

**Table S1** Mean (and range) number of days per year with freezing temperatures, given in intervals of 1 C. The mean (and range) total number of freezing days per year is also given. Temperature data are from hourly recordings at each of the parental field sites (Ågren & Schemske 2012) over 8 years (2003/2004 to 2010/2011).

Site	Temperature category	Soil		Air	
		Mean number of days	Range	Mean number of days	Range
<b>Italy</b>	0 to -1	0.1	(0-1)	6.0	(0-13)
	-1 to -2	0	-	4.0	(0-11)
	-2 to -3	0	-	1.8	(0-4)
	-3 to -4	0	-	0.5	(0-1)
	-4 to -5	0	-	0.3	(0-1)
	-5 to -6	0	-	0.3	(0-1)
	-6 to -7	0	-	0	-
	< -7	0	-	0	-
Freezing total	0.1	(0-1)	13	(0-24)	
<b>Sweden</b>	0 to -1	61.8	(18-114)	26.0	(12-49)
	-1 to -2	46.8	(15-119)	23.9	(14-35)
	-2 to -3	15.8	(0-62)	19.8	(12-31)
	-3 to -4	3.4	(0-10)	17.3	(12-29)
	-4 to -5	1.5	(0-6)	12.4	(7-18)
	-5 to -6	1.1	(0-7)	11.4	(6-19)
	-6 to -7	0.6	(0-4)	9.5	(5-13)
	< -7	0.9	(0-7)	38.1	(18-53)
Freezing total	132	(81-167)	158	(128-175)	

**Table S2** Results of simulations estimating the number of genomic co-localizations between freezing tolerance, survival, and fitness QTL expected by chance. The table gives the number of co-localizations found in 10000 replicate draws randomly placing 7 freezing tolerance QTL and 5 field survival QTL (observed numbers of each type) at unique positions along a 480 cM genome at 1 cM intervals. The genome was then divided into 80 “boxes”, each 6 cM wide. The total genome size and marker spacing used is roughly equivalent to the total length of the linkage map for all five chromosomes, and average marker spacing, respectively. Six cM is approximately the average width of the "boxes" containing fitness QTL in Figure 2. The number of co-localizations for each draw is defined as the number of times both a freezing tolerance QTL and fitness QTL occur in the same “box”.

Number of co-localization events	Frequency (i.e. $P$ )
0	0.6342
1	0.3141
2	0.0484
3	0.0033
>3	0

**Table S3** Benefits/costs of the Swedish genotype at each parental site (Italy and Sweden) for each of the three freezing tolerance QTL that co-localize with field fitness (mean fruits per RIL) and survival (%). Cell entries are expressed as percentage difference of the Swedish genotype relative to the Italian genotype.

	FrzT1:2		FrzT4:1		FrzT5:2	
	Italy	Sweden	Italy	Sweden	Italy	Sweden
Fitness	-13	-2	-11	+4	-18	+6
Survival	-4	+1	-2	+4	-5	+5

**Table S4** Candidate genes underlying adaptive freezing tolerance QTL, annotated with “cold” in the GO terms using experimental evidence (TAIR; [www.Arabidopsis.org](http://www.Arabidopsis.org)). Genes with predicted amino acid differences between the parents (Mckay *et al.*, unpublished data) are indicated in bold. The Italian allele for CBF2 has a deletion that is expected to result in a non-functional protein (Dong 2012).

QTL	Gene	Alias	Description
FrzT 1:2	<b>AT1G68090</b>	<b>ANNAT5</b>	calcium-binding protein annexin
	AT1G68530	CER6	member of the 3-ketoacyl-CoA synthase family involved in the biosynthesis of very long chain fatty acids
FrzT 4:1	AT4G24190	HSP90.7	ortholog of GRP94, an ER-resident HSP90-like protein and is involved in regulation of meristem size and organization.
	AT4G24280	cpHsc70-1	involved in protein import into chloroplasts during early developmental stages
	<b>AT4G24500</b>	<b>SIC</b>	proline-rich protein required for development and abiotic stress tolerance and involved in microRNA biogenesis
	AT4G24770	RBP31	chloroplast RNA-binding protein, a substrate of the type III effector HopU1 required for editing and stability of specific chloroplast mRNAs
	<b>AT4G25380</b>	<b>SAP10</b>	stress-associated protein, functions in DNA binding and zinc ion binding
	<b>AT4G25470</b>	<b>CBF2</b>	member of the DREB subfamily A-1 of ERF/AP2 transcription factor family, contains one AP2 domain. Involved in response to low temperature, abscisic acid, and circadian rhythm
	AT4G25480	CBF3	member of the DREB subfamily A-1 of ERF/AP2 transcription factor family, contains one AP2 domain. Involved in response to low temperature and abscisic acid
	<b>AT4G25490</b>	<b>CBF1</b>	member of the DREB subfamily A-1 of ERF/AP2 transcription factor family, contains one AP2 domain. Involved in response to low temperature and abscisic acid

