. This is especially important in the
450 region of the *Ku* gene where there is still confusion over the true order of the markers
451 and consequently, the number of QTLs controlling the key traits of early vigour, days to
452 flowering and seed size.
453
454
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Figure Legends

Figure 1: Linkage groups 22 (L) and 23 (R) showing markers (RHS) in order and cumulative distances (LHS) in centiMorgans.

Figure 2: Character distribution for Early Vigour, Height at Maturity, Days to Flowering and Seed Weight. Data are grouped into intervals, based on two years results.

Figure 3: Quantitative Trait Loci showing the locus interval, range and QTL position as well as gene effects and epistatic interactions on and between linkage groups (LG). Note: The upper figures denote the marker numbers without the prefix (DAWA) or the suffix (denoting the approximate marker length). The lower figures are the marker positions (cM) as on the Linkage map by Boersma *et al.* 2005 or, as in Figure 1. The light coloured bar indicates the QTL range and the estimated position and type of QTL is indicated by the 'button'. Epistatic genes are linked with a line.

Figure 3a: Early Vigour

Figure 3b: Height at Maturity

Figure 3c: Days to Flowering

Figure 3d: Seed Weight

Figure 4: The effect of two epistatic genes located on LG17 and LG23 on the early vigour of *L. angustifolius*.

621 Note: The first allele in each gene combination is located on LG17 and the second on
622 LG 23, with ‘A’ representing the allele originating from the domesticated parent and ‘B’
623 that from the wild parent.

