

Marker positions

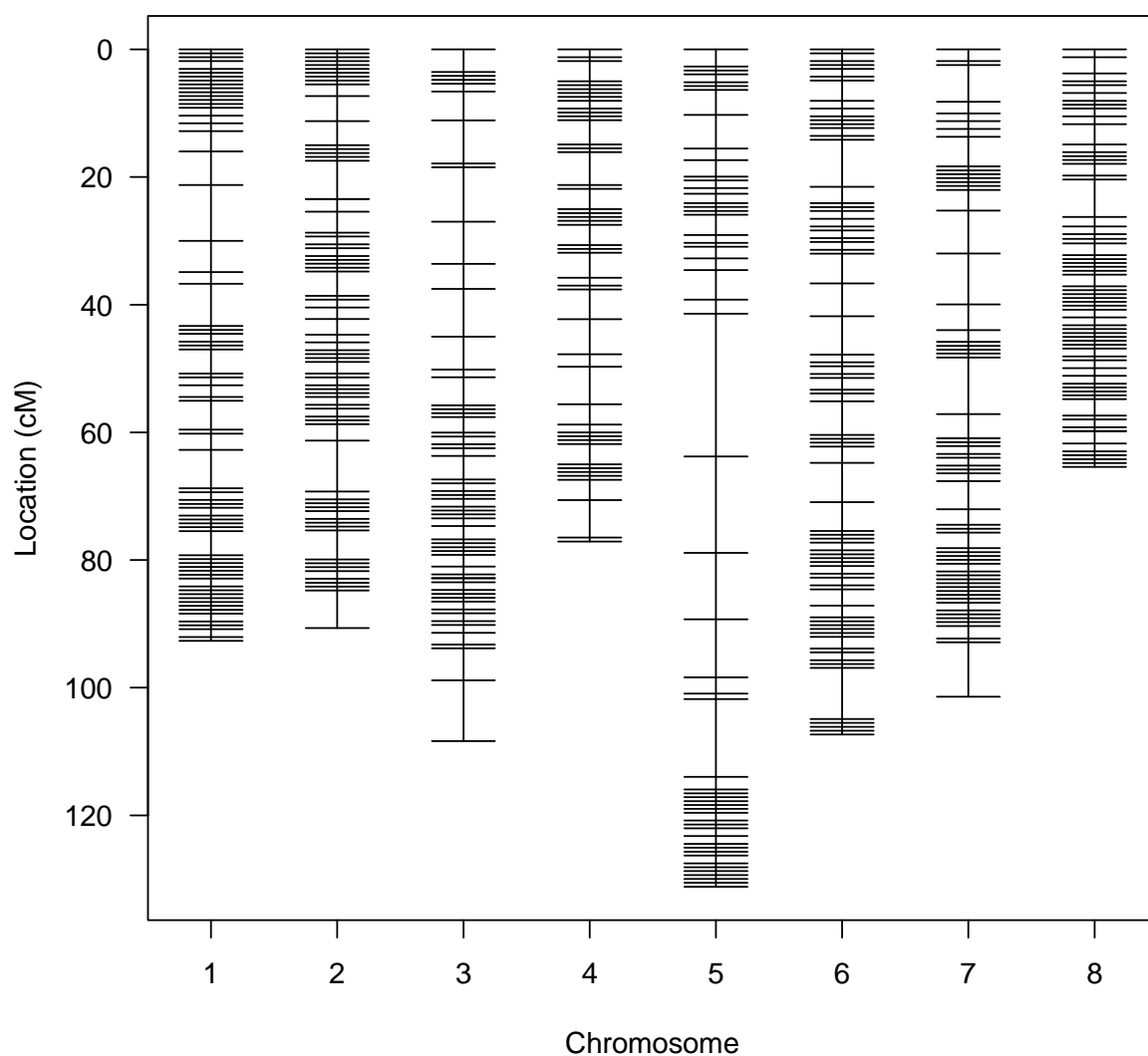


Figure S1. Distribution of 501 genetic markers in the *Erysimum cheiranthoides* Konstanz x Elbtalaue F2 population genetic map. Figure was generated using the `plotMap()` function in R/ql.

