White Rose Research Online

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk
Universities of Leeds, Sheffield and York

Deposited via The University of Sheffield.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/id/eprint/239228/

Version: Supplemental Material

Article:

de Carvalho, C.F., Planidin, N.P., Villoutreix, R. et al. (2025) Linking DNA methylation to localised genetic differentiation in Timema cristinae stick insects. Molecular Ecology, 34 (22). e70049. ISSN: 0962-1083

https://doi.org/10.1111/mec.70049

© 2025 The Authors. Except as otherwise noted, this author-accepted version of a journal article published in Molecular Ecology is made available via the University of Sheffield Research Publications and Copyright Policy under the terms of the Creative Commons Attribution 4.0 International License (CC-BY 4.0), which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/

Reuse

This article is distributed under the terms of the Creative Commons Attribution (CC BY) licence. This licence allows you to distribute, remix, tweak, and build upon the work, even commercially, as long as you credit the authors for the original work. More information and the full terms of the licence here: https://creativecommons.org/licenses/

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

UNIVERSITY OF LEEDS
University of Sheffield
UNIVERSITY of York

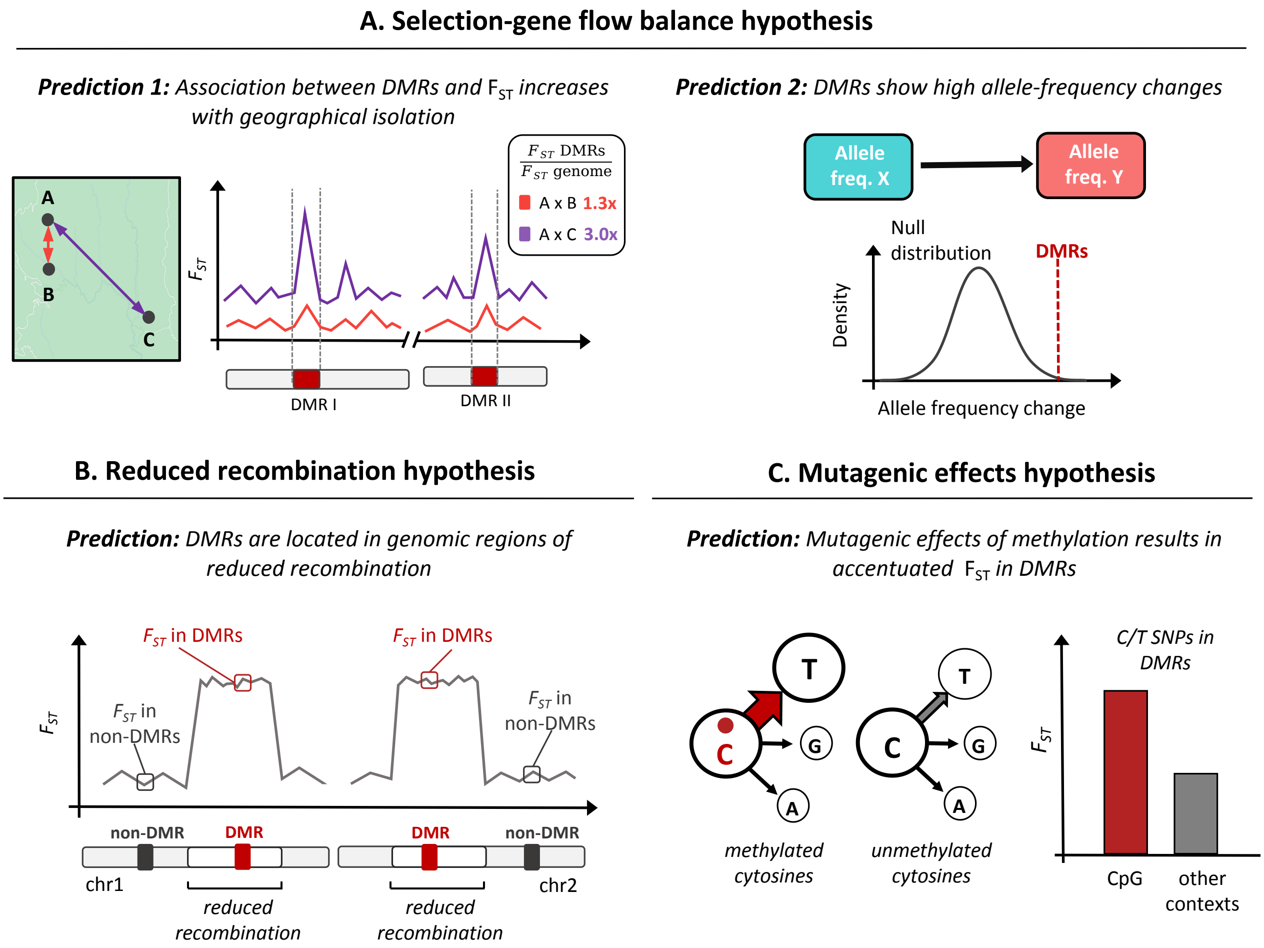


Figure 1. Patterns, hypotheses and predictions for investigating the association between accentuated FST in differentially methylated regions (DMRs). (**A**) The selection-gene flow balance hypothesis posits that different levels of selection and gene flow on DMRs and associated loci could generate an association between accentuated F_{ST} in DMRs. Two predictions can be raised with this hypothesis. First, that