Brief Communication

Title: `RADpainter` and `fineRADstructure`: population inference from RADseq data

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Abstract:

Powerful approaches to inferring recent or current population structure based on nearest neighbor haplotype 'coancestry' have so far been inaccessible to users without high quality genome-wide haplotypes. With a boom in non-model organism genomics, there is a pressing need to bring these approaches to communities without access to such data. Here we present `RADpainter`, a new program designed to infer the 'coancestry' matrix from restriction-site-associated DNA sequencing (RADseq) data. We combine this program together with a previously published MCMC clustering algorithm into `fineRADstructure` - a complete, easy to use, and fast population inference package for RADseq data (`https://github.com/millanek/fineRADstructure`).

Main Text:

Understanding of shared ancestry in genetic datasets is often key to their interpretation. The `fineSTRUCTURE` package (Lawson et al. 2012) represents a powerful model-based approach to investigating population structure using genetic data. It offers especially high resolution in inference of recent shared ancestry, as shown for example in the investigations of worldwide human population history (Hellenthal et al. 2014) and of genetic structure of the British population (Leslie et al. 2015). Further advantages when compared with other model-based methods, such as STRUCTURE (Pritchard et al. 2000) and ADMIXTURE (Alexander et al. 2009), include the ability to deal with a very large number of populations, explore relationships between them, and to quantify ancestry sources in each population.

The high resolution of `fineSTRUCTURE` and related methods derives from utilizing haplotype linkage information and from focusing on the most recent coalescence (common ancestry) among the sampled individuals - deriving a 'coancestry matrix', *i.e.* a summary of nearest neighbor haplotype relationships in the dataset. However, the existing pipeline for coancestry matrix inference was designed for large scale human genetic SNP datasets, where chromosomal location of the markers are known, haplotypes are typically assumed to be correctly phased (although it is possible to perform the analysis without this assumption), and missing data also needs to have been imputed. Therefore, these methods have so far been generally inaccessible for investigations beyond model organisms.

Therefore, we have developed `RADpainter`. With no requirements for prior genomic information (*e.g.* no need for a reference genome) and relatively low cost, RADseq data are fuelling a boom in ecological and evolutionary genomics, especially for non-model organisms (Andrews et al. 2016), where questions on population structure and relative ancestry are among the most commonly asked. `RADpainter` is designed to infer the coancestry matrix from RADseq data,

taking full advantage of the sequence of all the SNPs from each RAD locus to find (one or more) closest relatives for each allele. Then information about the nearest neighbours of each individual is summed up into the coancestry similarity matrix. By default, individuals are assumed to be diploid. However, we have also implemented an option to handle tetraploid species, a feature likely to be of particular use to the plant research community. We package `RADpainter` together with the `fineSTRUCTURE` MCMC clustering algorithm into an easy to use population inference package for RADseq data called `fineRADstructure`.

Summation across loci is done based on the assumptions of perfect linkage within each RAD sequence and frequent recombination between different RAD sequences. However, we at least partially account for any linkage between RAD loci (*e.g.* pairs of loci on the two sides of each restriction site) by adjusting the scaling constant 'c' that is passed on to the `fineSTRUCTURE` clustering algorithm together with the co-ancestry matrix (Lawson et al. 2012).

Where the donor and/or the recipient alleles are missing, their coancestry is assumed to be proportional to the amount of coancestry observed between them in the rest of the data (*i.e.* 'missing data' coancestry is shared proportionally to 'observed data' coancestry). Unknown nucleotides (Ns) can be present within alleles and their positions are ignored in all pairwise sequence comparisons. `RADpainter` outputs missingness (the proportion of missing alleles) per individual. We recommend that outliers should be removed and users should avoid cases with large systematic differences in missingness between putative populations.

We applied `fineRADstructure` to a RAD dataset including 120 individuals from 12 populations of the alpine *Heliosperma pusillum* species complex sampled at 1,097 unmapped loci (Trucchi et al. 2016). The complicated network of relationships among these twelve populations belonging to two phylogenetically intertwined species (*H. pusillum*: P, *H. veselskyi*: V) with contrasting ecology and a post-glacial history of divergence in some of the six sampled localities (A to F) makes it an excellent case to study the performance of our approach.

The `fineRADstructure` results (a clustered coancestry matrix; Figure 1) make the presence of twelve populations immediately clear, with substructure suggested in some of the populations. The relationships between the populations are clearly not tree-like with strong evidence of heterogeneous gene flow between the species (Trucchi et al. 2016). A variable level of intra-population co-ancestry, likely related to different degree of isolation or effective population size, is visible across the populations. A major improvements of our approach over previous results is the clear identification, at the same time, of both a global structure produced by historical process and a local structure related to ecological divergence.