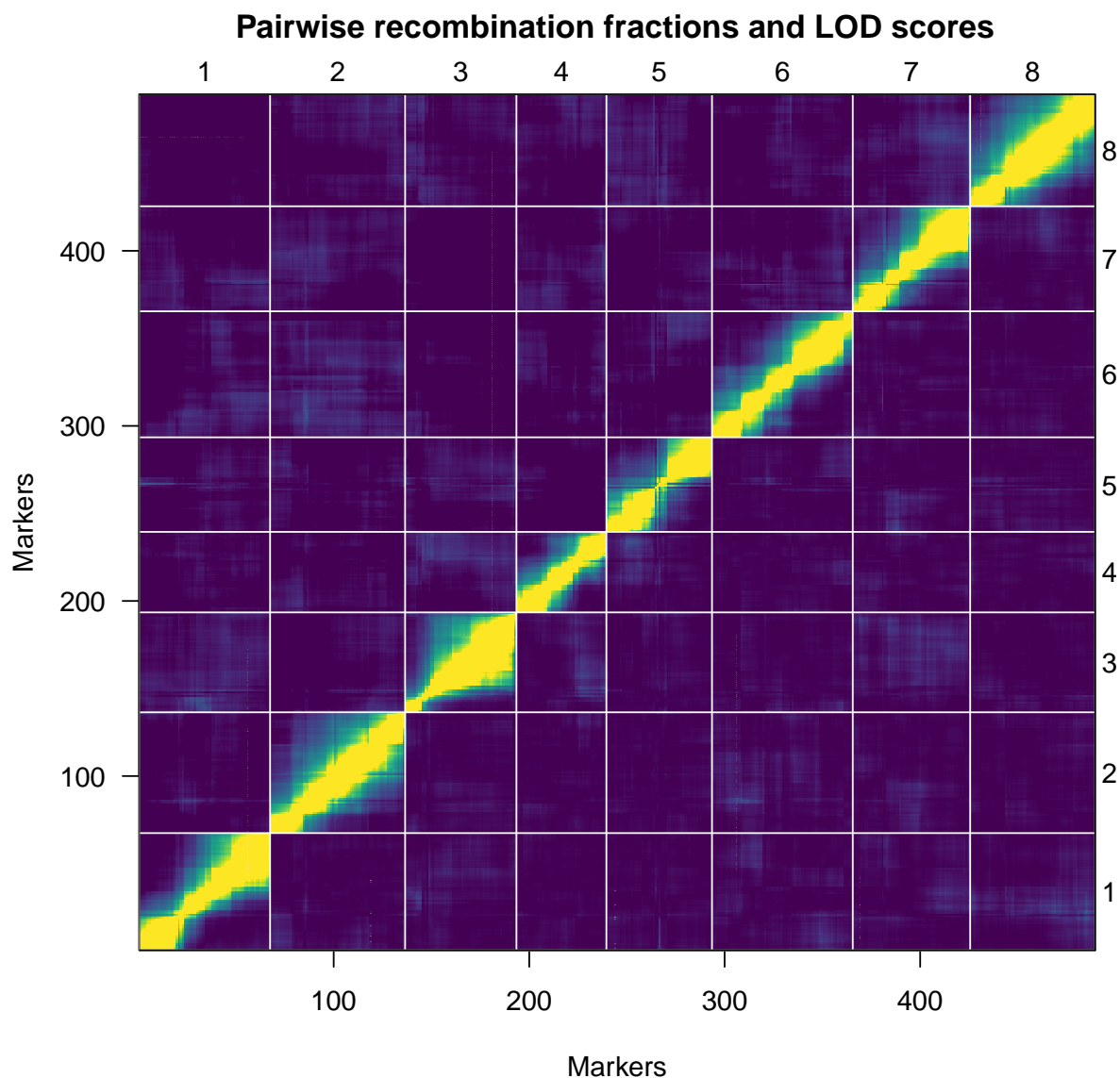


Figure S2. Genetic linkage of *Erysimum cheiranthoides* chromosomal markers. Pairwise recombination fractions (above diagonal) and LOD scores (below diagonal) indicating probability of genetic linkage for 501 genetic markers in the Konstanz x Elbtalaue genetic map. The 8 linkage groups correspond to the 8 chromosomes of *E. cheiranthoides*. Figure was generated using the plotRF() function in R/qtl.

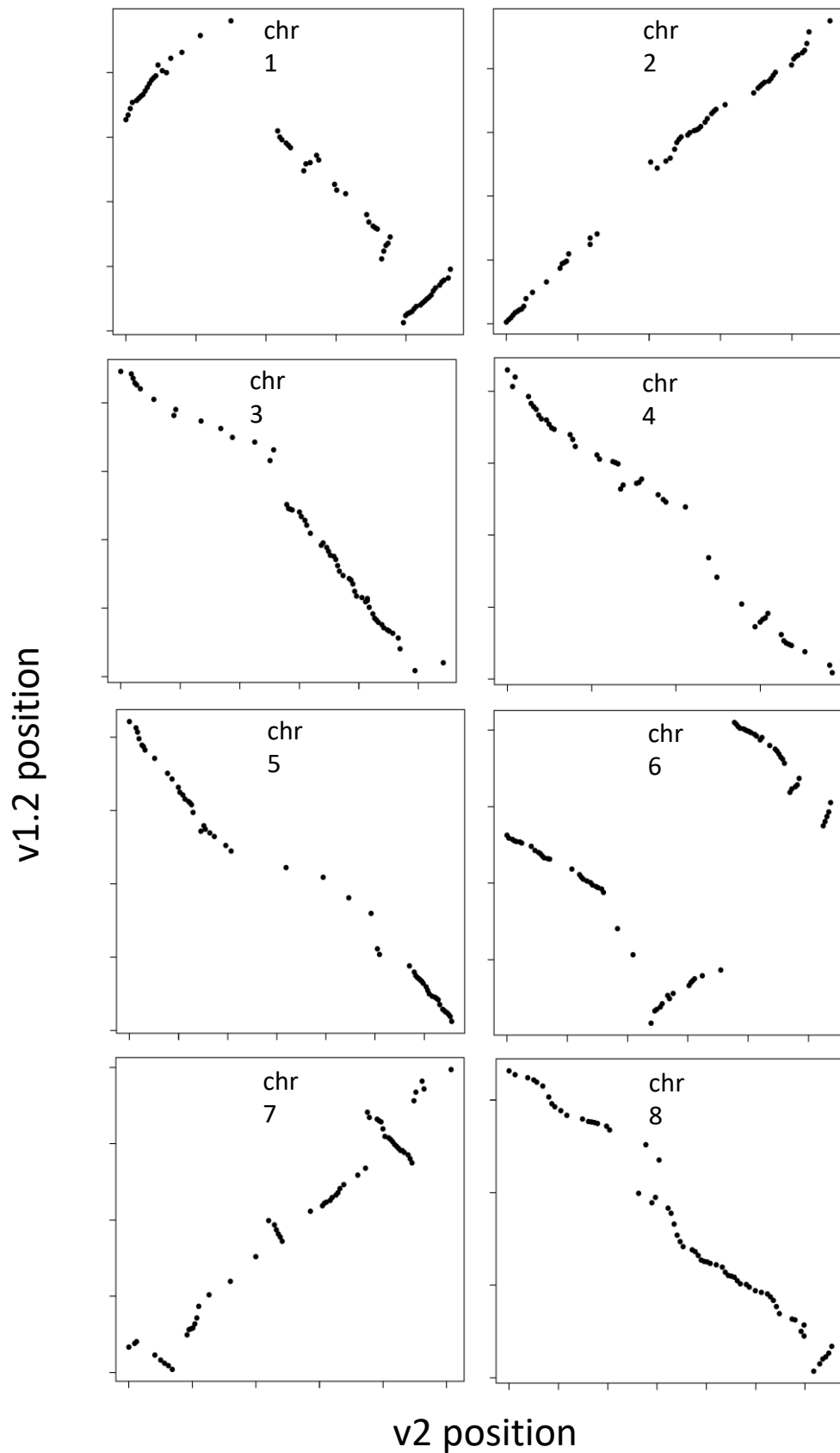


Figure S3. Position of genetic markers in *Erysimum cheiranthoides* genome v1.2 (Hi-C proximity guided assembly) and v2.0 (assembled using a classical genetic map). Large regions on chromosomes 1, 4, 6, 7, and 8 were inverted or incorrectly ordered in v1.2.

| | <i>E. cheiranthoides</i> v1.2 | <i>E. cheiranthoides</i> v2.0 |
|--|-------------------------------|-------------------------------|
| Total length (Mbp) | 177.2 | 174.1 |
| Number of chromosomes | 8 | 8 |
| Chloroplast assembly | no | yes |
| N50 (Mbp) | 22.4 | 21.9 |
| % of assembly anchored on chromosomes | 98.5 | 99.0 |
| Number of unanchored contigs | 216 | 123 |

Figure S4. Comparison of assembly statistics for *Erysimum cheiranthoides* genome v1.2 and v2.0. In addition to improved congruency with genetic mapping data (Figure 3), v2.0 includes a chloroplast assembly and fewer unanchored contigs. It is unclear why v2.0 is 3.1 Mbp shorter than v1.2, but the total length of v2.0 matches the total length of the PacBio contigs.

