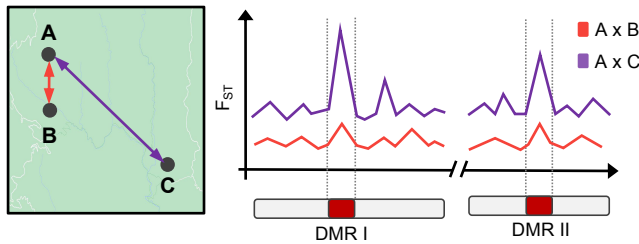


Figure 1. The *T. cristinae* study system. (A) *T. cristinae* and their host-plant ecotypes: *Adenostoma fasciculatum* and *Ceanothus spinosus*. Drawings from Rosa Ribas. **(B)** Map of study populations. The main map shows populations analyzed for differentially methylated regions (DMRs) from de Carvalho et al. (2023). The smaller scheme depicts the map of populations with whole-genome sequencing data from Soria-Carrasco et al. (2014), used to investigate genetic differentiation (*i.e.*, F_{ST}).

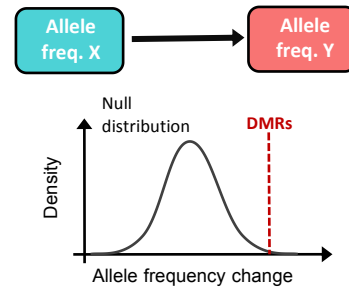
A. Geographical pattern of DMRs' F_{ST} enrichment

Pattern: DMRs' F_{ST} enrichment increases with geographical isolation



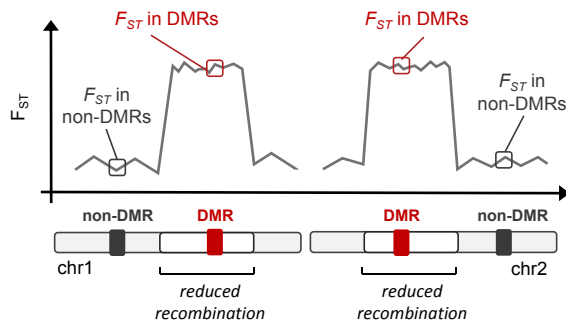
B. Selection gene-flow hypothesis

Prediction: DMRs show high allele-frequency changes



C. Reduced recombination hypothesis

Prediction: DMRs are located in genomic regions of reduced recombination



D. Mutagenic effects hypothesis

Prediction: Mutagenic effects of methylation results in DMRs' F_{ST} enrichment

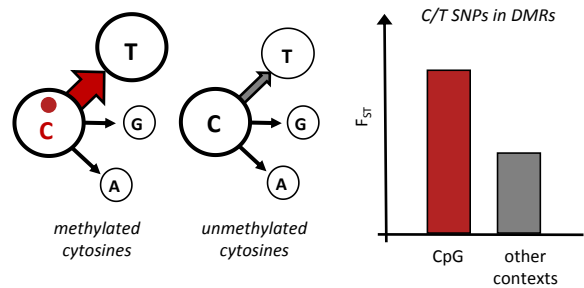


Figure 2. Patterns, hypotheses and predictions for investigating the enrichment of accentuated F_{ST} in differentially methylated regions (DMRs). (A) Expected geographical pattern of F_{ST} enrichment in DMRs. Populations with greater geographical isolation (e.g. A x C) are expected to show stronger F_{ST} enrichment in DMRs compared to geographically closer populations (e.g., A x B), due to differential gene flow effects on genetic differentiation. (B) The selection-gene flow balance hypothesis to explain the geographical patterns of F_{ST} enrichment in DMRs. This hypothesis posits that F_{ST} enrichment in DMRs increases with geographical isolation, reflecting stronger selection effects as gene flow decreases. One of the predictions of this hypothesis is that DMRs will show greater allele-frequency (freq.) shifts in transplant experiments between environments compared to other methylation tiles. (C) The reduced recombination hypothesis posits that the enrichment of accentuated F_{ST} in DMRs results from background selection in regions of reduced recombination. This predicts DMRs are concentrated in low-recombination regions. (D) The mutagenic DNA methylation hypothesis posits that DNA methylation increases F_{ST} enrichment by promoting specific mutations. Methylated cytosines (with the red dot) tend to mutate into thymines in higher rates than any other point mutation (Holliday & Grigg, 1993; Ossowski et al., 2010). This hypothesis predicts higher F_{ST} values for SNPs cytosine-to-thymine (C/T) SNPs in CpG contexts compared to other sequence contexts. Abbreviations: C=cytosine, T=thymine, G=guanine, A=adenine.