the magnitude of the association between F_{ST} and DMRs increases with geographical isolation, reflecting stronger selection effects as gene flow decreases. Second, that DMRs will show greater allele-frequency (freq.) shifts in transplant experiments between environments compared to other methylation tiles. (**B**) The reduced recombination hypothesis posits that the patterns of accentuated F_{ST} in DMRs result from background selection in regions of reduced recombination. This predicts DMRs are concentrated in low-recombination regions. (**C**) The mutatagenic DNA methylation hypothesis posits that DNA methylation elevates F_{ST} 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.

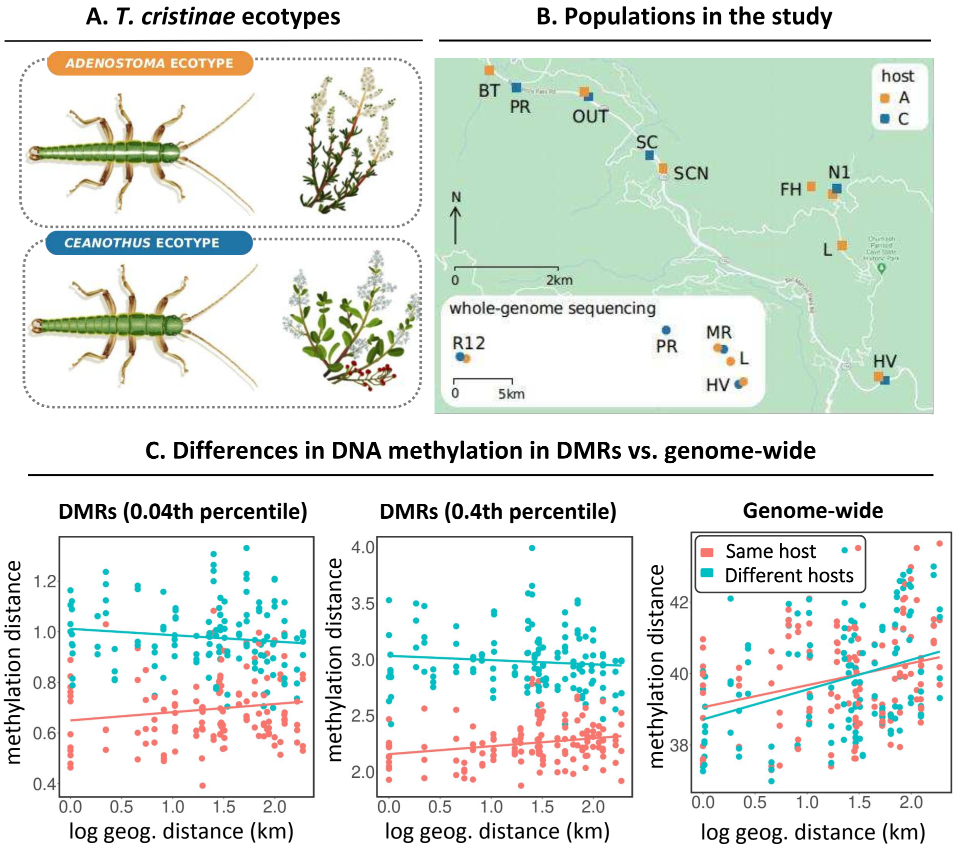


Figure 2. The *T. cristinae* study system and their differentially methylated genomic regions according to ecotype (DMRs). (**A**) *T. cristinae* and its two host-plant ecotypes: *Adenostoma fasciculatum* and *Ceanothus spinosus*. Illustrations by Rosa Ribas. (**B**) Sampling loactions of study populations. The main map shows populations analyzed for differentially methylated regions (DMRs) using from de Carvalho et al. (2023) using whole-genome bisulfite sequencing (WGBS). The inset depicts populations with whole-genome sequencing (WGS) data from Soria-Carrasco et al. (2014), used to assess genetic differentiation (F_{ST}). WGS data covers a broader geographical range, including additional populations (R12; see Supplementary Information). (**C**) Methylation distance patterns across ecotypes.Pairwise Euclidean methylation distances reveal that DMRs (0.04th and 0.4th *p-value* percentiles) are strongly associated with host ecotype, whereas genome-wide methylation follows an isolation-by-distance pattern (Figure from de Carvalho et al., 2023). Abbreviation: geog. = geographic