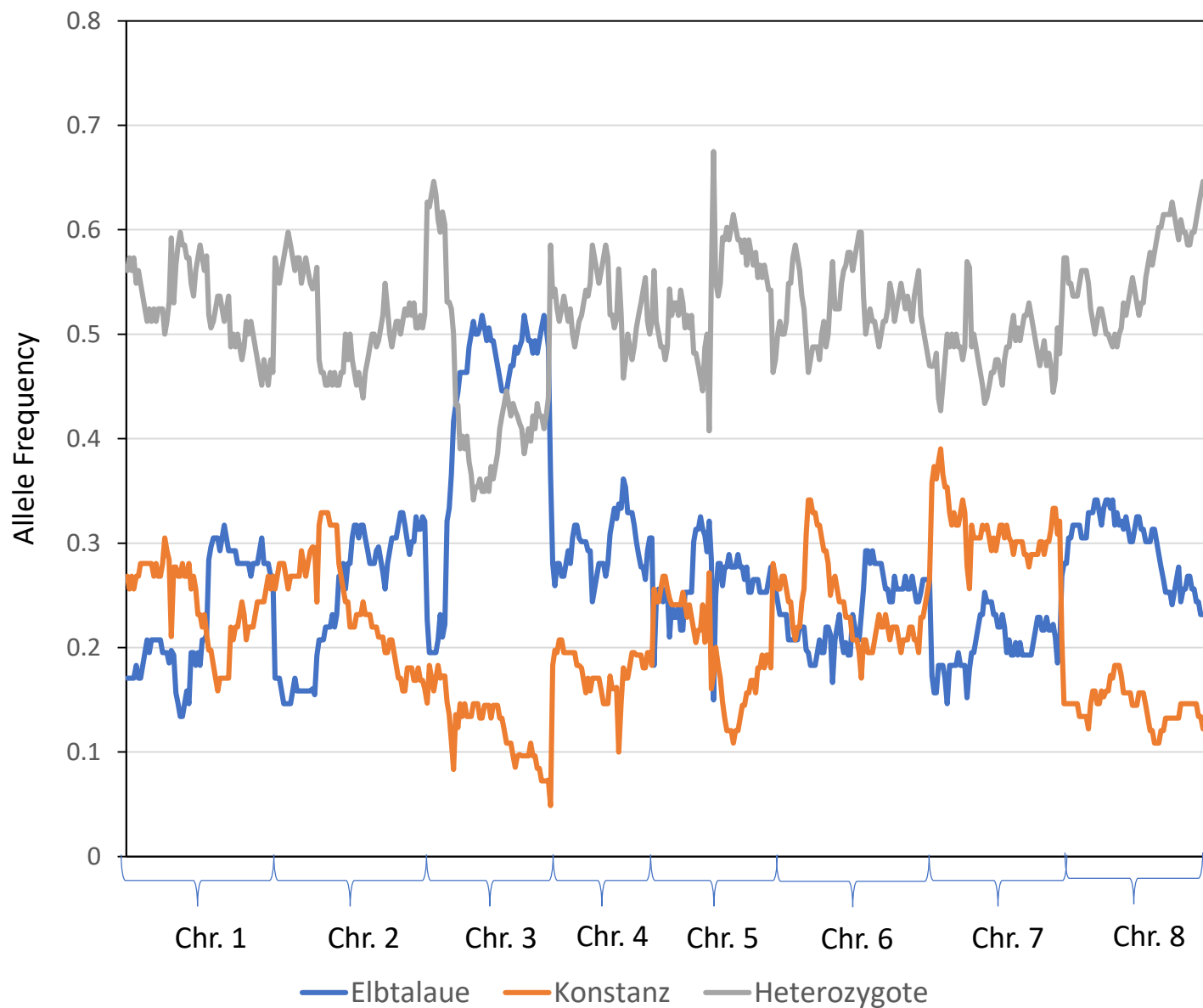


Figure S5. Allele frequencies across the *Erysimum cheiranthoides* F2 mapping population. The frequency of homozygous Elbtalaue, homozygous Konstanz, and heterozygous alleles for 501 molecular markers across 83 F2 lines is shown.

| | | |
|--------------------|--|----|
| Bo_CYP79F1 | MTMMILTSLPYPFQILLVFILSMASITLLGRILSRPTKTDRSRQLPPGPPGWPILGN | 60 |
| AT1G16400 | ---MMMKISFNTCFQILLGFIVFIASITLLGRIFSRPSKTDRCRQLPPGPPGWPILGN | 56 |
| Bs_BCMA2 | -MMMSLTSLPYPFQILLVFIVSMASIYILGRILSRPTKTDRSRQLPPGPPGWPILGN | 59 |
| Bs_BCMA1 | -MMMSLTSLPYPFQILLVFIVSMASISILGRILSRPTKAKDRSRHLPPSPPEWPILGN | 59 |
| Bs_BCMA3 | -MMMSLTSLPYPFQILLVFIVSMASISILGRILSRPTKTDRSRHLPPSPPEWPILGN | 59 |
| AT1G16410 | ---MMSFTSLPYPFHILLVFILSMASITLLGRILSRPTKTDRSCQLPPGPPGWPILGN | 57 |
| Erche01g017900_E1b | MMMMSLSTSLTYPFQILLVFVVSMAISILLRRILSKPTKTDRSRQLPPGPPGWPILGN | 60 |
| Erche01g017900_Kon | MMMMSLSTSLTYPFQILLVFVVSMAISILLRRILSKPTKTDRSRQLPPGPPGWPILGN | 60 |

| | | |
|--------------------|---|-----|
| Bo_CYP79F1 | LP E LM M TR R PR H K Y V D IAM K G Q K P DIAC F NFAG T HAI I I I NS D EIA R EAL K ER D AD F AD R PN | 120 |
| AT1G16400 | LP E LIM T TR R PR S K Y FHLAM K EL K T D IAC F NFAG T HT I T I INS D EIA R EAF R ER D AD L AD R PQ | 116 |
| Bs_BCMA2 | LP E LFMT R PR S K Y FHLVT K EL K T D IT C FN F AG V RAIT I NS D EIA R EAF R ER D AD L AD R PP | 119 |
| Bs_BCMA1 | LP E LFMT R PR S K Y FDLAM K EL K T D IAC F NFAG V RAIT I K S VEIA R EAF R ER D AD L AD R PH | 119 |
| Bs_BCMA3 | LP E LFMT R PR S K Y FDLAM K EL K T D IAC F NFAG V RAII I NS V EIA R EAF R ER D AD L AD R PH | 119 |
| AT1G16410 | LP E LFMT R PR S K Y FLAM K EL K T D IAC F NFAG I RAIT I NS D EIA R EAF R ER D AD L AD R PQ | 117 |
| Erche01g017900_E1b | LP E LFMT R PR A K Y FLAM K EL K T D IAC F NFAG V HAIT I HS D EIA R EAF R ER D AE L AD R PN | 120 |
| Erche01g017900_Kon | LP E LFMT R PR A K Y FLAM K EL K T D IAC F NFAG V HAIT I HS D EIA R EAF R ER D AE L AD R PN | 120 |