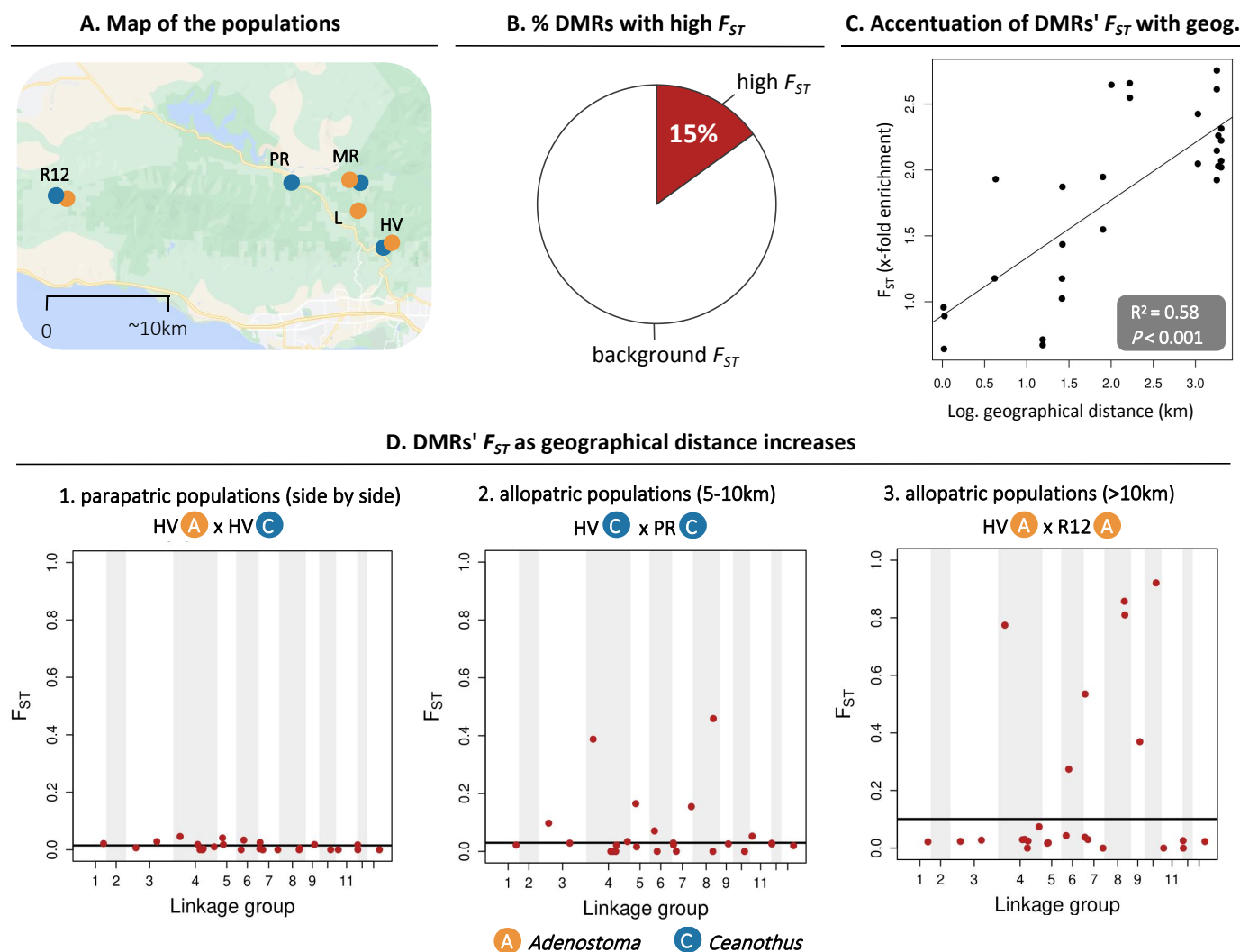


Figure 3. Genetic differentiation (F_{ST}) in DMRs across populations. (A) Map of the populations (n=8 populations) used in this study, of both *Adenostoma* and *Ceanothus* ecotypes (orange and blue points, respectively). This includes parapatric populations (side by side), and with different levels of geographical isolation (Soria-Carrasco et al., 2014). (B) Percentage of DMRs with accentuated F_{ST} ('high F_{ST} ' in the figure), a magnitude that is marginally more elevated than expected by chance (expected values: 5%, $P=0.077$). (C) Accentuation of F_{ST} in DMRs according to the pairwise geographical distance between populations (logarithmically transformed). X-fold enrichment expresses relative F_{ST} values in DMRs compared to the background levels. (D) F_{ST} values in DMRs between some population pairs separated by different degrees of geographical isolation. The black bar represents the mean F_{ST} across methylation tiles (null expectation) and the red dots represent the F_{ST} in DMRs. DMRs tend to exhibit particularly higher F_{ST} values with increasing levels of geographic isolation.

