Supplement

Genotype data

	M_1	M_2	$\cdots$	M_k
S_1	AA	AB	$\cdots$	AA
S_2	AA	AA	$\cdots$	AB
S_3	AB	AB	$\cdots$	AB
S_4	AB	AA	$\cdots$	AA
S_5	AA	AA	$\cdots$	AA
S_6	AB	AA	$\ldots$	AB
S_7	AB	AB	$\cdots$	AA
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
S_s	AA	AB	$\cdots$	AA

Phenotype data

	P_1	P_2	$\cdots$	P_T
S_1	1.5	3.6	$\cdots$	2.8
S_2	4.1	1.9	$\cdots$	2.2
S_3	3.2	0.6	$\cdots$	2.8
S_4	3.3	4.8	$\cdots$	4.2
S_5	4.1	2.9	$\cdots$	2.6
S_6	0.7	1.4	$\cdots$	2.5
S_7	2.2	3.4	$\cdots$	1.7
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
S_s	2.3	2.6	$\cdots$	2.9

$\Rightarrow$

Observed QTLs

	P_1	P_2	$\cdots$	P_T	Counts
L_1		•	$\cdots$		N_1
L_2	•		$\cdots$		N_2
L_3			$\cdots$	•	N_3
L_4	•	•	$\cdots$	•	N_4
L_5		•	$\cdots$		N_5
L_6			$\cdots$		N_6
L_7		•	$\cdots$		N_7
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
L_l			$\cdots$	•	N_l

$\Rightarrow$

Figure S 1: Hotspot analysis in a genetical genomics study. The data is composed by genotypes and phenotypes on s subjects, $S_1, \ldots, S_s$, from a segregating population. The genotype data is composed by the genotypes of k markers, $M_1, \ldots, M_k$. The phenotype data is composed by measurements on T quantitative phenotypes, $P_1, \ldots, P_T$. The output of the analysis is a QTL matrix, where rows represent l genomic positions, $L_1, \ldots, L_l$, and columns the phenotypes. A significant QTL is represented by a bullet, for example, phenotype P_1 maps to QTLs located at the L_2 and L_4 genomic positions. For each genomic position, $L_1, \ldots, L_l$, we count the number of significant QTLs, $N_1, \ldots, N_l$. We say we detected a significant hotspot at a genomic location, L_j, when the respective count, N_j, is higher than what is expected by chance at a pre-determined genome wide error rate.

Observed QTL matrix

	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9
L_1		•							•
L_2	•				•				
L_3								•	
L_4	•	•	•	•		•	•		•
L_5		•			•				
L_6							•	•	
L_7		•							•

Permutation 1

	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	Counts
L_1		•				•			•	3
L_2				•			•			2
L_3	•	•	•					•	•	5
L_4		•		•						2
L_5							•			1
L_6	•				•		•			3
L_7		•							•	2