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|--------------------|---|-----|
| Bo_CYP79F1 | L F N M R T I G G N H K S M G N S P Y G E Q F M K M K R V I T T E I M S V K T L N M L V A A R T I E A D N L L A Y L L S | 180 |
| AT1G16400 | L S I V E S I G D N Y K T M G T S S Y G E H F M K M K K V I T T E I M S V K T L N M L E A A R T I E A D N L I A Y I H S | 176 |
| Bs_BCMA2 | L F M I Q T I G D N Y K S M G S A P Y G G Q F M K M K K V I T T E I M S V K T L N M M V A A R T I E A D N L I A Y V H S | 179 |
| Bs_BCMA1 | L F I M E T I G D N Y K S M I V S P Y G E Q F M K M K R V I T T E I M S V K T L T M L A A A R S I E A D N L I A Y V H S | 179 |
| Bs_BCMA3 | L F I M E T I G D N Y K S M L I S P Y G E Q F M K M K R V I T T E I M S V K T L N M L A A A R S I E A D N L I A Y V H S | 179 |
| AT1G16410 | L F I M E T I G D N Y K S M G I S P Y G E Q F M K M K R V I T T E I M S V K T L K M L E A A R T I E A D N L I A Y V H S | 177 |
| Erche01g017900_E1b | L F I M E T I G D N Y K S M G I S P Y G E Q F M K M K R V I T T E I M S V K T L N M L V A A R T I E A D N L I A Y V H S | 180 |
| Erche01g017900_Kon | L F I M E T I G D N Y K S M A I S P Y G E Q F M K M K R V I T T E I M S V K T L N M L V A A R T I E A D N L I A Y V H S | 180 |
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|--------------------|--|-----|
| Bo_CYP79F1 | MYKRSETADVREFSRVYGYAVTMRLLFGRRHVTKENVFSDEGRLGQAEKDHLDAIFETLN | 240 |
| AT1G16400 | MYQRSETVDVRELSRVYGYAVTMRMLFGRRHVTKENMFSDDGRLGKAEKHHLEVIFNTLN | 236 |
| Bs_BCMA2 | MYQRSETVDVRELSRVYGYAVTMRMLFGRRHVTKENVFSDEGRLGKAERHLEAIFNTLN | 239 |
| Bs_BCMA1 | MYQRSETVDVRELSRVYGYAVTMRMLFGRRHVTKENVFSDEGRLGKAEKHHLEAIFNTLN | 239 |
| Bs_BCMA3 | MYQRSETVDVRELSRVYGYAVTMRMLFGRRHVTKENVFSDEGRLGKAEKHHLEAIFNTLN | 239 |
| AT1G16410 | MYQRSETVDVRELSRVYGYAVTMRMLFGRRHVTKENVFSDDGRLGNAEKHHLEVIFNTLN | 237 |
| Erche01g017900_E1b | MYQRSETVDVRELSRVYGYTVMRMLFGRRHVTKENVYSDDGRLGKAEKHHLEAIFNTLN | 240 |
| Erche01g017900_Kon | MYQRSETVDVRELSRVYGYTVMRMLFGRRHVTKENVYSDDGRLGKAEKHHLEAIFNTLN | 240 |
| | ***** | |

| Accession | Sequence | Score |
|--------------------|---|-------|
| Bo_CYP79F1 | CLPSFSPADYLEKWFGRWNIDGQEERVVMYCNKVRSYNNPIIDERVEIWRKGGKAAVED | 300 |
| AT1G16400 | CLPGFSPVDYVDRWLGGWNIDGEEERAKVNVNLVRSYNNPIIDERVEIWRKGGKAAVED | 296 |
| Bs_BCMA2 | SLPSFSPTDYMERWLKGNWIGGQEERVTVNCNIVRSYNNPIIDERVEIWRKGGKAAVED | 299 |
| Bs_BCMA1 | CFPSFSPADYVERWLKGNWIDGQEERVTVNCNIVRSYNNPIIDERVEIWRKGGKAAVED | 299 |
| Bs_BCMA3 | CLPSFSPADYVERWLKGNWIDGQEERVTMNCNIVRSYNNPIIDERVEIWRKGGKAAVED | 299 |
| AT1G16410 | CLPSFSPADYVERWLGNVNDGQEKRVTECNIVRSYNNPIIDERVQLWREEGGKAAVED | 297 |
| Erche01g017900_E1b | CLPAFSPADYVERWLGNVNDGQEQRVKVCNIVRSYNNRIIDERVEIWRKGGKAAVED | 300 |
| Erche01g017900_Kon | CLPAFSPADYVERWLGNVNDGQEQRVKVCNIVRSYNNRIIDERVEIWRKGGKAAVED | 300 |

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|--------------------|--|-----|
| Bo_CYP79F1 | WIDTFITLKDENGKYYITPDEIKACQVFCIAAIDNPANNMEWTLAEMLNPEILKKALK | 360 |
| AT1G16400 | WLDTFITLKDQNGNYLVTPEIKACQVFCIAAIDNPANNMEWTLGEMLNPEILRKALK | 356 |
| Bs_BCMA2 | WLDTFITLKDQNGNYMVTPEIKAQYVFCIAAIDNPANNMEWTLAEMLNPEILRKALE | 359 |
| Bs_BCMA1 | WLDTFITLKDQNGKYVVTPEIKACQVFCIAAFDNPANNMEWTLAEMLNPEILRKALK | 359 |
| Bs_BCMA3 | WLDTFITLKDQNGKYVVTPEIKACQVFCIAAFDNPANNMEWTLAEMLNPEILKKALK | 359 |
| AT1G16410 | WLDTFITLKDQNGKYLVTPEIKACQVFCIAAIDNPANNMEWTLGEMLNPEILRKALK | 357 |
| Erche01g017900_E1b | WLDTFITLKDQNGKYLVTPEIKACQVFCIAAIDNPANNMEWTLAEMLNPEILKKALK | 360 |
| Erche01g017900_Kon | WLDTFITLKDQNGKYLVTPEIKACQVFCIAAIDNPANNMEWTLAEMLNPEILKKALK | 360 |
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|--------------------|--|-----|
| Bo_CYP79F1 | ELDEVVGDRLVQESDIPNLNYLKACCRETFRIHPSAHYVPTHVARQDTTLGGYFIPKGS | 420 |
| AT1G16400 | ELDEVVGKDRLVQESDIRNLNYLKACCRETFRIHPSAHYVPPHVARQDTTLGGYFIPKGS | 416 |
| Bs_BCMA2 | ELEEVVGKDRLVQESDIPNLNYLKACCRETFRIHPSAHYVPPHVARQDTTLGGYFIPKGS | 419 |
| Bs_BCMA1 | ELDEVVGKDRLVQESDIPNLNYLKACCRETFRIHPSSHYAPPHVARQDTTLGGYFIPKGS | 419 |
| Bs_BCMA3 | ELDEVVGKDRLVQESDIPNLNYLKACCRETFRIHPSTHYVPTHVARQDTTLGGYFIPEGs | 419 |
| AT1G16410 | ELDEVVGDRLVQESDIPNLNYLKACCRETFRIHPSAHYVPSHLARQDTTLGGYFIPKGS | 417 |
| Erche01g017900_Elb | ELDEVVGKDRLVQESDIPNLTYLKACCRETFRIHPSAHYVPPHVARQDTTLGGYFIPKGS | 420 |
| Erche01g017900_Kon | ELDEVVGKDRLVQESDIPNLTYLKACCRETFRIHPSAHYVPPHVARQDTTLGGYFIPKGS | 420 |
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| Bo_CYP79F1 | HIHVGPRGIGRSSKIWTDLVYKPERHLEGDGISKELSLVETELRFVSFGTGRRCVGVK | 480 |
| AT1G16400 | HIHVCPRGLGRNPKIWKDPLAYEPERHLQGDGITKEVTLVETEMRFVSFGTGRRCVGVK | 476 |
| Bs_BCMA2 | HIHVCPRGLGRNPKIWKDPLVYQPERHLQGDGITKEVTLVEAEMRFVSFGTGRRCIGVK | 479 |
| Bs_BCMA1 | HIHVCRRGLGQNPKIWKDPLLYKPERHLQGDGITQEVTLVETEMRFVSFGTGRRCIGVK | 479 |
| Bs_BCMA3 | HIHVCRRGLGQNPKIWKDPLVYKPERHLQGDGITQEVTLVETEMRFVSFGTGRRCIGVK | 479 |
| AT1G16410 | HIHVCPRGLGRNPKIWKDPLVYKPERHLQGDGITKEVTLVETEMRFVSFGTGRRCIGVK | 477 |
| Erche01g017900_Elb | HIHVGPRGLGRNPKIWKDPLVYKPERHLQGDGITKEVTLVETEMRFVSFGTGRRCIGVK | 480 |
| Erche01g017900_Kon | HIHVGPRGLGRNPKIWKDPLVYKPERHLQGDGITKEVTLVETEMRFVSFGTGRRCIGVK | 480 |
| | **** *:***. ****.*** *:*****:*** *:***:****:*****:*** | |
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| Bo_CYP79F1 | VGTIMMVIMLARFLQAFNWKLHPGYGPLSLEDDA-LLMAKPLLLVEPRLAPNLYPKFC | 539 |
| AT1G16400 | VGTIMMAMMLARFLQGFNWKLHRDFGPLSLEDDASLLMAKPLLLVEPRLASNLYPKFR | 536 |
| Bs_BCMA2 | VGTIMMVMLARFIQGFNWKLHQDFGPLSLEDDDESLLMAKPLLLVEPRLAPNLYPKFR | 539 |
| Bs_BCMA1 | VGTIMMVMLARFIQGFNWKLHQDFGPLSLEDDASLLMAKPLLVSVELRLAPHFYKKIR | 539 |
| Bs_BCMA3 | VGTIMMVMLARFIQGFNWKLHQDFGPLSLEDDASLLMAKPLLLVEPRLAPNLYKKFR | 539 |
| AT1G16410 | VGTIMMVMLARFLQGFNWKLHQDFGPLSLEDDASLLMAKPLLHLSVEPRLAPNLYPKFR | 537 |
| Erche01g017900_Elb | VGTIMMVMLARFLQAFNWKLHQDFGPLSLEDDA-LLMAKPLLLVEPRLAPNLYPKFR | 539 |
| Erche01g017900_Kon | VGTIMMVMLARFLQAFNWKLHQDFGPLSLEDDA-LLMAKPLLLVEPRLAPNLYPKFR | 539 |
| | *****.:***:*.*****.:*****:*****:*** **.:*** | |
| | | |
| Bo_CYP79F1 | P | 540 |
| AT1G16400 | P | 537 |
| Bs_BCMA2 | H | 540 |
| Bs_BCMA1 | P | 540 |
| Bs_BCMA3 | P | 540 |
| AT1G16410 | P | 538 |
| Erche01g017900_Elb | P | 540 |
| Erche01g017900_Kon | P | 540 |

