# Epigenetic signatures of parental effects in soil mites

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Understanding the causes and consequences of trans-generational parental effects in natural populations is increasingly a major focus in ecology and evolution.

The potential that epigenetic cytosine methylation can mediate maternal effects has been relatively underexplored in non-model systems, partly because methods for characterising genome-wide CpG methylation are either preclusively expensive (bisulphite sequencing) or yield insufficient resolution (msAFLP).

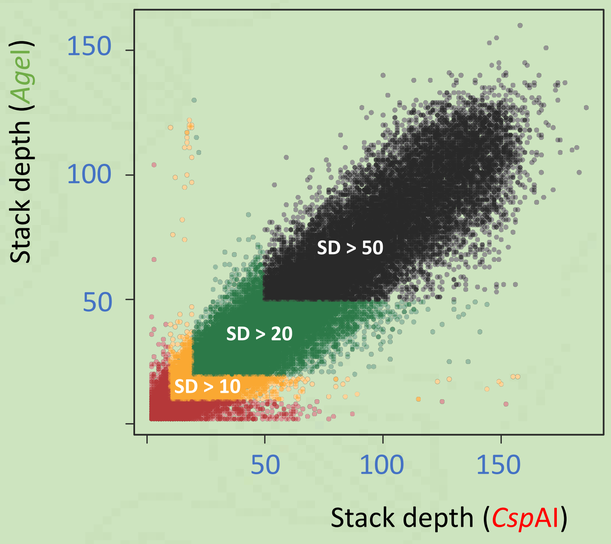
Here we: 1) demonstrate proof-of-concept of methylation sensitive RAD-genotyping, and; 2) use this approach to resolve very different landscapes of genome-wide CpG methylation in the offspring of soil mites maintained under contrasting food regimes.

*Sancassania berlesei* is a common detritivorous soil mite that has been used extensively in microcosm experiments to demonstrate the influence of maternal effects on life history evolution and population dynamics.

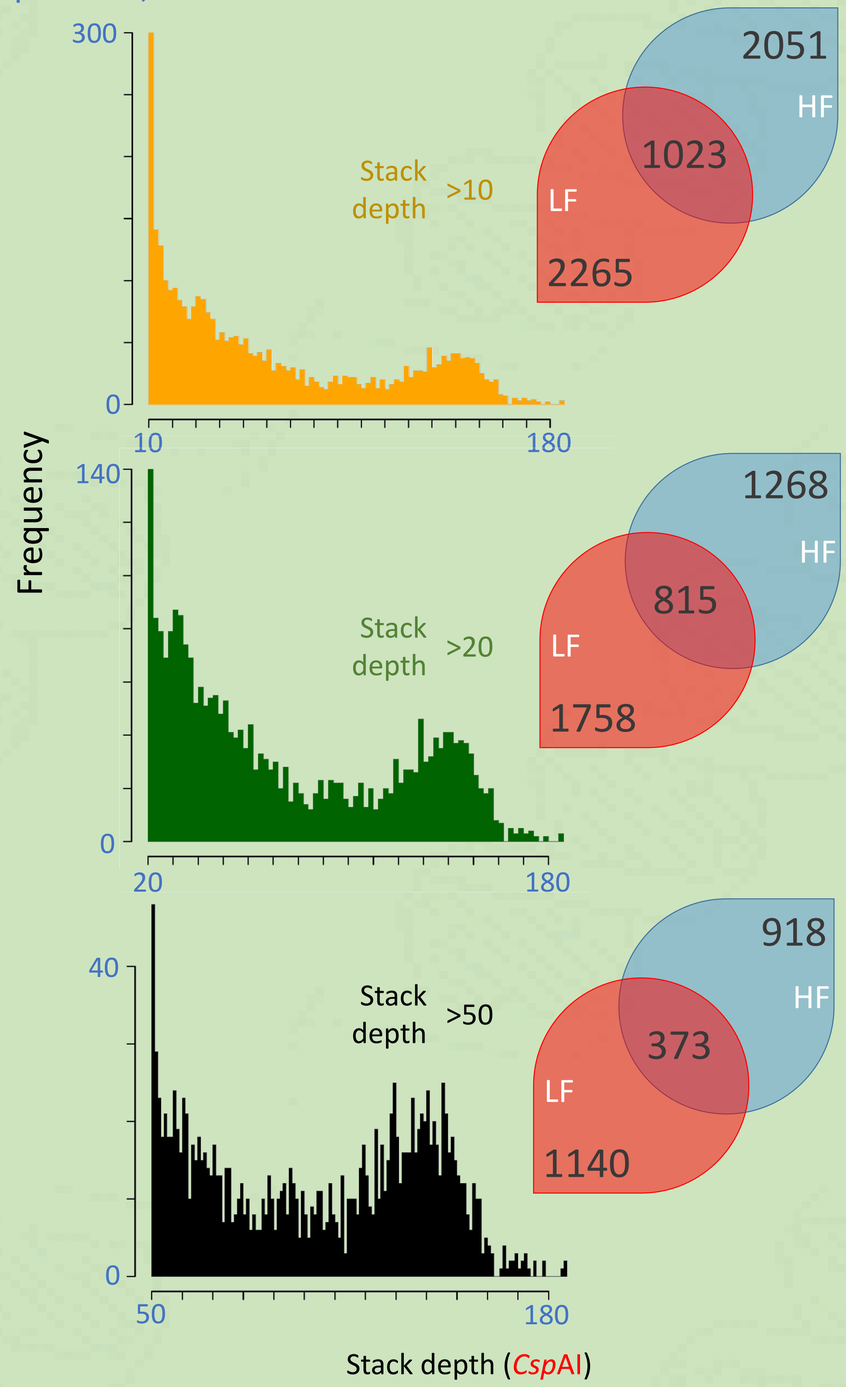
Here we bred soil mites from a single stock population and reared same-age eggs to adulthood under either **high** or **low** food levels. The eggs of these individuals were then themselves reared under common-garden conditions of high food.