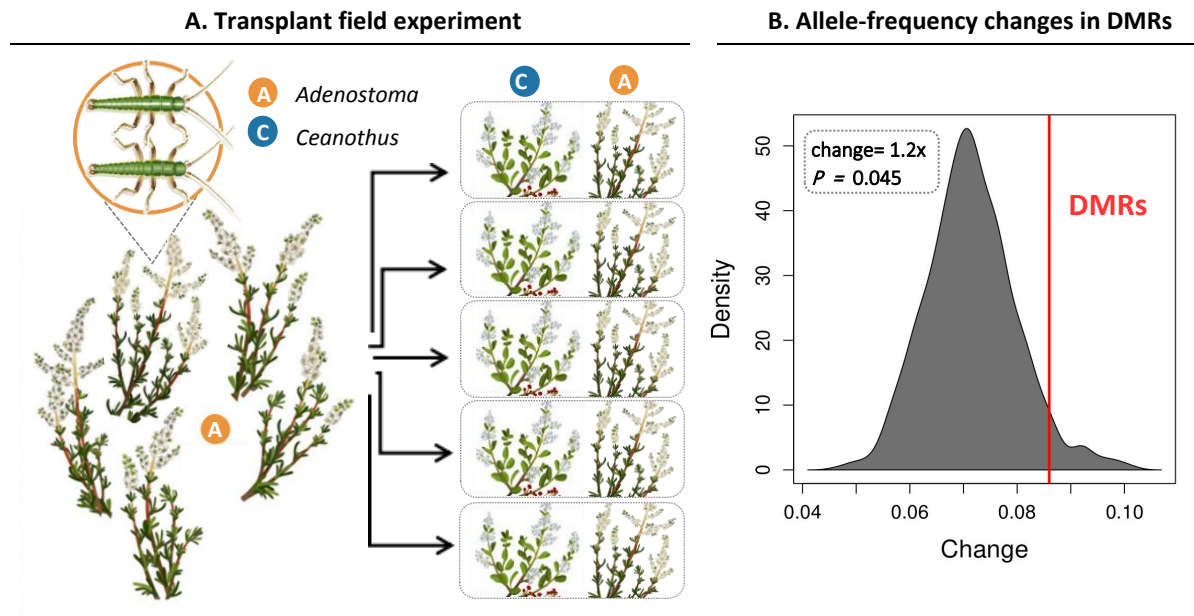


Figure 4. Field experiment testing for allele-frequency changes in DMRs. (A) Design of field experiment, where 491 *T. cristinae* specimens to experimental bushes of *Adenostoma* or *Ceanothus*. Individuals were collected, tissue-sampled, and then transplanted. Survivors were recaptured after eight days, allowing comparison of genetic changes between the released and surviving individuals (Gompert, Comeault, et al., 2014). The analysis in this study focused on whether genetic changes were more pronounced in DMRs than expected by chance. (B) DMRs show a weak yet statistically significant enrichment in allele-frequency changes between release and recapture in a field survival experiment, compared to a null expectation obtained by random sampling (observed $\Delta p=0.086$; null $\Delta p=0.071$; $P=0.045$). The analysis shown here was performed on 1kb windows located within genes, and DMRs were delimited using the cut-off of $P < 0.0001$.

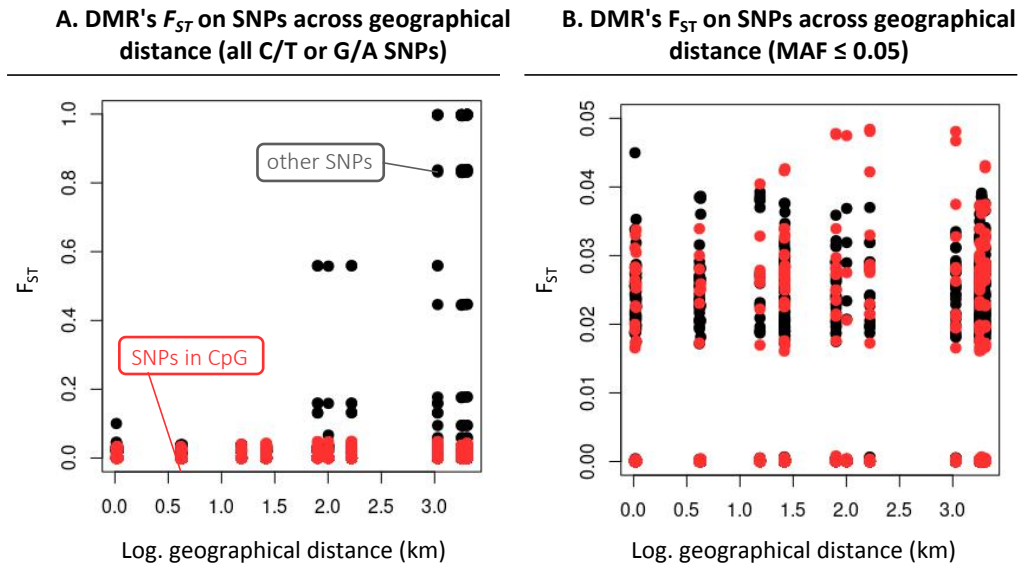


Figure 5. Mutagenic effects of DNA methylation on SNPs within DMRs. (A) C/T or G/A SNPs within DMRs and their corresponding F_{ST} between all pairwise comparisons, separated at different levels of geographic isolation. Geographical distance was logarithmically transformed. SNPs that could be caused by mutagenic effects of DNA methylation in cytosines (thus in cytosines followed by guanines, CpG, context) are represented in red. (B) Same graph as in (A), but controlling for minor-allele frequencies (MAF) below or equal to 0.05, since a mean of 95.2% of SNPs across all 28 population pairs show $MAF \leq 0.05$. Abbreviations: C=cytosine, T=thymine, G=guanine, A=adenine.