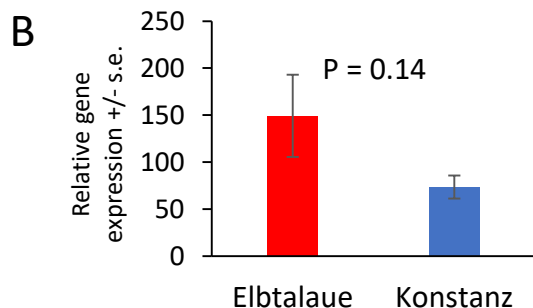


Figure S6. Alignment of CYP79F sequences and gene expression. (A) CYP79F protein sequences from *Erysimum cheiranthoides* (Erche), *Arabidopsis thaliana* (AT), *Boechera stricta* (Bs), and *Brassica oleracea* (Bo) were aligned using Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). Red arrows indicate positions with amino acid variation in *B. stricta* that has been associated with the differences in the preferential incorporation of methionine (BCMA2) or branched chain amino acids (BCMA1 and BCMA3) into glucosinolates side chains (Prasad et al, 2012, *Science* 337:1081-1084). The *E. cheiranthoides* Elbtalaue (Elb) and Konstanz (Kon) alleles differ at one of these positions, having glycine and serine, respectively at position 51. (B) Expression levels of Erche01g017900 in the Elbtalaue and Konstanz accessions. Mean +/- s.e. of N = 5; P value is from a two-tailed *t*-test.

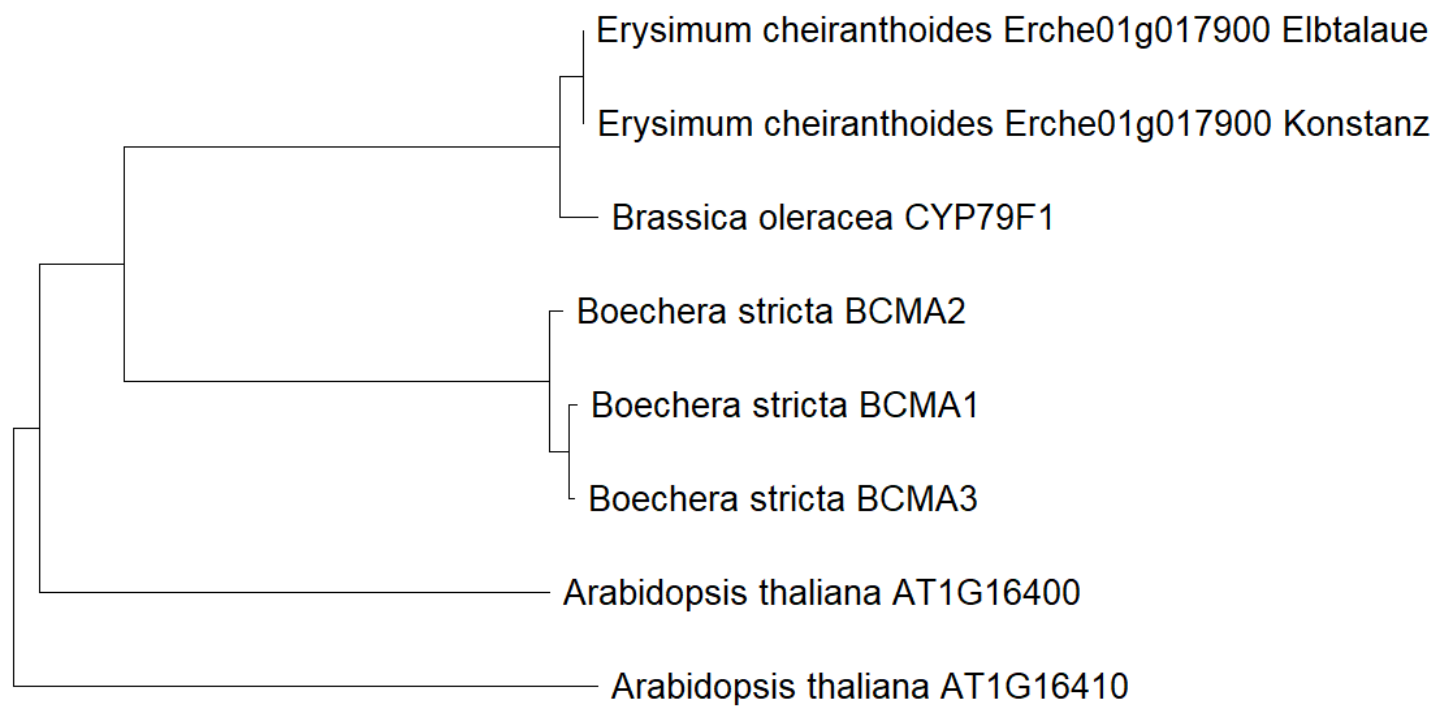


Figure S7. Neighbor-joining tree of CYP79F protein sequences. CYP79F protein sequences from *Erysimum cheiranthoides*, *Arabidopsis thaliana*, *Boechera stricta*, and *Brassica oleracea* were aligned using the Muscle algorithm and a neighbor-joining tree was constructed using MEGA11.