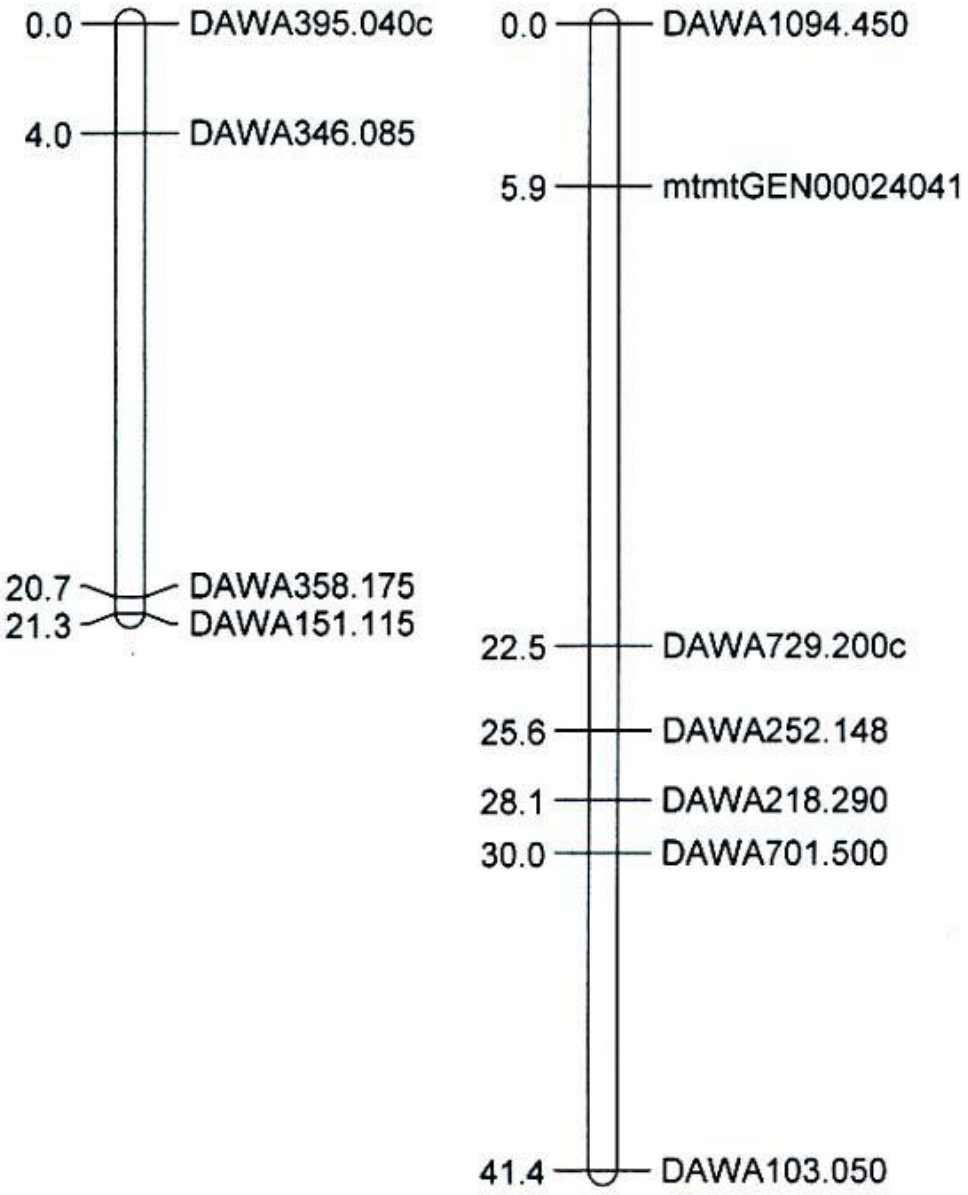
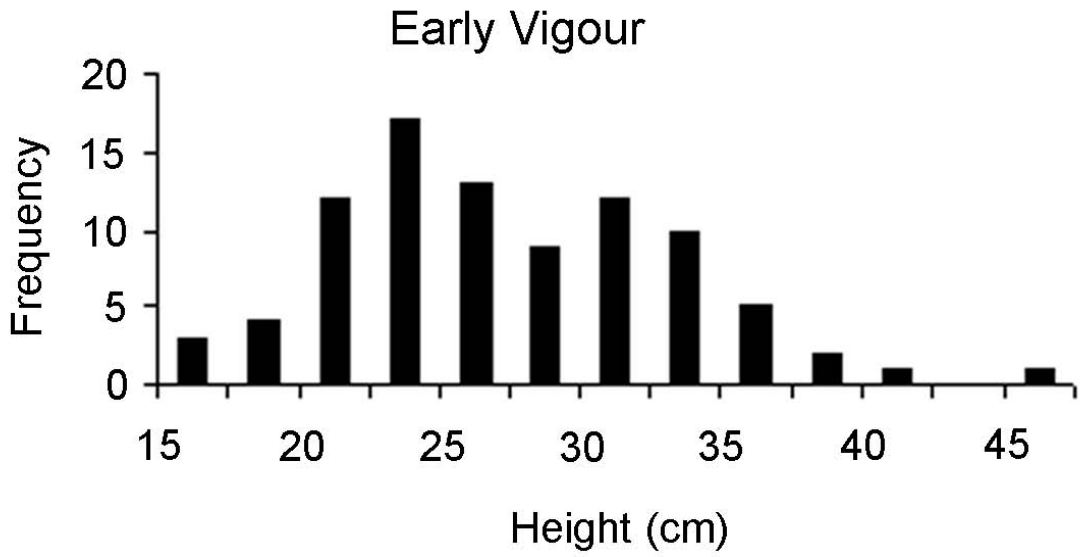
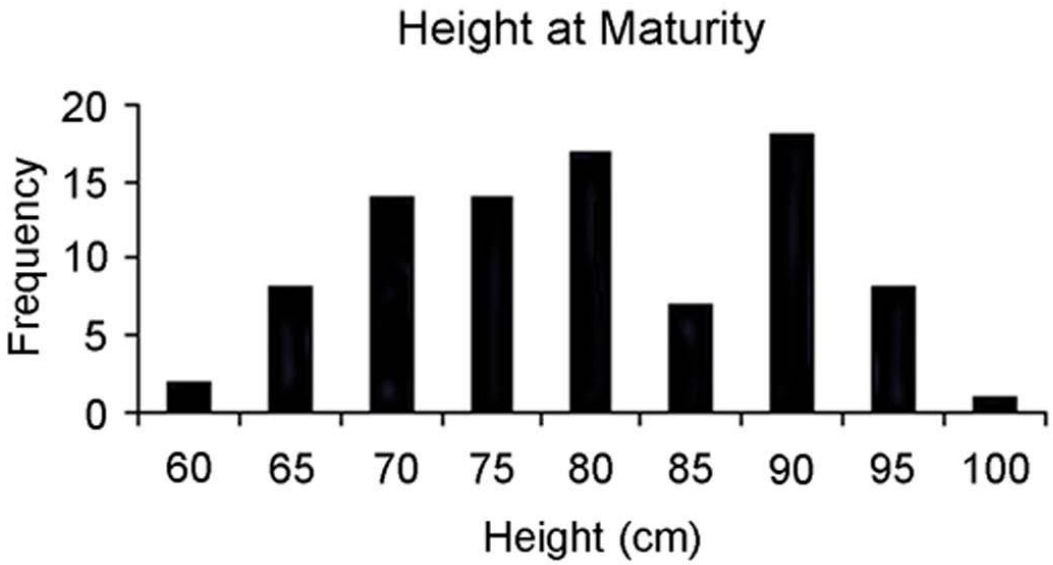