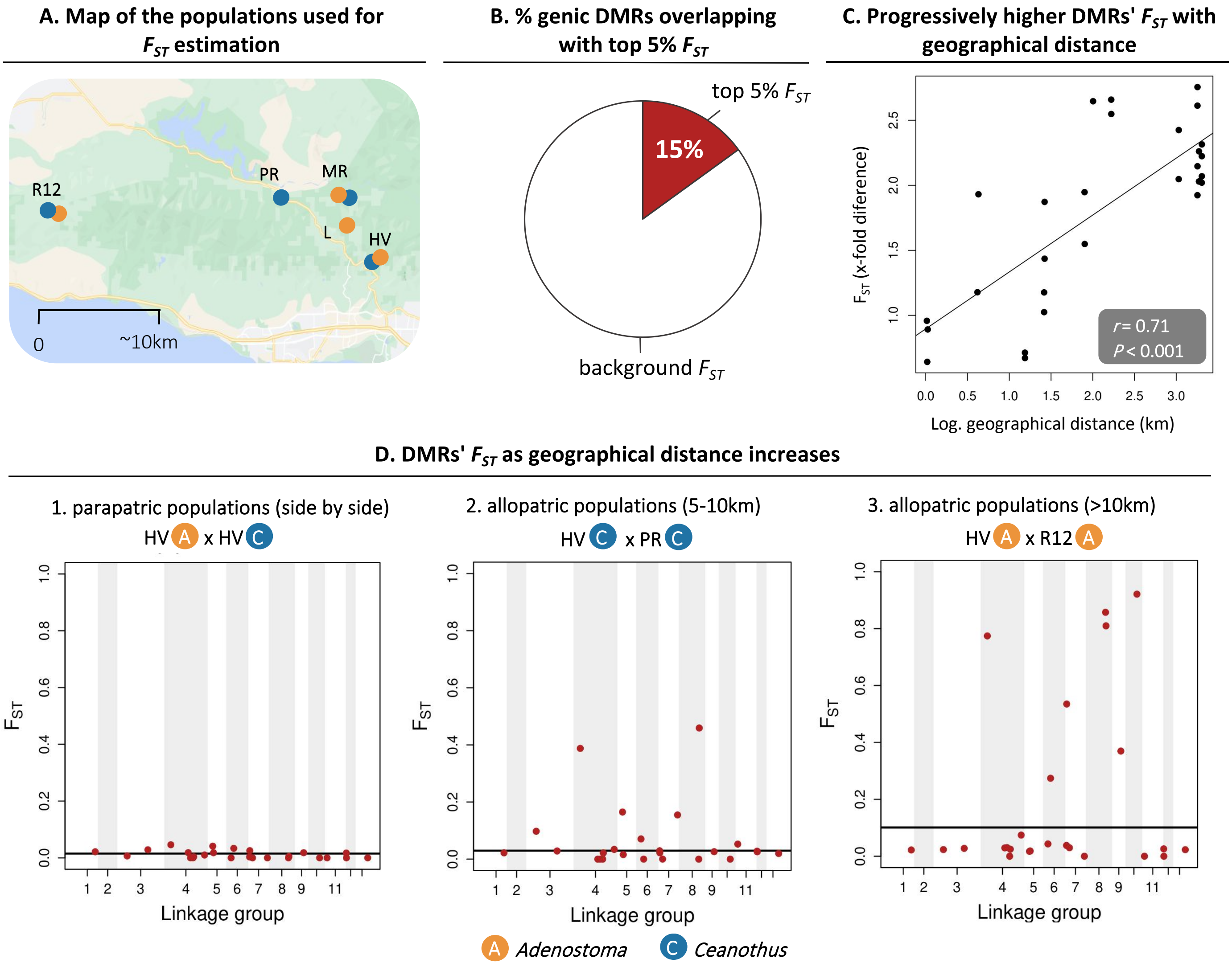
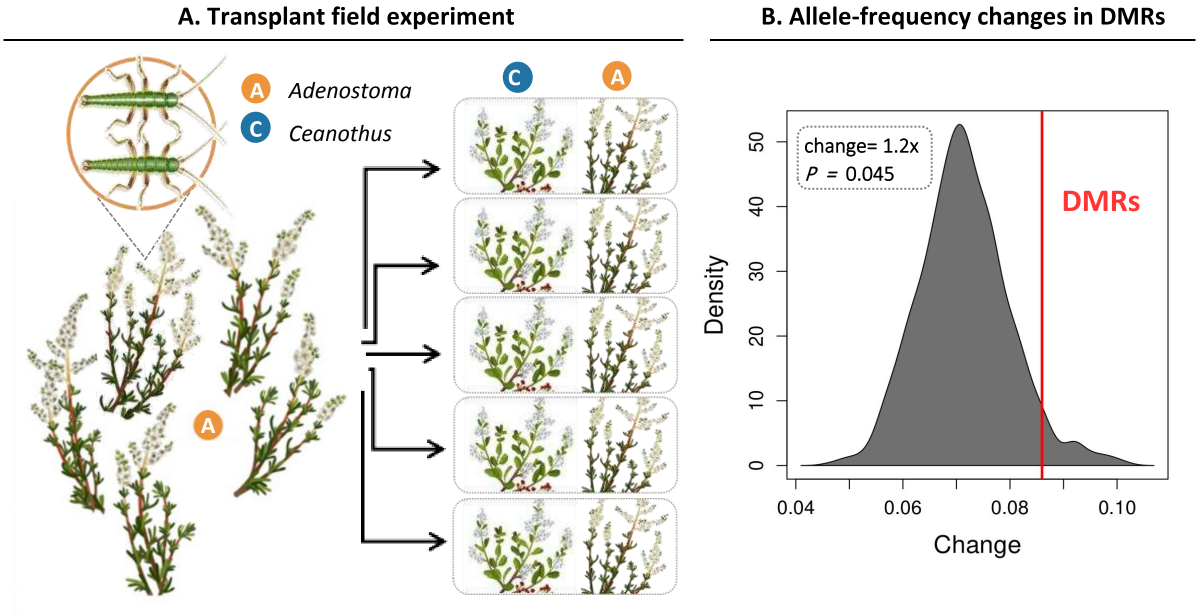


