Hi-C

In-situ was performed on plant tissue using previously described methods (Louwers *et al.*, 2009; Rao *et al.*, 2014). Young Pinot Noir (VCR18) grapevine leaves were collected from either Azienda Agraria A. Servedei (Udine, UD, Italy) or Vivai Cooperativi Rauscedo (Rauscedo, PN, Italy). For each Hi-C experiment, approximately 2.0 grams of aerial tissue were fixed with 2% formaldehyde in 0.5x Nuclei Isolation Buffer (10mM HEPES, pH 8.0, 125 mM sucrose, 0.5 mM $MgCl_2$, 2.5 mM KCl, 20% (v/v) glycerol, 0.125% Triton X-100, 0.5% (v/v) β-mercaptoethanol. Leaves were fixed for one hour at room temperature under a vacuum. Fixation was quenched with the addition of glycine to 125 mM and an additional 5-minute incubation at room temperature. Leaves were washed three times with ddH2O, flash-frozen in liquid nitrogen, and ground to a fine powder with a mortar and pestle. Cells were lysed with the addition of 10 mL of ice-cold 1x Nuclei Isolation Buffer (20 mM HEPES, pH 8.0, 250 mM sucrose, 1.0 mM $MgCl_2$, 5.0 mM KCl, 40% (v/v) glycerol, 0.25% Triton X-100, 1.0% (v/v) β-mercaptoethanol) plus 50 uL of protease inhibitor cocktail for plants (Sigma-Aldrich, P9599) and the liquefied sample was filtered through 3 layers of Miracloth. The nuclei suspension was centrifuged at 4°C for 15 minutes at 3000 x g. The supernatant was discarded and the pellet was resuspended in 1 mL of 1x Nuclei Isolation Buffer containing 5 uL protease inhibitor cocktail. Resuspended nuclei were transferred to a 2 mL Eppendorf tube and centrifuged at 4°C for 5 minutes at 1900 x g. The supernatant was discarded and this wash was repeated. A final wash was performed with the same centrifuge conditions with 1 mL of 1x NEBuffer2 (New England Biolabs, B7002). Nuclei were resuspended in 100 uL of 0.5% SDS and incubated at 62°C for 10 minutes. Following this, 145 uL of ddH2O and 50 uL of 10% Triton X-100 were added and the sample was gently mixed and incubated at 37°C for 15 minutes. Next, 25 uL of 10x NEBuffer 2 and either 100 U of MboI (NEB, R0147) or 400 U of HindIII (NEB, R0104) restriction enzyme were added to digest chromatin. Samples were incubated overnight while slowly rotating at 37°C. Digestion reactions were incubated at 62°C for 20 minutes and cooled to room temperature. To each tube was added 50 uL of the biotinylation mixture (37.5 uL of 0.4 mM biotin-14-dCTP (ThermoFisher Scientific, 19518018), 1.5 uL each of 10 mM dATP, dGTP, and dTTP (Euroclone, EMR27X025), and 8 uL of 5U/uL DNA Polymerase I, Large (Klenow) Fragment (NEB, M0210). Reactions were incubated at 37°C for approximately one hour with slow rotation. Next, to each tube was added 900 uL of ligation mix (663 uL ddH2O, 120 uL of 10x NEB T4 DNA ligase buffer (NEB, B0202), 100 uL of 10% Triton X-100, 12 uL of 10 mg/mL Bovine Serum Albumin (NEB, B9000) and 5 uL of 400 U/uL T4 DNA Ligase (NEB, M0202)). Tubes were mixed by inversion and incubated at room temperature for 4

hours with slow rotation. Following ligation, nuclei were pelleted at 1900 x g at room temperature for five minutes and resuspended in 450 uL of 1x TE. To degrade proteins, 50 uL of 20 mg/mL Proteinase K (NEB, P8107) and 40 uL of 10% SDS were added, and samples were incubated at 65°C overnight. An additional 50 uL of 20 mg/mL Proteinase K was added to the samples, and tubes were incubated for 90 minutes at 65°C. DNA was extracted with the addition of 500 uL of a 25:24:1 phenol:chloroform:isoamyl alcohol mixture (Sigma-Aldrich, P2069), vortexing for three seconds, and centrifugation at 16,000 x g for 5 minutes. The aqueous layer was transferred to a new tube and the extraction was repeated. To the extracted aqueous layer was added 1/10 volume of 3.0 M sodium acetate, 2 uL of 20 mg/mL glycogen, and 2.5 volumes of 100% ethanol. Tubes were incubated at -80°C for one hour and then -20°C for one hour, followed by centrifugation at 16,000 x g at 4°C for 20 minutes. Pellets were washed once with 70% ethanol and dried at 65°C for two minutes, then resuspended for 30 minutes at 37°C in 45 uL of 10 mM Tris buffer and 5 uL of 1mg/mL RNaseA (Sigma-Aldrich, R6513). DNA concentrations were determined using a Qubit Fluorometer (ThermoFisher Scientific) and approximately 5 ug of DNA was used for sonication. To bring the sample volume to 100 uL, 10 mM Tris buffer ph 8.0 was added to the 5 ug of resuspended DNA and the sample was transferred to a 0.5 mL sonication tube (Diagenode). Samples were sonicated in a Diagenode Bioruptor for five cycles of 15 seconds on, 90 seconds off on High. Approximately 200 ng each of pre- and post-sonication DNA aliquots were loaded on a 1.4% agarose gel to confirm DNA quality and sonication efficiency. For each sample, 150 uL of 10 mg/mL Dynabeads MyOne Streptavidin C1 (ThermoFisher Scientific, 65001) were washed with 400 uL of Tween Wash Buffer (5 mM Tris buffer, pH 7.5, 0.5 mM EDTA, 1 M NaCl, 0.05% Tween 20). Beads were resuspended in 100 uL of 2x binding buffer (10 mM Tris buffer pH 7.5, 1.0 mM EDTA, 2.0 M NaCl) and the sonicated Hi-C DNA was added to the beads. Tubes were slowly rotated at room temperature for 15 minutes. Beads were separated on a magnet and the supernatant was discarded. Beads were washed 2x with 600 uL of Tween Wash Buffer and shaking at 55°C for 2 minutes at 300 rpm in a Thermomixer (Eppendorf). Beads were resuspended in 100 uL 1x NEB T4 ligase buffer and transferred to a new tube, and were then collected again on a magnet. The supernatant was discarded and the beads were resuspended in 100 uL of end-repair mix (88 uL of 1x NEB T4 DNA ligase buffer supplemented with 10 mM ATP, 4 uL of 10 mM dNTP mix, 5 uL of 10 U/uL T4 Polynucleotide Kinase (NEB M0201), 4 uL of 3U/uL T4 DNA polymerase I (NEB, M0203), and 1 uL of 5U/uL DNA Polymerase I, Large (Klenow) fragment (NEB, M0210). Reactions were incubated at room temperature for 30 minutes, then placed on a magnet and the supernatant was