**Table S4** *Continued*

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FrzT 5:2	AT5G63980	SAL1	bifunctional protein that has 3'(2'),5'-bisphosphate nucleotidase and inositol polyphosphate 1-phosphatase activities, involved in the response to cold, drought, and ABA. Involved in degradation of small mRNAs. Regulates light-dependent repression of hypocotyl elongation and flowering time via its 3'(2'),5'-bisphosphate nucleotidase activity
	<b>AT5G64220</b>	<b>CAMTA2</b>	calmodulin-binding transcription activator protein with CG-1 and Ankyrin domains
	<b>AT5G65020</b>	<b>ANNAT2</b>	calcium-binding protein annexin
	AT5G65940	CHY1	hydrolyzes beta-hydroxyisobutyryl-CoA
	<b>AT5G66400</b>	<b>RAB18</b>	dehydrin protein family, ABA- and drought-induced.
	AT5G67320	HOS15	WD-40 protein involved in histone deacetylation in response to abiotic stress. Functions as a repressor of cold tolerance induced genes.
	AT5G67450	ZF1	zinc-finger protein, mRNA levels are elevated in response to low temperature, cold temperatures and high salt. The protein is localized to the nucleus and acts as a transcriptional repressor.
	AT5G67590	FRO1	high similarity to the 18-kD Fe-S subunit of complex I (NADH dehydrogenase, EC 1.6.5.3) in the mitochondrial electron transfer chain.

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**Table S5** Summary of studies that have mapped the genetic basis of freezing tolerance in *Arabidopsis thaliana*. Those that used multiple mapping populations are represented by multiple rows, as are single mapping populations subjected to different environmental conditions. The magnitude of difference between parental lines in freezing tolerance is given for each study, and is categorical for studies that score freezing tolerance in damage categories, or by electrolyte leakage assays. Mapping population type (Cross), size (# lines) and marker information (type and #) are given, as well as a summary of how many QTL were found. Potential overlap between the freezing tolerance QTL found in these studies and the three adaptive QTL (FrzT1:2 , FrzT4:1, or Frz5:2) was visually assessed based on LOD profile plots, as it is difficult to compare locations between different mapping populations with different linkage maps.

Study	Mapping parents	Difference in parental mean		Cross	# lines	Marker type	# markers	# QTL	FrzT1:2?	FrzT4:1?	FrzT5:2?
		frz. Tol.									
Alonso-Blanco <i>et al.</i> 2005	Cvi x Ler	> 70%		RIL	132	AFLP, CAPs, SSRs	99	5	yes	yes	yes
Alonso-Blanco <i>et al.</i> 2005	Cvi x Ler	> 70%		RIL	53	AFLP, CAPs, SSRs	99	4	maybe	yes	no
Gery <i>et al.</i> 2011	Can x Col	Large		RIL	164	SNPs	84	5	no	yes	no
Gery <i>et al.</i> 2011	Bur x Col	None		RIL	164	SNPs	87	2	maybe	no	no
Kang <i>et al.</i> 2013	SXcgx x CQtlx	~40%		F2	78	Indels	58	2	no	yes	maybe
Meissner <i>et al.</i> 2013	Te x C24	Large		RIL	250	SNPs	69	3	no	no	no