Figure 3. Genetic differentiation (F_{ST}) in DMRs across populations. (**A**) Map of the populations (n=8 populations) used in this study, of both *Adenostoma* and *Ceanothus* ecotypes (orange and blue points, respectively). This includes parapatric populations (side by side), and with different levels of geographical isolation (Soria-Carrasco et al., 2014). (**B**) Percentage of genic DMRs overlapping with top 5% F_{ST} values, a magnitude that is marginally more elevated than expected by chance (expected values: 5%, P=0.077). (**C**) Mean F_{ST} relative to the genomic background gets progressively higher with geographical distance between populations (logarithmically transformed). X-fold difference 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.



A. Transplant field experiment

B. Allele-frequency changes in DMRs

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 Δp=0.086; null Δp=0.071; *P*=0.045). The analysis shown here was performed on 1kbp windows located within genes, and DMRs were delimited using the cut-off of *P* < 0.0001.

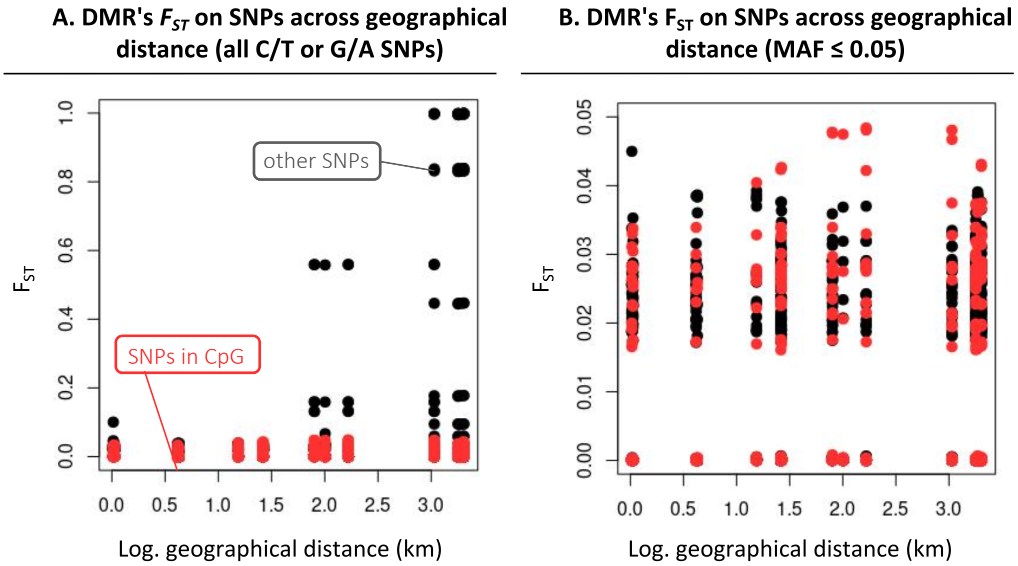


Figure 5. Mutagenic effects of DNA methylation on SNPs within DMRs. (**A**) C/T or G/A SNPs within DMRs and their corresponding F_{ST} between all pairwise comparisons, separated at different levels of geographic isolation. Geographical distance was logarithmically transformed. SNPs that could be caused 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 ≤ 0.05. Abbreviations: C=cytosine, T=thymine, G=guanine, A=adenine.