$\Rightarrow \quad N_{max}^1 = 5$

$\vdots$

Permutation 1000

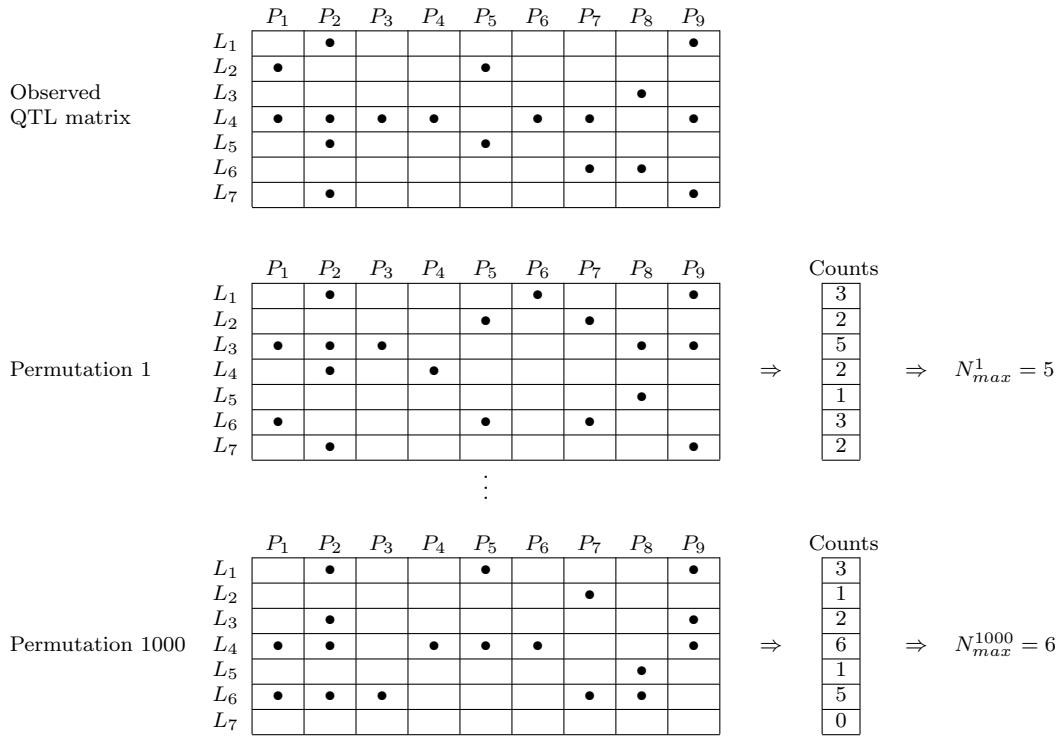|	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	Counts
L_1		•			•				•	3
L_2							•			1
L_3		•							•	2
L_4	•	•		•	•	•			•	6
L_5								•		1
L_6	•	•	•				•	•		5
L_7										0

$\Rightarrow \quad N_{max}^{1000} = 6$

Figure S 2: Permutation scheme adopted by West et al. (2007) and Wu et al. (2008). In this permutation scheme we take the QTL matrix and, for each fixed phenotype (column in the QTL matrix), we permute the QTL locations (the row cells at each fixed column). This figure depicts the result of two permutations of the observed QTL matrix. The permutation null distribution of hotspot sizes is derived as follows. For each one of the, say 1000, permutations we: (i) permute the genomic positions of the QTLs for each one of the phenotypes separately; (ii) for each genomic location we record the number of QTLs; (iii) record the maximum count N_{max}^{per}. The permutation null distribution (for the λ threshold used to derive the observed QTL matrix) is then given by the distribution of the 1,000 N_{max}^{per} values.
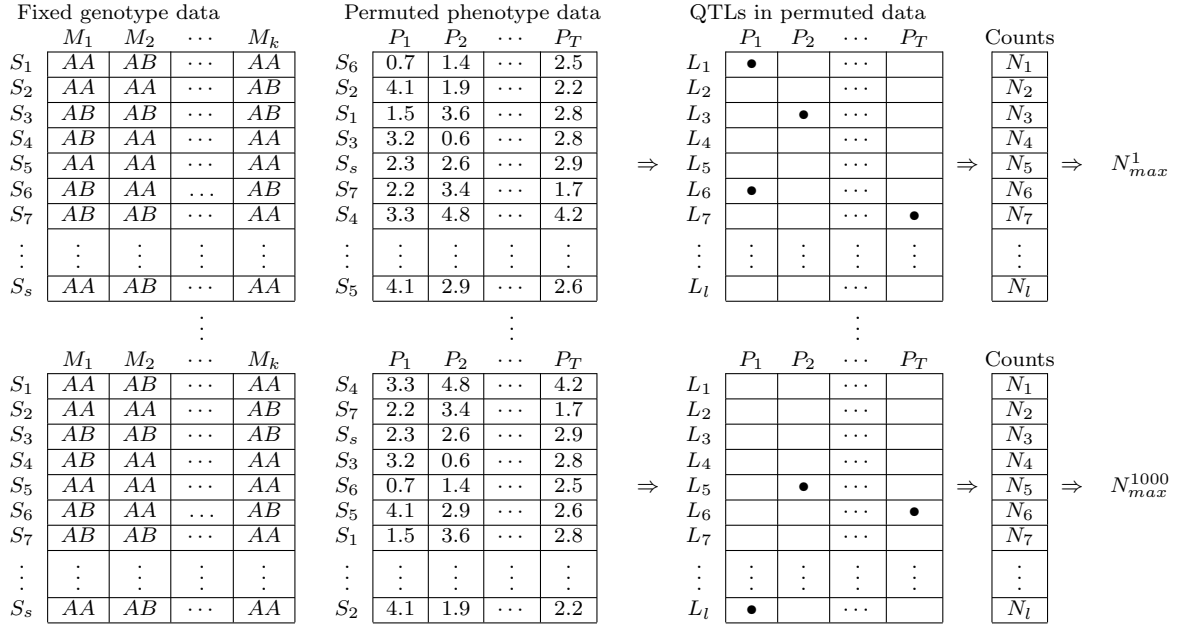
Fixed genotype data