0.0
DAWA395.040c
4.0
DAWA346.085
20.7
DAWA358.175
21.3
DAWA151.115
0.0
DAWA1094.450
5.9
mtmtGEN00024041
22.5
DAWA729.200c
25.6
DAWA252.148
28.1
DAWA218.290
30.0
DAWA701.500
41.4
DAWA103.050

Figure 1



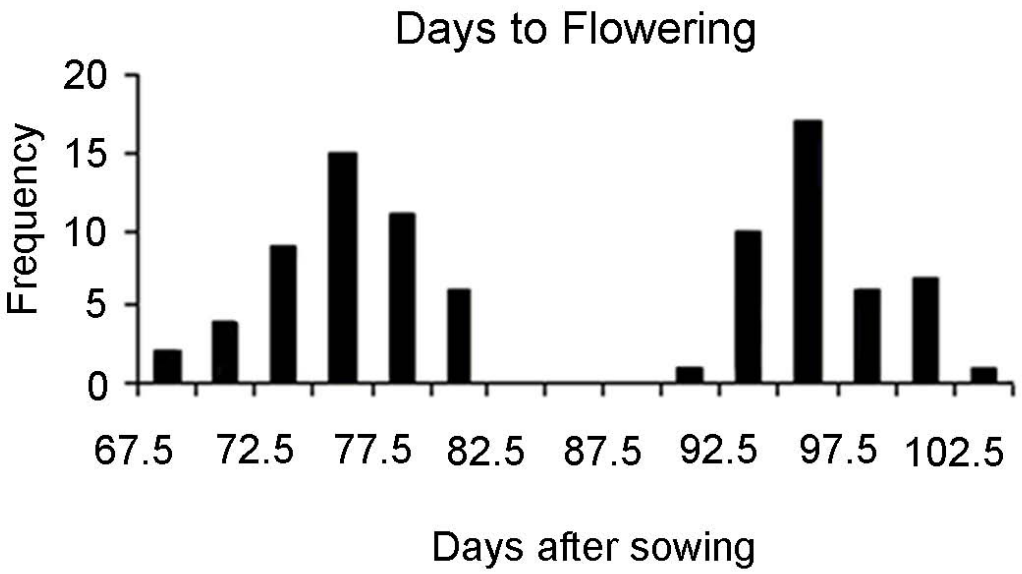
Parent A = 46.7 cm
Parent B = 20.4 cm

Figure 2a



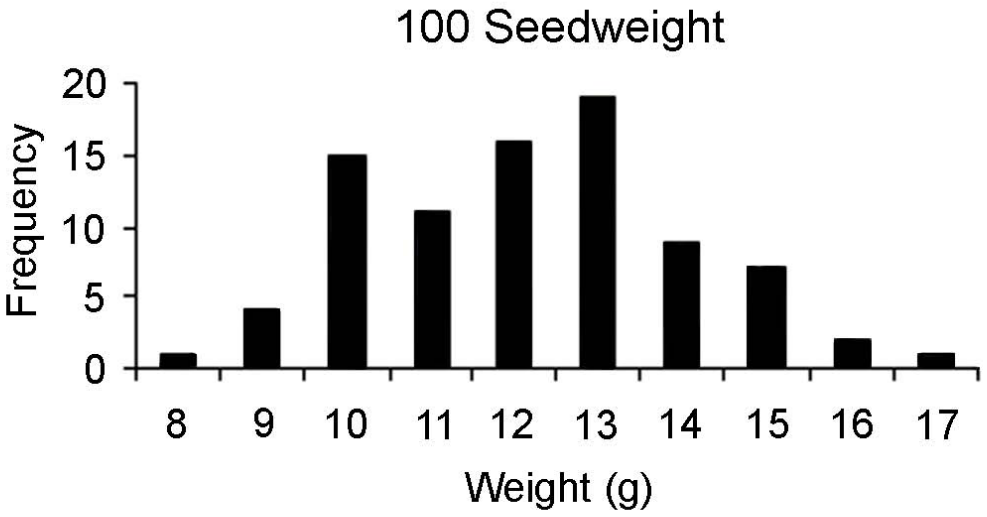
Parent A = 86.7 cm
Parent B = 77.4 cm

Figure 2b



Parent A = 72.5 d
Parent B = 96 d

Figure 2c



Parent A = 15.0 g
Parent B = 11.4 g (year 1 data only)