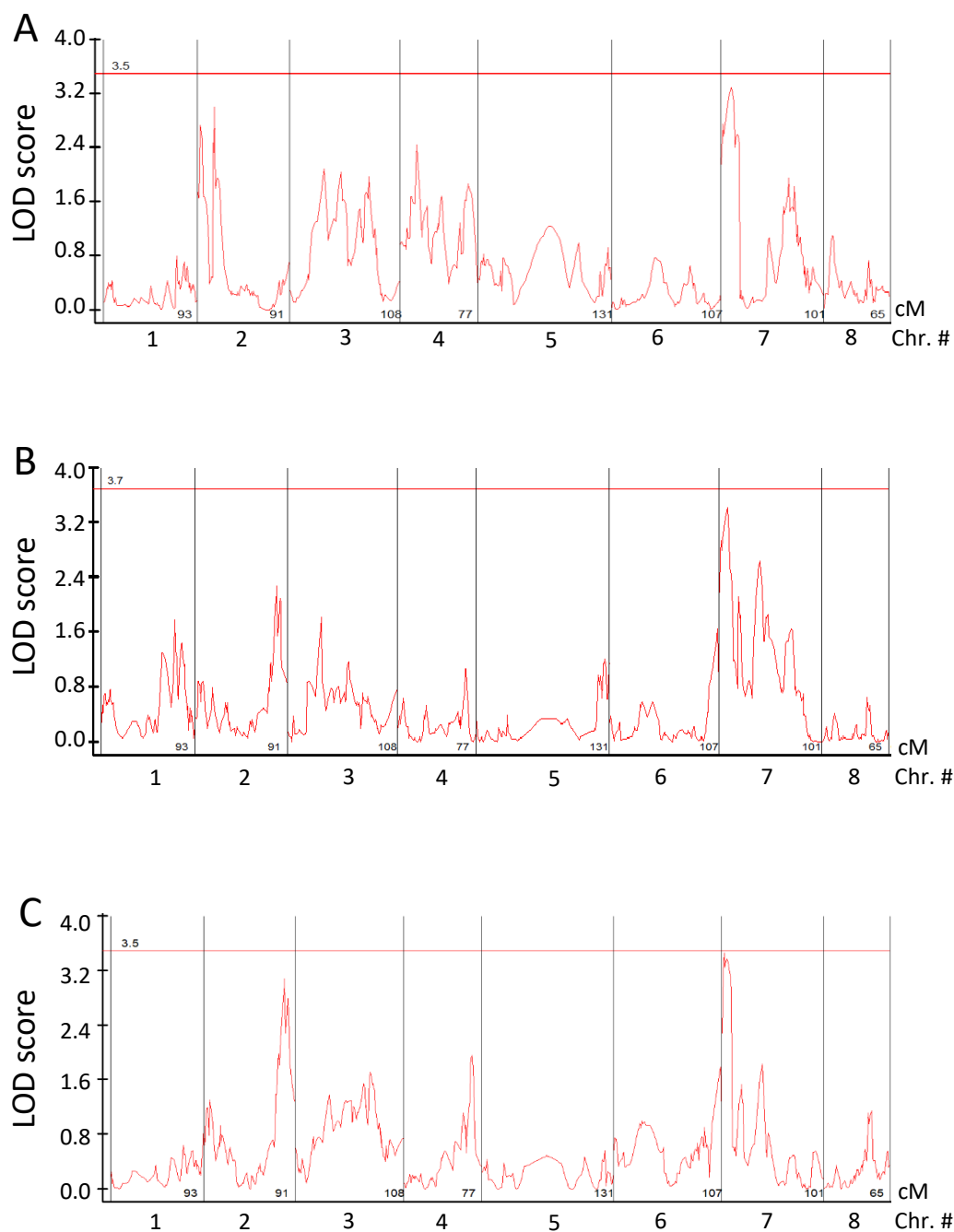


Figure S8. Quantitative trait loci (QTL) affecting glucosinolate abundance in *Erysimum cheiranthoides*. LOD plots of (A) 3-methylsulfinylpropylglucosinolate (3MSIP), (B) 3-methylsulfonylpropylglucosinolate (3MSOP), and (C) 4-methylsulfonylbutylglucosinolate (4MSOB) abundance in an Elbtalau x Konstanz F2 population are shown. Horizontal lines in are 95% confidence levels, calculated based on 500 permutations of the data. Graphs were created using Windows QTL Cartographer.