	M_1	M_2	$\cdots$	M_k
S_1	AA	AB	$\cdots$	AA
S_2	AA	AA	$\cdots$	AB
S_3	AB	AB	$\cdots$	AB
S_4	AB	AA	$\cdots$	AA
S_5	AA	AA	$\cdots$	AA
S_6	AB	AA	$\ldots$	AB
S_7	AB	AB	$\cdots$	AA
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
S_s	AA	AB	$\cdots$	AA

Permuted phenotype data

	P_1	P_2	$\cdots$	P_T
S_6	0.7	1.4	$\cdots$	2.5
S_2	4.1	1.9	$\cdots$	2.2
S_1	1.5	3.6	$\cdots$	2.8
S_3	3.2	0.6	$\cdots$	2.8
S_s	2.3	2.6	$\cdots$	2.9
S_7	2.2	3.4	$\cdots$	1.7
S_4	3.3	4.8	$\cdots$	4.2
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
S_5	4.1	2.9	$\cdots$	2.6

$\Rightarrow$

QTLs in permuted data

	P_1	P_2	$\cdots$	P_T
L_1	•		$\cdots$	
L_2			$\cdots$	
L_3		•	$\cdots$	
L_4			$\cdots$	
L_5			$\cdots$	
L_6	•		$\cdots$	
L_7			$\cdots$	•
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
L_l			$\cdots$	

$\Rightarrow$

Counts

N_1
N_2
N_3
N_4
N_5
N_6
N_7
$\vdots$
N_l

$\Rightarrow \quad N_{max}^1$

	M_1	M_2	$\cdots$	M_k
S_1	AA	AB	$\cdots$	AA
S_2	AA	AA	$\cdots$	AB
S_3	AB	AB	$\cdots$	AB
S_4	AB	AA	$\cdots$	AA
S_5	AA	AA	$\cdots$	AA
S_6	AB	AA	$\ldots$	AB
S_7	AB	AB	$\cdots$	AA
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
S_s	AA	AB	$\cdots$	AA

	P_1	P_2	$\cdots$	P_T
S_4	3.3	4.8	$\cdots$	4.2
S_7	2.2	3.4	$\cdots$	1.7
S_s	2.3	2.6	$\cdots$	2.9
S_3	3.2	0.6	$\cdots$	2.8
S_6	0.7	1.4	$\cdots$	2.5
S_5	4.1	2.9	$\cdots$	2.6
S_1	1.5	3.6	$\cdots$	2.8
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
S_2	4.1	1.9	$\cdots$	2.2

$\Rightarrow$

	P_1	P_2	$\cdots$	P_T
L_1			$\cdots$	
L_2			$\cdots$	
L_3			$\cdots$	
L_4			$\cdots$	
L_5		•	$\cdots$	
L_6			$\cdots$	•
L_7			$\cdots$	
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$
L_l	•		$\cdots$	

