**Supplementary Text: full materials and methods**

***Copy number variations shape genomic structural diversity underpinning ecological adaptation in the wild tomato Solanum chilense***

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**Full Materials and Methods**

**Sequence Read Processing**

We used 35 whole-genome paired-end Illumina data for seven populations of *S. chilense* (five plants for each population) representing different geographic groups available on European Nucleotide Achieve (ENA) BioProject PRJEB47577. We performed the same pipeline of read processing as the previous study (Wei, et al. 2023) for quality trimming and mapping to reference genome of *S. chilense* (Silva-Arias, et al. 2023).SNPs were also called and filtered based on the *S. chilense* genome using the same pipeline in a previous study (Wei, et al. 2023).

**Identification and genotyping of copy number variations**

To obtain high-confidence CNVs, including deletions (DELs) and duplications (DUPs), we chose four CNV detection tools to perform CNV calling based on a comprehensive evaluation of structural variation detection algorithms (Kosugi, et al. 2019). They enumerated potential good algorithms for each SV category, among which LUMPY (Layer, et al. 2014), Manta (Chen, et al. 2016), Wham (Kronenberg, et al. 2015) and DELLY (Rausch, et al. 2012) are better algorithms in deletion or duplication categories. These tools are combined with multiple algorithms to detect CNVs using whole-genome sequencing data, including read depth, paired-end mapping, split read and *de novo* assembly approaches.

In 35 samples, for Lumpy v0.3.1, we first extracted the discordant paired-end reads with abnormal insertion size from mapped results using the *view* function of Samtools v1.7 (Wysoker, et al. 2009), and the split-read alignments also were extracted using ‘extractSplitReads\_BwaMem’ script in Lumpy package. The output BAM files were sorted using the *sort* function of Samtools. We then run Lumpy using the mapped reads, discordant paired-end reads, and split reads as inputs to detect CNVs. DELLY v0.7.6 was run using default parameters, and then outputted bcf file was converted into a vcf file using bcftools v1.9 (Danecek, et al. 2011; Danecek, et al. 2021). Furthermore, Manta v1.6 and Wham v1.8 were run using default parameters. For each accession, CNV call sets from LUMPY, DELLY, Manta and Wham were then merged with SURIVOR v1.0.7 (Jeffares, et al. 2017). We set minimum CNV length as 50bp, maximum CNV length as 1Mb, minimum distance of 1,000bp, and types must match. Only CNVs called by at least two of the four tools were retained. The merged CNV set was inputted to SVTyper v0.7.0 to call genotypes, respectively, for population genetics analysis using a Bayesian algorithm (Chiang, et al. 2015). SVTyper performs breakpoint genotyping of structural variants using whole genome sequencing data. It assesses discordant and concordant reads from paired-end and split-read alignments to infer genotypes at each site. The pipeline included all command lines and parameters of CNV calling, merging, and genotyping, which can be found on our Gitlab repository: https://gitlab.lrz.de/population\_genetics/s\_chilense\_cnv.

To verify the sensitivity and accuracy of our pipeline of CNV calling, we simulated NGS data using a Python script ‘CNV-Sim’ obtained on <https://github.com/NabaviLab/CNV-Sim>. It extends the functionality of existing NGS read simulators to introduce CNVs in the generated reads. We run CNV-Sim v0.9.2 in whole genome, which utilises the functionality of ART (Huang, et al. 2012) to introduce CNVs in the genome. We simulated 1,000 duplication and 1,000 deletion regions ranging from 50bp to 1Mb based on 150 bp short-reads. Then, these simulated reads were inputted into the same pipelines to identify CNVs. The command lines of simulation can be found at: https://gitlab.lrz.de/population\_genetics/s\_chilense\_cnv/-/blob/main/CNVs\_simulation.