| | | |
|-----------------|---|-----|
| AT5G53810_IGMT5 | MANHLQVPLTKPDRVKEEQVEEEARLLARRLANAAASPMVLKAAELGVIDTITTV-GG | 59 |
| R_sativus_ | MGFPFEETLSSNPKTQTVDNDELGLMAVRLANAAAFPMVLKASLELGVFDTLHAEAR | 60 |
| B_oleracea_ | MGILIEETLSANSNTQTVIDDDELGLMAVRLANAAAFPMVLKAAELGVFDTLYAEAR | 60 |
| Erche01g022144 | MGYLFEEVSSNPKTTIVTDDNDELGLMAVRLANAAAFPMVLKAAELGVFDTLYAEASR | 60 |
| Erche01g022140 | MGFLFEESLSSNSKTPIVDDNDELGLMAVRLANAAAFPMVLKAAELGVFDTLYAETSS | 60 |
| Erche01g022141 | MGFLFEESLSSNSKTPIVDDNDELGLMAVRLANAAAFPMVLKAAELGVFDTLYAEASR | 60 |
| AT1G21130_IGMT4 | MGYLLLEETLSSNSKTPIVIDDDELGLMAVRLANAAAFPMVLKAAELGVFDTLYAEASR | 60 |
| AT1G21100_IGMT1 | MGYLFQETLSSNPKTPIVDDNDELGLMAVRLANAAAFPMVLKAAELGVFDTLYAAASR | 60 |
| AT1G21120_IGMT2 | MGYLFEEETLSSNPKTPIVDDNDELGLMAVRLANAAAFPMVLKASLELGVFDTLYAEASR | 60 |
| AT1G21110_IGMT3 | MGYLFEEETLSSNPKTPIVDDNDELGLMAVRLANAAAFPMVLKASLELGVFDTLYAEASR | 60 |
| | *. : : : . : : : * : * : * : * : * : * : * : * : * | |
| AT5G53810_IGMT5 | GDLWLSPEIALRLPTKPCNLEAPALLDRMLRFLVSHSVLKCRTVIEENGQTGKVERVYA | 119 |
| R_sativus_ | TDAFLSPSEIASRLPTTPRNPAPVLLDRMLRLLASYSMVCKDKA-----GKGERTYR | 113 |
| B_oleracea_ | TNSFLSPSEIASRLPTTPRNPAPVLLDRMLRLLASYSMVCKGVSS-----GKVERVYR | 115 |
| Erche01g022144 | TDSFLSPSEIASRLPTTPRNPAPVLLDRMLRLLASYSMVCKGTISA-----GKGERVYR | 115 |
| Erche01g022140 | TDSFLSPSEIASRLPTTPRNPAPVLLDRMLRLLASYSMVCKGVLE-----GKGERVYK | 115 |
| Erche01g022141 | TDSFLSPSEIASRLPTTPRNPAPVLLDRMLRLLASYSMVCKGVLE-----GKGERVYK | 115 |
| AT1G21130_IGMT4 | SDSFLSPSEIASRLPTTPRNPAPVLLDRMLRLLASYSVVKCGKVSE-----GKGERVYR | 115 |
| AT1G21100_IGMT1 | TDSFLSPYIEIASRLPTTPRNPAPVLLDRMLRLLASYSMVCKGKALS-----GKGERVYR | 115 |
| AT1G21120_IGMT2 | TDSFLSPSEIASRLPTTPRNPAPVLLDRMLRLLASYSMVCKEKVSV-----GKGERVYR | 115 |
| AT1G21110_IGMT3 | TDSFLSPSEIASRLPTTPRNPAPVLLDRMLRLLASYSMVCKEKVSV-----GKEQVRVYR | 115 |
| | : : * * * : * * * : * * * : * * * : * * * : * * * : * * * : * | |
| AT5G53810_IGMT5 | AEPVCKYLLNKSDVSGSFASLFMLDLSDFVIKTWTHLEDVILEGRDAFSSAH-GMKLFE | 178 |
| R_sativus_ | AEPICRFFLKDNIQDIGSLASQVIVNFDVFLNTWAQLKDVVLEGGDAFGRAHGGMKLFD | 173 |
| B_oleracea_ | AEPICRFFLKDNIQDIGSLASQVIVNFDVFLNTWAQLKDVVLEGGDAFGRAHGGMKLFD | 175 |
| Erche01g022144 | AEPICRFFLKDNIQDIGSLASQVIVNFDVFLNTWAQLKDVVLEGGDAFGRAHGGMKLFD | 175 |
| Erche01g022140 | AEPICRFFLKNNIQDIGSLASQVIVNFDVFLNTWAQLKDVVLEGGDAFGRAHGGMKLFD | 175 |
| Erche01g022141 | AEPICRFFLKDNIQDIGSLASQVIVNFDVFLNTWAQLKDVVLEGGDAFGRAHGGMKLFD | 175 |
| AT1G21130_IGMT4 | AEPICRFFLKDNIQDIGSLASQVIVNFDVFLNTWAQLKDVVLEGGDAFGRAHGGMKLFD | 175 |
| AT1G21100_IGMT1 | AEPICRFFLKDNIQDIGSLASQVIVNFDVFLNTWAQLKDVVLEGGDAFGRAHGGMKLFD | 175 |
| AT1G21120_IGMT2 | AEPICRFFLKNNIQDIGSLASQVIVNFDVFLNTWAQLKDVVLEGGDAFGRAHGGMKLFD | 175 |
| AT1G21110_IGMT3 | AEPICRFFLKNNIQDIGSLASQVIVNFDVFLNTWAQLKDVVLEGGDAFGRAHGGMKLFD | 175 |
| | * * * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * | |
| AT5G53810_IGMT5 | YIQADERFGKVFNRAMLESSTMVTEKVLKFYEGFKDVKTLVDVGGGLGNTLGLITSKYPH | 238 |
| R_sativus_ | YMGTDERFSKLFNQGTGFT--IADVKKALEVYQGFEDVDVLVDVGGGVGNTLGVVTSKYPN | 231 |
| B_oleracea_ | YMGTDERFSKLFNQGTGFT--IADVKKALEVYQGFEDVDVLVDVGGGVGNTLGVVTSKYPN | 233 |
| Erche01g022144 | YMGTDERFSKLFNQGTGFT--IADVKKALEVYEGFKGVNVLVDVGGGVGNTLGVVTSKYPN | 233 |
| Erche01g022140 | YMGTDERFSKLFNQGTGFT--IADVKKALEVYEGFKGVNVLVDVGGGVGNTLGVVTSKYPN | 233 |
| Erche01g022141 | YMGTDERFSKLFNQGTGFT--IADVKKALEVYEGFKGVNVLVDVGGGVGNTLGVVTSKYPN | 233 |
| AT1G21130_IGMT4 | YMGTDERFSKLFNQGTGFT--IADVKKALEVYQGFEDVDVLVDVGGGVGNTLGVVTSKYPN | 233 |
| AT1G21100_IGMT1 | YMGTDERFSKLFNQGTGFT--IADVKKALEVYEGFKGVNVLVDVGGGVGNTLGVVTSKYPN | 233 |
| AT1G21120_IGMT2 | YMGTDERFSKLFNQGTGFT--IADVKKALEVYQGFEDVDVLVDVGGGVGNTLGVVTSKYPN | 233 |
| AT1G21110_IGMT3 | YMGTDERFSKLFNQGTGFT--IADVKKALEVYQGFEDVDVLVDVGGGVGNTLGVVTSKYPN | 233 |
| | * : * * * : * : * : * : * : * : * : * : * : * : * : * : * | |
| AT5G53810_IGMT5 | LIGINFDLAPVLANAHSYPGVNHVAGDMFIKIPKGDIAFMKWILHDWTEQCVAAILKNCW | 298 |
| R_sativus_ | IKGINFDLTALAQAPSYPGVEHVAGDMFVDVVPKGDAMILKRILHDWTEDECVKILKNCW | 291 |
| B_oleracea_ | IKGINFDLTALAQAPSYPGVEHVPGDMFVDVVPKGDAMILKRILHDWTEDECVKILKNCW | 293 |
| Erche01g022144 | IRGINFDLTALAQAPSYPGVEHVAGDMFVDIPTGDAMILKRILHDWTEDECVKILKNCW | 293 |
| Erche01g022140 | IKGINFDLICALAQAPSYPGVEHVAGDMFVDVPTGDAMILKRILHDWTEDECVKILKNCW | 293 |
| Erche01g022141 | IKGINFDLICALAQAPSYPGVEHVAGDMFVDVPTGDAMILKRILHDWTEDECVKILKNCW | 293 |
| AT1G21130_IGMT4 | IKGINFDLTALAQAPSYPGVEHVAGDMFVDVPTGDAMILKRILHDWTEDECVKILKNCW | 293 |
| AT1G21100_IGMT1 | IKGINFDLTALAQAPSYPGVEHVAGDMFVDVPTGDAMILKRILHDWTEDECVKILKNCW | 293 |
| AT1G21120_IGMT2 | IKGINFDLTALAQAPSYPGVEHVAGDMFVDVPTGDAMILKRILHDWTEDECVKILKNCW | 293 |
| AT1G21110_IGMT3 | IKGINFDLTALAQAPTYPGVEHVAGDMFVDVPTGNAMILKRILHDWTEDECVKILKNCW | 293 |
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|-----------------|--|-----|
| AT5G53810_IGMT5 | KSLEENGKLIIVEMVTPVEAKSGDICSNIIVFGMDMTLTQCSGGKERDLYEFENLAYASG | 358 |
| R_sativus_ | KSLPENGKVVVIELVTPDDAENG DINANIAFDMMLMFTQCSGGKERSRAEFEALAAASG | 351 |
| B_oleracea_ | KSLPENGKVVVIELVTPDDAENG DINANIAFDMMLMFTQCSGGKERSRAEFEALAMEAG | 353 |
| Erche01g022144 | KSLPENGKVVVIELVTPDDAENG DINANIAFDMMLMFTQCSGGKERSRAEFEALAAASG | 353 |
| Erche01g022140 | KSLPENGKVVVIELVTPDDAENG DINANIAFDMMLMFTQCSGGKERSRAEFEALAAASG | 353 |
| Erche01g022141 | KSLPENGKVVVIELVTPDDAENG DINANIAFDMMLMFTQCSGGKERSRAEFEALAAASG | 353 |
| AT1G21130_IGMT4 | KSLPESGKVVVIELVTPDEAENG DINANIAFDMMLMFTQCSGGKERSRAEFEALAAASG | 353 |
| AT1G21100_IGMT1 | KSLPENGKVVVIELVTPDEAENG DINANIAFDMMLMFTQCSGGKERSRAEFEALAAASG | 353 |
| AT1G21120_IGMT2 | KSLPENGKVVVIELVTPDEAENG DINANIAFDMMLMFTQCSGGKERSRAEFEALAAASC | 353 |
| AT1G21110_IGMT3 | KSLPQNGKVVVIELVTPDEAENG DINANIAFDMMLMFTQCSGGKERSRAEFEALAAASG | 353 |
| | *** :.***::*:*** :*:*** :*:*.*** :*****. *** ** : | |
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| AT5G53810_IGMT5 | FSRCAIVCAVYFVSVEIYK--- | 378 |
| R_sativus_ | FTHCKFVCQAYHCWIIIEFCKENA | 374 |
| B_oleracea_ | FTHCKFVCQAYHCWIIIEFCK--- | 373 |
| Erche01g022144 | FTHCNFVCQAYHCWIIIEFCK--- | 373 |
| Erche01g022140 | FTHCKFVCQAYHCWIIIEFCK--- | 373 |
| Erche01g022141 | FTHCKFVCQAYHCWIIIEFCKGNV | 376 |
| AT1G21130_IGMT4 | FTHCKFVCQAYHCWIIIEFCK--- | 373 |
| AT1G21100_IGMT1 | FTHCKFVCQAYHCWIIIEFCK--- | 373 |
| AT1G21120_IGMT2 | FTHCKFVCQAYHCWIIIEFCK--- | 373 |
| AT1G21110_IGMT3 | FSHCQFVCQAYHCWIIIEFCK--- | 373 |
| | *::* :** .* :*** : | |