$\Rightarrow$

Counts

N_1
N_2
N_3
N_4
N_5
N_6
N_7
$\vdots$
N_l

$\Rightarrow \quad N_{max}^{1000}$

Figure S 3: A permutation scheme that preserves the correlation among the phenotypes. Breitling et al. (2008) proposed a permutation scheme where the rows of the phenotype data matrix are permuted, while the genotype data matrix is kept intact. The idea is to break the connection between the genotype and phenotype data, but to preserve the correlation structure among the phenotypes. The permutation null distribution of hotspot sizes is derived as follows. For each one of the, say 1000, permutations we: (i) permute the rows of the phenotype data matrix, while keeping the genotype data intact (note the different row orderings of the permuted phenotype data matrices in relation to original phenotype matrix in Figure 1); (ii) perform mapping analysis of the T phenotypes, using a predetermined LOD threshold, λ, to determine a new QTL matrix (note that all QTLs detected with the permuted data are false positives); (iii) for each genomic location $L_1, \ldots, L_l$ we record the number of QTLs, $N_1, \ldots, N_l$; (iv) we record the maximum count $N_{max}^{per} = \max\{N_1, \ldots, N_l\}$. The permutation null distribution for the chosen λ threshold is then given by the distribution of the 1,000 N_{max}^{per} values.
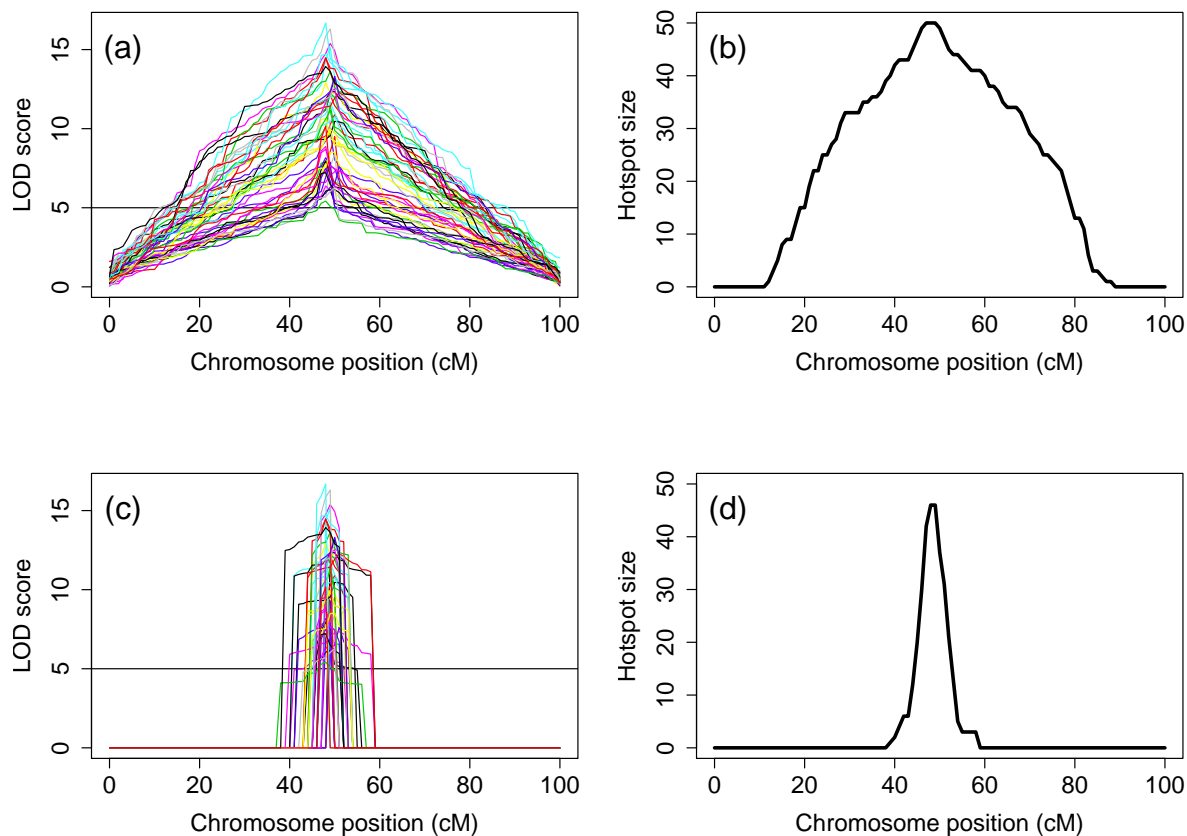