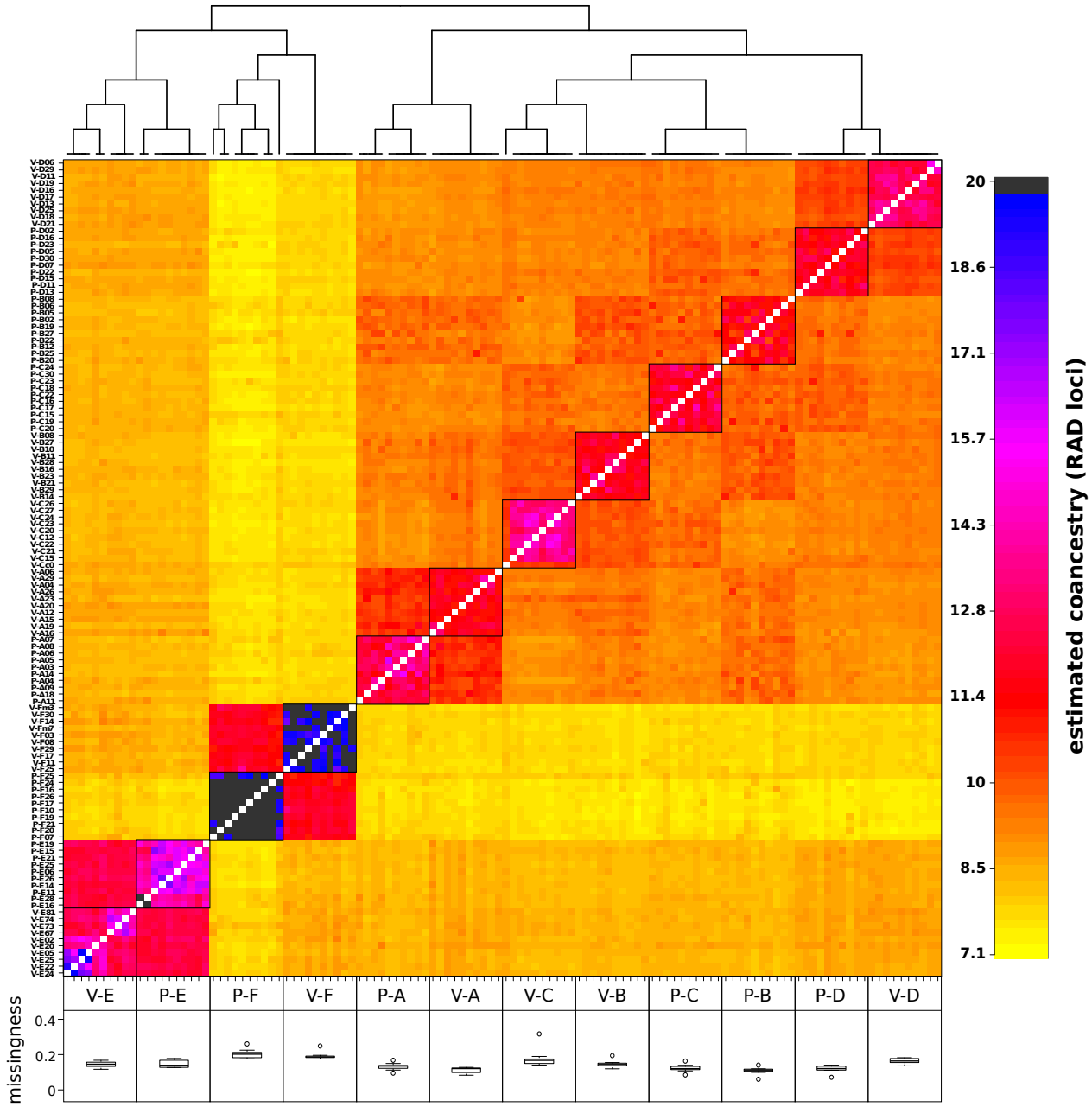

Figure 1: Clustered fineRADstructure coancestry matrix. Individuals within the twelve populations corresponding to the two sibling species (*H. pusillum*: P, *H. veselskyi*: V) and six localities (A to F) share more coancestry with each other than between populations. Hierarchical structure among and between localities is clearly inferred - populations at localities B and C cluster by species, whereas populations at localities A, D, E, and F cluster by locality. Missingness is shown below each population label, confirming that it does not contain strong outliers and does not vary systematically between inferred populations.

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