Figure S9. Alignment of indole glucosinolate methyltransferase (IGMT) protein sequences. Sequences of predicted IGMT proteins from *Erysimum cheiranthoides* (Erche01g022140.a, Erche01g022140.b, and Erche01g022140.d), *Arabidopsis thaliana* (AT1G21100, AT1G21110, AT1G21120, AT1G21130, and AT5G53810), radish (*Raphanus sativus*), and cabbage (*Brassica oleracea*) were aligned using Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>)

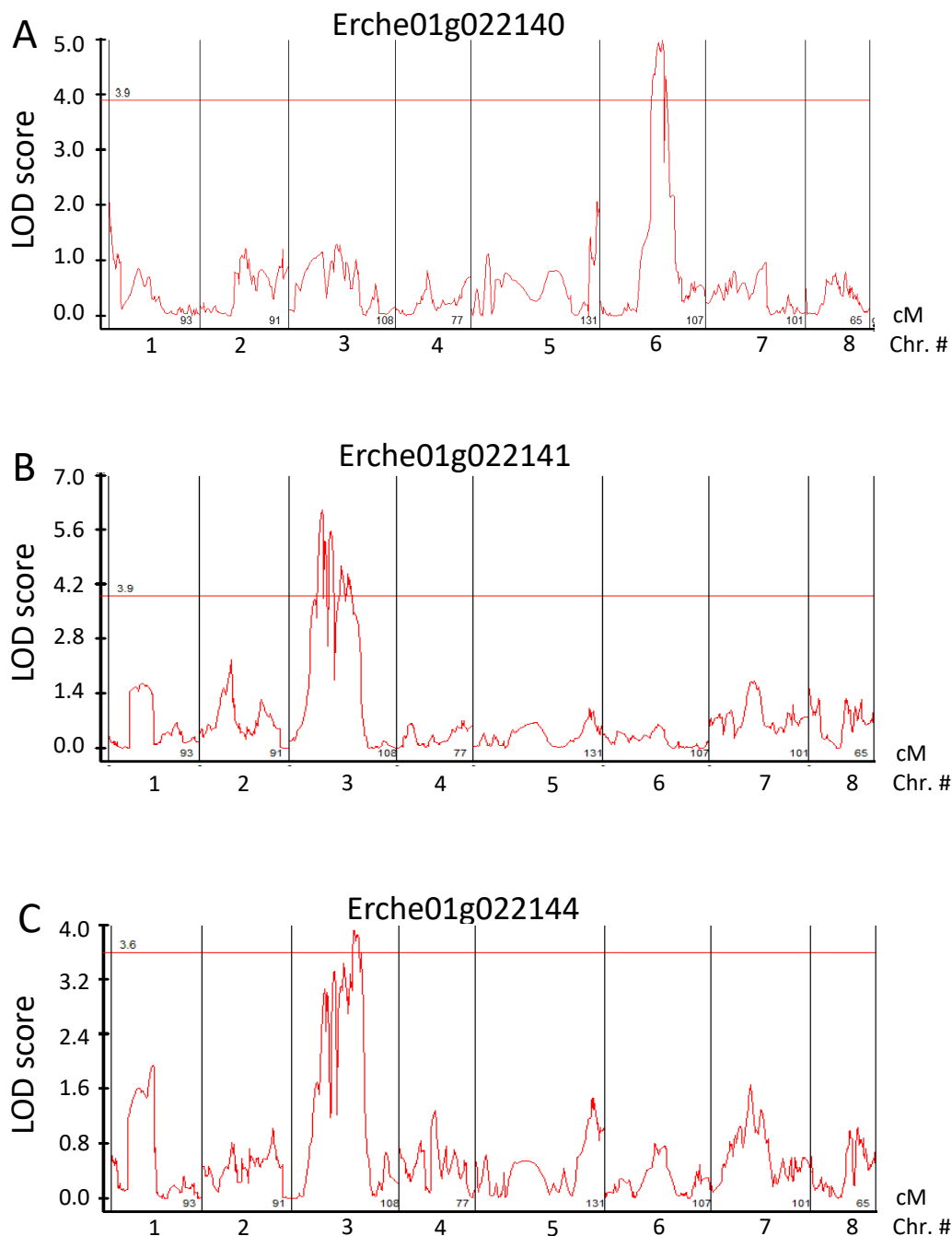


Figure S10. Quantitative trait loci (QTL) affecting indole glucosinolate methyltransferase gene expression in *Erysimum cheiranthoides*. LOD plots of (A) Erche01g022140.a, (B) Erche01g022140.b, and (C) Erche01g022140.c expression levels in an Elbtalau x Konstanz F2 population are shown. Horizontal lines in are 95% confidence levels, calculated based on 500 permutations of the data. Graphs were created using Windows QTL Cartographer.