Figure 2d

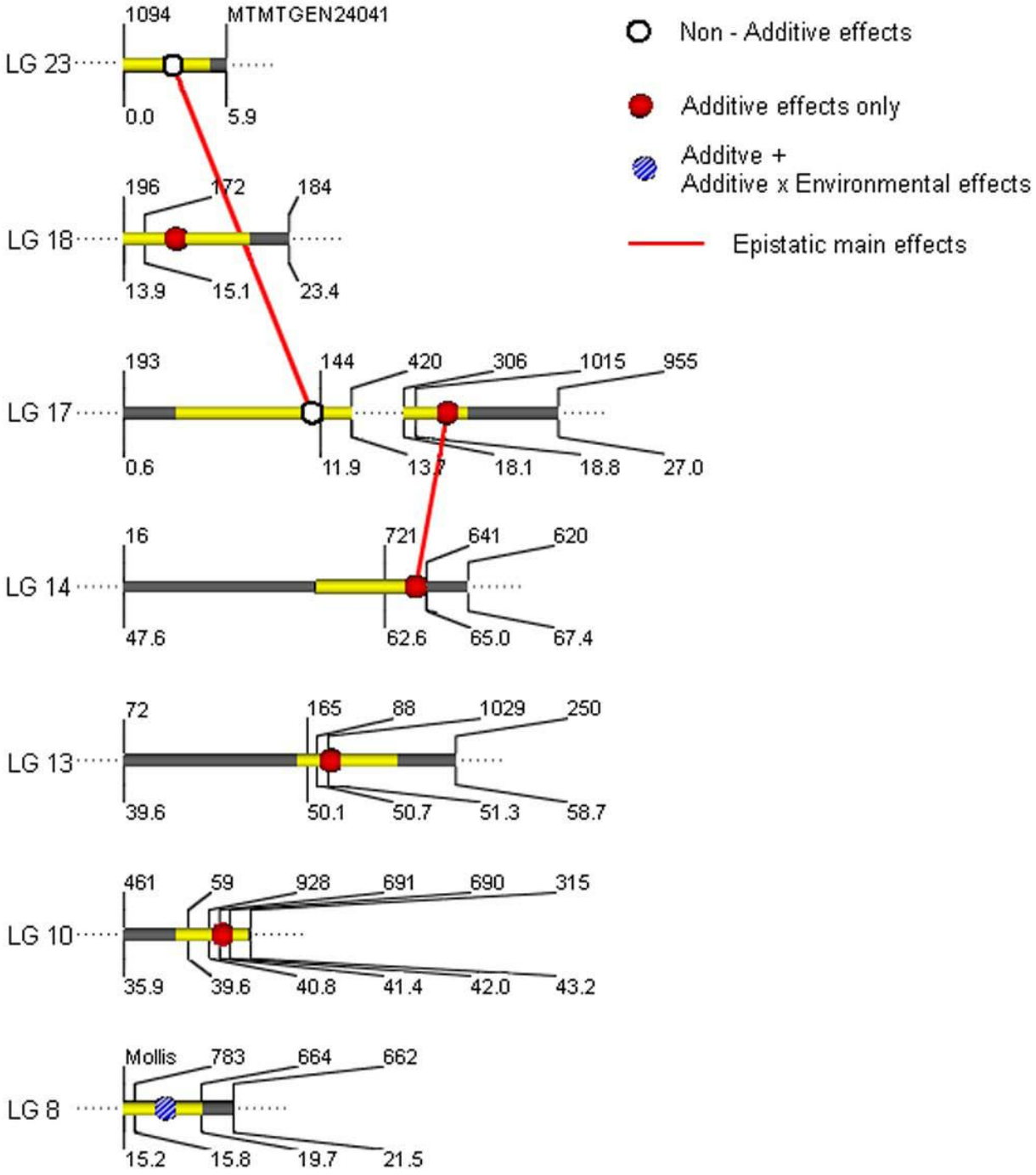

Non - Additive effects
Additive effects only
Additve +
Additive x Environmental effects
Epistatic main effects
LG 23
1094
MTMTGEN24041
0.0
5.9
LG 18
196
172
184
13.9
15.1
23.4
LG 17
193
144
420
306
1015
955
0.6
11.9
13.7
18.1
18.8
27.0
LG 14
16
721
641
620
47.6
62.6
65.0
67.4
LG 13
72
165
88
1029
250
39.6
50.1
50.7
51.3
58.7
LG 10
461
59
928
691
690
315
35.9
39.6
40.8
41.4
42.0
43.2
LG 8
Mollis
783
664
662
15.2
15.8
19.7
21.5