**Population structure analysis**

The population structure was constructed using all SNPs and genotyped CNVs from SVTyper, respectively. The principal component analysis (PCA) was performed to seek a summary of the clustering pattern among sampled genomes using GCTA v1.91.4 (Yang, et al. 2011). We first converted VCF format to plink format using VCFtools v1.17 (Danecek, et al. 2011), then plink format was converted to a binary format using PLINK v1.9 (Purcell, et al. 2007) with parameters ‘--noweb --make-bed’ to generate input of GCTA. The inference of population structure was performed using the program ADMIXTURE v1.3.0 (Alexander, et al. 2009). Six scenarios (ranging from K = 2 to K = 7) were assessed for genetic clustering using the same input with PCA analysis.

**Quantification of gene copy number**

We employed two strategies to quantify gene copy number (CN). First, we used the read-depth-based method implemented in Control-FREEC v11.6 to estimate copy numbers (CNs) by 10 kb windows with 1 kb step size across the entire genome (Boeva, et al. 2012). We used the following parameters in Control-FREEC: ploidy=2, breakPointThreshold = 0.8, degree=3, minExpectedGC = 0.3, maxExpectedGC = 0.55, and telocentromeric=0. We then obtained gene CN from the Control-FREEC outputs, and gene coordinates in the genome. However, some genes were observed to have more than one CN estimate. These events may be due to imperfect estimation of breakpoints using our window size and sliding window. We calculated the average CN if one gene corresponds to multiple CNs.

Additionally, we also employed another strategy to calculate gene CN. For each sample, we estimated read depth using Mosdepth v0.3.2 (Pedersen and Quinlan 2018) by 1,000 bp sliding windows, and gene read depth was calculated from gene coordinates. We then used median read-depth values of all windows and genes as a normalising factor to obtain the final window and gene CN estimate, respectively, and the formula as CN = (read depth / median value) × 2.

**Identification of candidate genes associated with population differentiation**

We calculated VST to identify genes with divergent CN profiles among seven populations. The VST measurement, analogous to FST, is applied to identify loci that differentiate by CN between populations (Redon, et al. 2006). Both VST and *F*ST consider how genetic variation acts on the differentiation of populations or closely related species and range from 0 (no differentiation) to 1 (complete differentiation). Using a sliding window-based approach, we first calculated pairwise FST and VST to compare the efficacy of population differentiation estimated by SNPs and CNVs. The FST was calculated for each pair of populations using VCFtools over a 1 kb non-overlapping sliding window. VST for each pair of populations was calculated by considering (VT-VS)/VT, where VT is the total variance among all individuals, and VS is the average variance within each population, weighted for sample size (Redon, et al. 2006). VST was also calculated based on CNs of 1kb sliding windows across the reference genome. In addition, we calculated two VST data sets from two different CN quantitative strategies for each pair of populations, respectively.

After assessing the strength of the effect of copy number changes on population differentiation, we identified candidate genes related to population differentiation based on strategy from Rinker et al. 2019. Similar to the sliding window-based method, the VST value of each gene was independently calculated based on two CN quantifications. An R script shows the pipeline of VST calculation and identification of candidate genes (R script available at [https://gitlab.lrz.de/population\_genetics/s\_chilense\_cnv/-/blob/main/VST.R).](https://github.com/weikai-320722/CNV_35genomes/blob/main/VST.R).) We performed permutation tests on the CN counts to identify which genes displayed the most significant degree of observed inter-population CN variation that was likely not due to sampling bias. Here, we randomly permuted gene CNs of each gene for 35 individuals and calculated a new VST for every permutation and every gene, respectively. This process (permutation test) was repeated 1,000 times, creating a random distribution of VST values for each gene. We then selected candidate genes that observed VST fell above the 95th and 99th percentile of the permuted VST distribution. These candidate genes displayed substantial intra-population CN homogeneity and high degrees of inter-population differentiation. Finally, genes were considered significant when observed VST values were above the maximum 95% (differentiated) or 99% (extremely differentiated) confidence interval cutoff in both gene CN estimate methods.