DNA was extracted from pools of individuals derived from high food (HF) and low food (LF) parents and used to produce parallel RAD-genotyping libraries that were 100 base paired end sequenced using *HiSeq*. The first library was cut using the restriction enzyme *Csp*AI, whilst the second was cut using the methylation-sensitive isoschizomer *Age*I. As such, CpG methylation of a specific ACCGGT cut-site will lead to a RAD-tag being identified in the *Csp*AI derived sequences, but this will be absent from the *Age*I sequences. Such loci were identified using Stacks software.

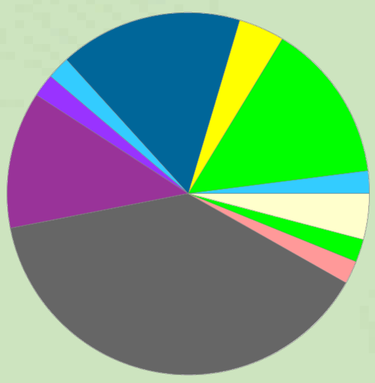
Stack depth was comparable for 157,722 unmethylated RAD-tag homologues that were found in both the *Csp*AI and *Age*I libraries, suggesting that the absence of RAD-tags that infers methylated loci is likely due to CpG methylation of the cut site rather than variance in read depth or quality across the libraries.




Stack depth for putatively methylated loci with RAD-tags present in the *Csp*AI library and absent from the *Age*I library varied considerably, but even at Stack depth >50, numerous loci were identified. The Venn diagrams highlight the number of methylated loci that were identified in only the high food (HF) or low food (LF) population, or were common to both.




The methylated loci found in both high food and low food populations with a stack depth of >100 reads matched 49 known proteins from the *Drosophila* genome. The pie diagram below summarises their Gene Ontology (GO) Biological Process categories.



- Biological adhesion (GO:0022610)
- Biological regulation (GO:0065007)
- Cellular component organization (GO:0071840)
- Cellular process (GO:0009987) explode
- Developmental process (GO:0032502)
- Immune system process (GO:0002376)
- Localization (GO:0051179)
- Metabolic process (GO:0008152)
- Multicellular organismal process (GO:0032501)
- Reproduction (GO:0000003)
- Response to stimulus (GO:0050896)

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- Methylation-sensitive RAD can resolve genome-wide patterns of CpG methylation.
- Parental conditions affect the epigenetic landscape of their offspring, consistent with CpG methylation being a mechanism promoting transgenerational epigenetic inheritance in soil mites.
- The dominant signature of CpG methylation is in metabolic processing, consistent with expectations that constitutively expressed genes are hyper-methylated.

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