Figure 3a

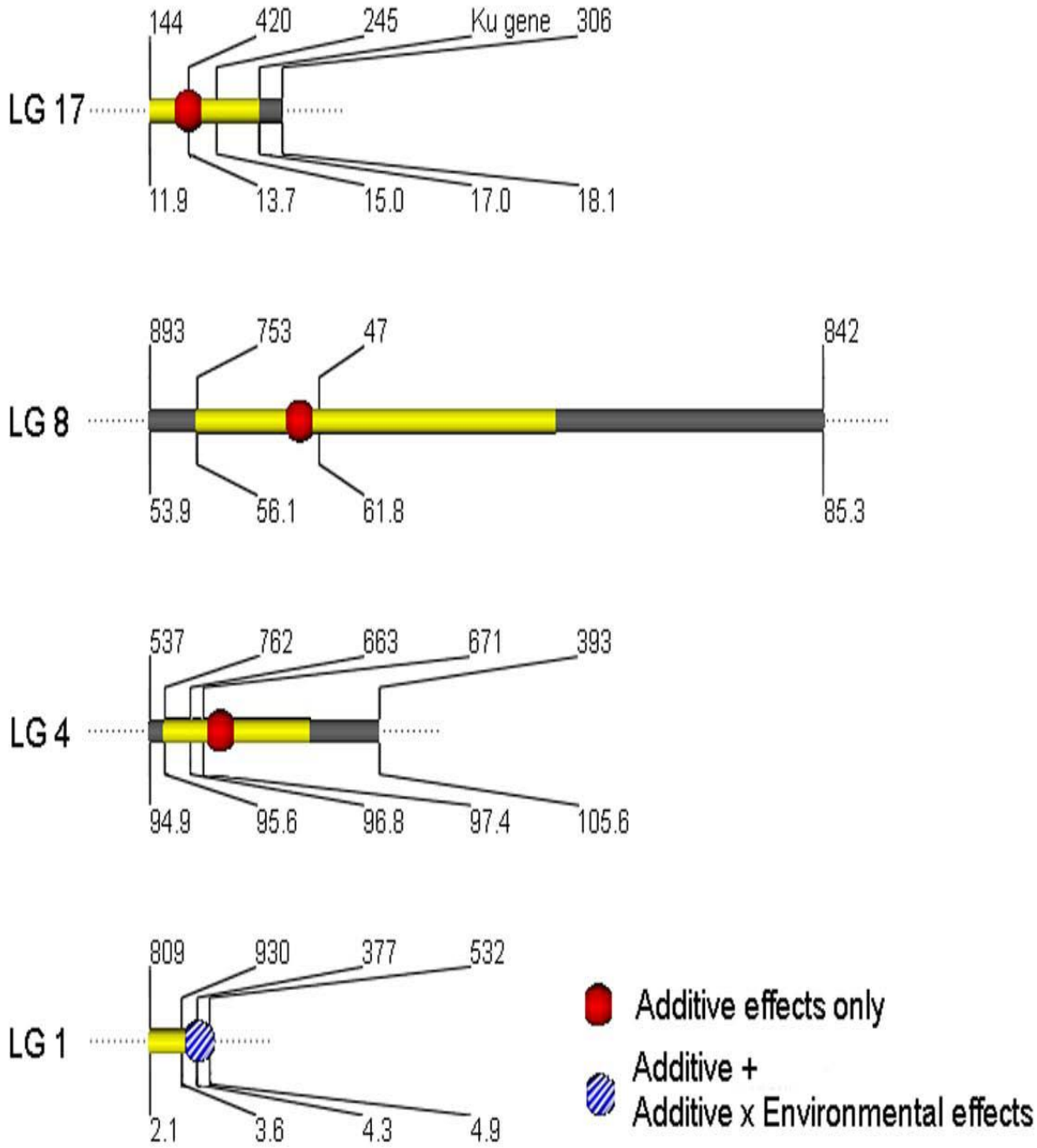

LG 17
144
420
245
Ku gene
306
11.9
13.7
15.0
17.0
18.1
LG 8
893
753
47
842
53.9
56.1
61.8
85.3
LG 4
537
762
663
671
393
94.9
95.6
96.8
97.4
105.6
LG 1
809
930
377
532
2.1
3.6
4.3
4.9
Additive effects only
Additive +
Additive x Environmental effects

Figure 3b