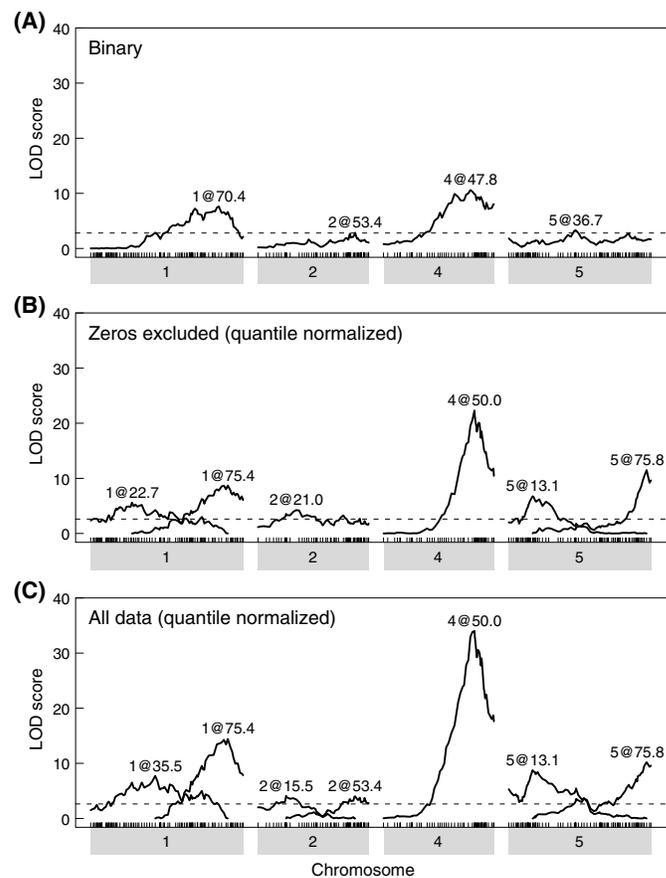


Fig. S1 QTL LOD profile plots from the stepwise function in R/qtl using different approaches for analyzing freezing tolerance. Numbers above peaks give chromosome and (@) position in cM. Dashed lines indicate LOD thresholds based on 10,000 permutations. Results from a binary analysis of freezing tolerance where RILs with zero freezing tolerance (100% mortality) were scored as 0, and all others scored as 1 (A). Results using quantile normalized RIL mean freezing tolerance omitting 80 RILs with a mean of 0 (B). Results for the QTL model presented in the paper using a normal distribution model on all the data after quantile normalization (C).

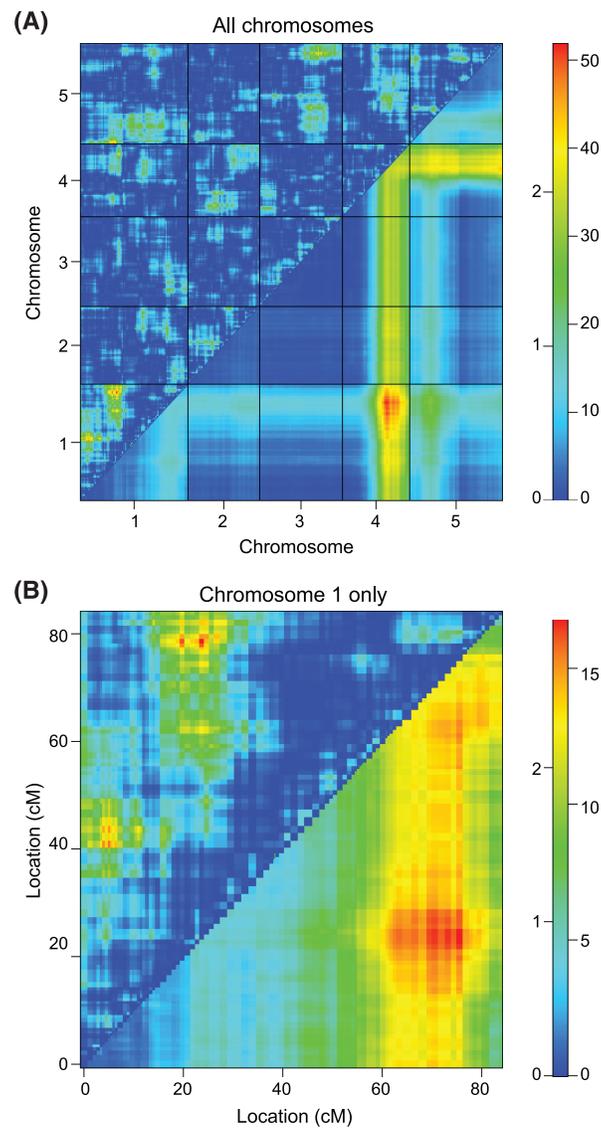


Fig. S2 Heat maps illustrating all pairwise (Scan-two) additive and epistatic LOD scores from R-QTL (Broman & Sen 2009). The LOD score for the full model (two additive markers and their interaction) of each pairwise marker combination is plotted below the diagonal. Above the diagonal is the LOD score of only the epistatic (interaction) term in the model: the difference between the LOD score of the full model and the additive-only model. The legends for the epistatic and full model LOD scores are plotted on the left and right side, respectively, of the vertical bar adjacent to each plot. All 5 chromosomes (A), expansion of chromosome 1 (B) to highlight areas of modest, but non-significant, epistasis. Note that the range of LOD scores, as indicated by the colors, is different for the two panes.