removed. Beads were washed 2x with 600 uL of 1x Tween Wash Buffer for 2 minutes at 55°C at 300 rpm. Beads were resuspended in 100 uL of 1x NEBuffer 2 and transferred to a new tube and were placed on a magnet and the supernatant was discarded. Beads were then resuspended in 100 uL of A-tailing mixture (90 uL of 1x NEBuffer 2, 5 uL of 10 mM dATP, and 5 uL of 5 U/uL Klenow exo- enzyme (NEB, M0212). Tubes were incubated at 37°C for 30 minutes, then placed on a magnet and the solution was discarded. Beads were washed 2x with 600 uL of 1x Tween Wash Buffer for 2 minutes at 55°C at 300 rpm. Beads were then resuspended in 100 uL of 1x T4 DNA ligase buffer (NEB, B0202), then placed on a magnet and the supernatant was discarded. Beads were resuspended in the adapter annealing mix (39 uL 1x T4 DNA ligase buffer supplemented with 5.0% PEG 8000 (Sigma-Aldrich, P5413), 1 uL H2O, 1 uL 10x T4 DNA ligase buffer, 1 uL of 50% PEG 8000, 5 uL T4 DNA ligase, 5 uL of an Illumina Truseq adapter) and incubated for 2 hours at room temperature. Beads were then washed 2x with 600 uL of 1x Tween Wash Buffer for 2 minutes at 55°C at 300 rpm, and 1x with 200 uL NEBuffer 2. Finally, beads were resuspended in 50 uL of NEBuffer 2. Bead-bound Hi-C DNA was amplified using Q5 polymerase (NEB, M0491) and Illumina TruSeq primer cocktail under the following conditions: **1x** 98°C 1 min; **12x** 98°C 10 sec, 65°C 30 sec, 72°C 30 sec; **1x** 72°C 3 min. Reactions were pooled and separated from the C1 beads, then purified by adding 0.7x volume of AmpureXP beads (Beckman Coulter, A63880) and incubating for 10 minutes at room temperature. Beads were placed on a magnet and washed twice with 70% ethanol, then dried 10 minutes. Beads were resuspended in 20 uL of 10 mM Tris, and the supernatant was removed to a new tube. Size and molarity of fragments was determined via Bioanalyzer (Agilent) or Caliper (Perkin-Elmer), and samples were sequenced for paired-end, 125 bp reads on an Illumina HiSeq 2500 sequencer by IGA Technology Services (Udine, Italy).

Adapters were removed from the reads using Cutadapt version 1.5 (Martin, 2011) and low quality bases were trimmed and contaminant sequenced were filtered out by ERNE version 1.4 (Del Fabbro *et al.*, 2013). The clean and trimmed reads were processed with HOMER version 4.9 (Heinz *et al.*, 2010). We used the homerTools *trim* utility to find the chimeric reads and trim each read from the duplicated restriction site to the 3' end, keeping only trimmed products longer than 20 bp. For each library, the trimmed read1 and read2 were independently aligned to the *Vitis vinifera* reference (PN40024, version 3, http://services.appliedgenomics.org/pub/grape-assembly/vitis_12xV3.fasta) using bwa-mem version 0.7.10 (Li and Durbin, 2009) with default parameters and reads mapping with low quality (MAPQ<10) were filtered out using Samtools version 0.1.19 (Li *et al.*, 2009). The aligned reads were used to create the unfiltered Tag directory,

with –tbp (maximum tags per base pair) set to 1. The tag directory was then filtered to remove self-ligation products and reads starting on a restriction fragment, and reads from regions with 5 times more reads than the average (spikes) were removed. Whole genome contact maps were generated at 50 kb resolution and normalized for coverage and distance (*-norm*). PC1 values were generated from a PCA performed using the HOMER utility *runHiCpca.pl*, and A and B compartments were assigned to each 50 kb window according to the sign of the first component (PC1) values, where positive values identified A compartments and negative values identified B compartments. Since the PC1 eigenvectors sign may be inconsistent across chromosomes, a manual correction of the signs was performed by direct inspection of the contact map.

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