Figure S 4: Using LOD support intervals to reduce the spread of QTL hotspots. Panel (a) shows the LOD profile curves of 50 traits showing peaks around 50cM. Panel (b) shows how many traits have LOD score above the LOD threshold 5 (horizontal line) for each genomic location. Panel (c) shows the processed LOD curves where, for each trait, we computed the 1.5 LOD support interval and set the LOD scores outside the interval to zero. Panel (d) shows the counts based on the processed LOD profiles. Note how the spread of the hotspot location is drastically reduced from panel (b) to panel (d).
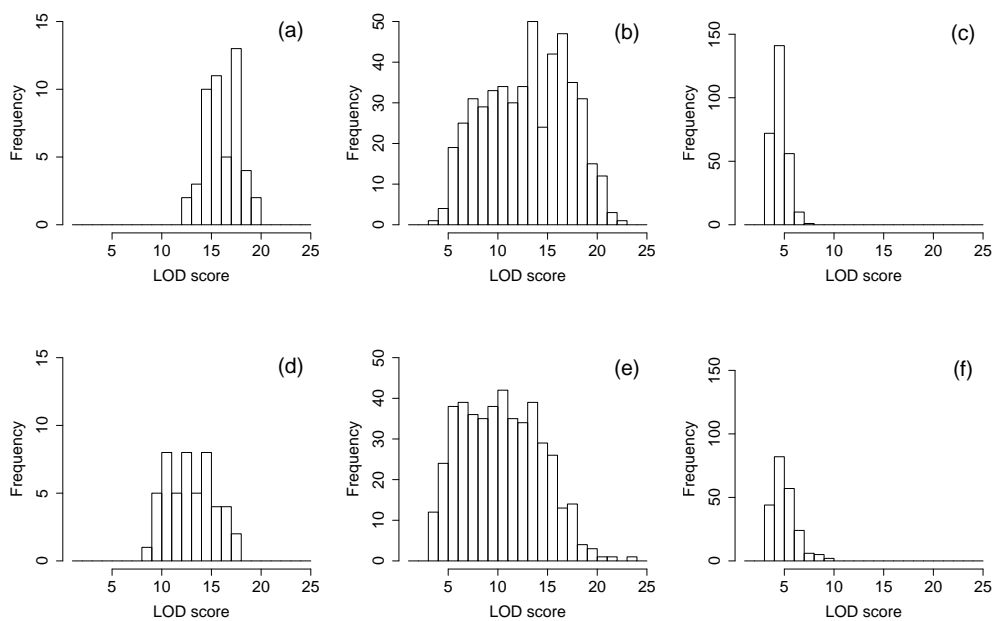
Figure S 5: Hotspot LOD score distributions for simulated examples 1 and 2. Panels (a) and (d) show the LOD score distribution for the hotspot on chromosome 3 for simulated example 1 and 2, respectively. Panels (b) and (e) show the LOD score distribution for the hotspot on chromosome 7 for simulated example 1 and 2, respectively. Panels (c) and (f) show the LOD score distribution for the hotspot on chromosome 15 for simulated example 1 and 2, respectively. The histograms show the distribution of the LOD scores of the traits composing the hotspot at the hotspot peak location.
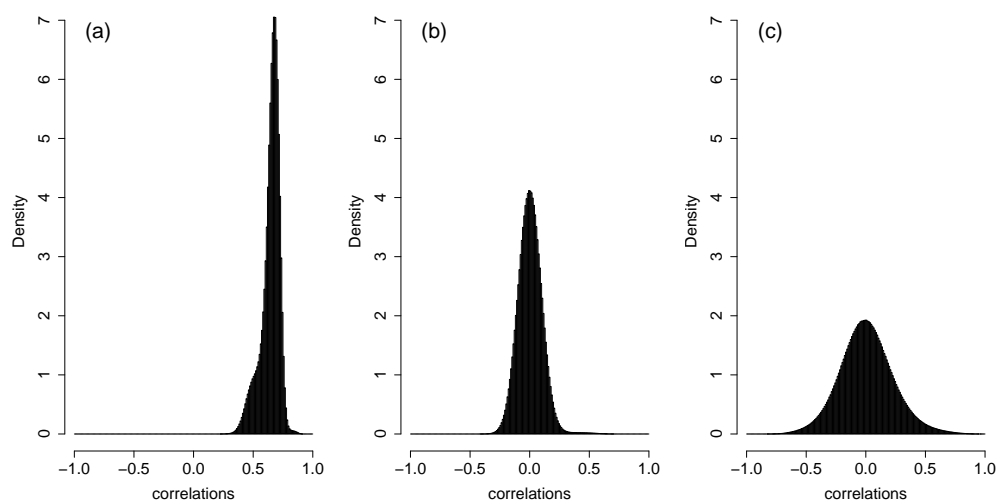
Figure S 6: Pairwise correlations among phenotypes. Panels (a) and (b) show the results for simulated examples 1 and 2, respectively. Panel (c) shows the distribution of the pairwise correlations for the yeast data-set.