**Gene ontology (GO) analysis**

We first performed a blast of our genes to the *A. thaliana* dataset TAIR10 (e-value cutoff was 10-6) (Camacho, et al. 2009; Berardini, et al. 2015). The most matching entry (lowest e-value) was selected as the target homologue for enrichment analysis. We used the R package clusterProfiler to perform GO enrichment analysis using the *A. thaliana* annotation database as the background (Yu, et al. 2012). The Benjamini-Hochberg method, a false discovery rate (FDR) method, was used to calibrate initial *P* values, and calibrated *P* values smaller than 0.05 were used as the cutoff for a significant level to obtain final GO terms.

**Expansion and contraction of gene copy number**

To gain insight into how copy numbers of these differentiated genes vary across populations, we analysed gene CNs expansion and contraction with 3,359 differentiated genes. We first calculated the mean CN for each gene for each population. We then constructed a population-based phylogenetic tree using SNPs by TreeMix v1.13 (Pickrell and Pritchard 2012), and finally the ultrametric tree was generated based on *force.ultrametric* function of phytools R package (Revell 2012). The ultrametric tree can be obtained at our GitLab repository <https://gitlab.lrz.de/population_genetics/s_chilense_cnv/-/blob/main/ultrametric_tree.nwk.>

Finally, we analyse gene CN expansion and contraction in different groups using CAFE v4.2.1 (Han, et al. 2013) with the same *lambda* (the rate of change of evolution in a tree). We first run CAFE for genes with CN less than 100 to calculate an accurate *lambda* value (λ=0.00206736781311 in this study) because genes with large CNs can lead to non-informative parameter estimates. We then run CAFE for genes with CN larger than 100 using the same *lambda* value. The Viterbi method obtains the branch-specific P values with the randomly generated likelihood distribution. A low p-value indicates a rapidly evolving branch. Viterbi *P* values were computed for each significant gene to assess significant expansion or contraction along a specific branch. We set a p-value smaller than 0.05 to detect gene CN with a significantly greater rate of evolution (expansion or contraction) in different groups/populations.

**Associated analysis between gene copy number and climatic conditions**

The environmental data include 37 climatic layers obtained from two public databases, WorldClim2 (Fick and Hijmans 2017) and ENVIREM (Title and Bemmels 2018) (Dataset S6). To evaluate the relative contribution of the abiotic environment to explaining patterns of genetic variation, we first used the Redundancy Analysis (RDA) (Capblancq and Forester 2021) to associate CNs of 3,539 differentiated genes with climatic variables. RDA analyses were performed with an individual-based approach, using as input CNs for each differentiated gene for each sample. RDA was performed using the *rda* function from the vegan package as implemented in R (Forester, et al. 2018), modelling CNs as a function of predictor variables and producing constrained axes and representative predictors (climatic variables). All variables were centred and scaled before running the CN-environment association test. Multi-collinearity between representative predictors was assessed using the variance inflation factor (VIF), and since all predictor variables showed VIF < 10, none were excluded. The loadings of the CNs in the ordination space determined which genes were candidates for being under local adaptation. The CN loadings were stored as specified in the RDA object. The significance of RDA-constrained axes was assessed using the *anova.cca* function (*P* < 0.001).

Unlike RDA, which is a multivariate ordination technique that can analyse many loci and environmental predictors simultaneously, LFMM (latent factor mixed models) is a univariate test (Frichot, et al. 2013; Caye, et al. 2019), which means that it builds a model for each gene or SNP and each predictor variable. We first performed the lfmm\_ridge function implemented in the R library LFMM to obtain an object that contains the latent variable score matrix using a K value of four latent factors (as evaluated from analysis of population structure) based on CNs of 3,539 differentiated genes and six representative climate variables (as obtained from RDA). Then, we perform association testing using the *lfmm\_test* function. The Benjamini-Hochberg method was used to calibrate the P-value and set 0.01 as the significance threshold to obtain candidate genes associated with climatic variables.

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