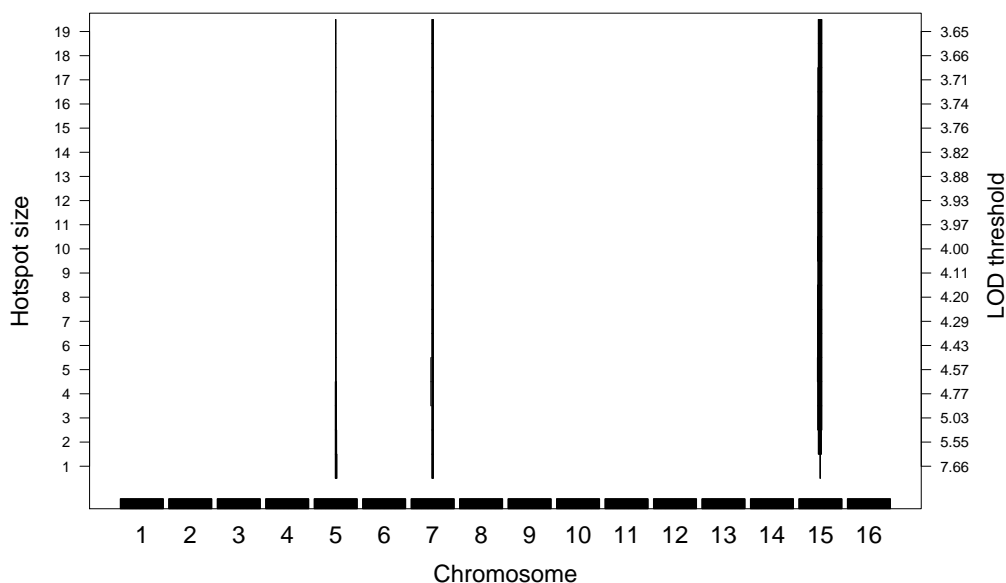
Figure S 7: Hotspot size significance profile derived with the NL-method for simulated example 2. For each genomic location (i.e., x-axis position) this figure shows the hotspot sizes at which the hotspot was significant, that is, at which the hotspot locus had more traits mapping to it with a LOD score higher than the threshold on the right, than expected by chance. The scale in the left shows the range of spurious hotspot sizes investigated by our approach. The scale in the right shows the respective LOD thresholds associated with the spurious hotspot sizes in the left. The range is from 7.66, the conservative empirical LOD threshold associated with a spurious "hotspot of size 1", to 3.65, the single trait empirical threshold, associated with a spurious hotspot of size 19. All permutation thresholds were computed targeting GWER $\leq$ 0.05, for $n = 1, \ldots, 19$. Note that this figure does not show the actual size of the hotspots. For instance, at the single trait LOD threshold of 3.65 this plot indicates that more traits mapped to the middle regions of chromosomes 5, 7 and 15 than what would be expected by chance (19 traits), but do not show the actual hotspot sizes (50, 464 and 220, respectively, as depicted in Figure **??**a).