

1 Going down the rabbit hole: a review on how to link genome-wide data with
2 ecology and evolution in natural populations

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4

5 Abstract

6 Characterizing species history and assessing the nature and extent of local adaptation is
7 crucial in conservation, agronomy, functional ecology and evolutionary biology. The ongoing
8 and constant improvement of next-generation sequencing (NGS) techniques has facilitated the
9 production of an increasingly growing amount of genetic markers across genomes of non-
10 model species. The study of variation at these markers across natural populations has
11 deepened the understanding of how population history and selection act on genomes.
12 However, this improvement has come with a burst of analytical tools that can confuse naïve
13 users. This confusion can limit the amount of information effectively retrieved from complex
14 genomic datasets. In addition, the lack of a unified analytical pipeline impairs the diffusion of
15 the most recent analytical tools into fields like conservation biology. This requires efforts be
16 made in providing introduction to these methods. In this paper I describe possible analytical
17 protocols and recent methods dealing with analysis of genome-scale datasets, clarify the
18 strategy they use to infer demographic history and selection, and discuss some of their
19 limitations.

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28 Introduction

29 Genetic makeup of populations is shaped by multiple historical and selective factors. The
30 advent of Next-Generation Sequencing (NGS) in the last 20 years has enhanced our
31 understanding on how intermingled these factors are, and how they can impact genomic
32 variation. Important results have been gathered on model species, or species with an
33 economical interest. Such results include, among other examples, an improved perspective on
34 human history of migrations, admixture and adaptation (e.g. Sabeti *et al.*, 2002; Abi-Rached
35 *et al.*, 2011; Li and Durbin, 2011), elucidating the origin of domesticated species (e.g.
36 Axelsson *et al.*, 2013; Schubert *et al.*, 2014), or characterizing the genetic bases of local
37 adaptation in model or near-model species (e.g. Legrand *et al.*, 2009; Kolaczkowski *et al.*,
38 2011; Roux *et al.*, 2013; Kubota *et al.*, 2015). These studies have brought insights at an
39 unprecedented scale on the links between genotype, phenotype and environment. Most of
40 these studies relied on a precise knowledge of both population history and patterns of
41 selection, together with functional validation of variants associated to selected phenotypes.

42 Translation of these methods into non-model species is part of a shift in evolutionary sciences
43 that aims at better understanding biological diversity at various scales (Mandoli and
44 Olmstead, 2000; Jenner and Wills, 2007; Abzhanov *et al.*, 2008). Recent breakthroughs
45 brought by the study of initially non-model species (e.g. White *et al.*, 2010; Ellegren *et al.*,
46 2012; Weber *et al.*, 2013; Poelstra *et al.*, 2014) have confirmed the value of population
47 genomics from this perspective. These advances are needed to broaden our view about the
48 evolutionary process and improve sampling of distant clades. Ultimately, this process should
49 provide a more balanced picture than the one brought by the study of a few model species
50 (Abzhanov *et al.*, 2008). Genomic approaches also have the potential to improve conservation
51 genetic inference by scaling up the amount of data available (Shafer *et al.*, 2015).

52 However, the widespread use of sophisticated analytical tools remains challenged by the lack
53 of communication between fields (Shafer *et al.*, 2015), little user-friendliness of software and
54 the ever-increasing amount of tools made available. Much effort has been put recently in
55 addressing these issues, but a lack of clarity subsists and many uncertainties remain. The
56 application of sometimes complex methods to species with little background has nonetheless
57 become more accessible, and has the potential to bring valuable information.

58 In this paper, I propose various methods and suggestions to deal with usual questions in
59 population genomics and genetics of adaptation in natural populations. I begin with a succinct

60 review of methods available to obtain genome-wide polymorphism data before focusing on i)
61 methods devoted to the study of population demographic history (Figure 1) and ii) methods
62 aiming at detecting signatures of selection (Figure 2).

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64 Glossary

65 SNP: single nucleotide polymorphism.

66 Variant calling: identifying confidently genomic variants from alignment data (in SAM/BAM
67 format, see Li *et al.*, 2009). Classical SNP callers include the Genome Analysis Toolkit or
68 GATK (McKenna *et al.*, 2010), freebayes (Garrison and Marth, 2012), samtools (Li *et al.*,
69 2009) or Platypus (Rimmer *et al.*, 2014). Other tools call large-scale variants such as
70 inversions, translocations or copy-number variation (see main text).

71 Phasing: a process which identifies the alleles that are co-located on the same chromosome
72 copy.

73 Pooled sequencing: a protocol where tens or hundreds samples are pooled in a single library
74 prior sequencing (Futschik and Schlötterer, 2010). This prevents any individual identification
75 of each sample.

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77 Obtaining genetic markers and linking them to a genome

78 **Common sequencing methods**

79 I consider here two main ways of dealing with genomics in non-model species: reduced
80 representation (Davey *et al.*, 2011) and whole-genome resequencing. Reduced representation
81 allows sampling homogeneously variants across the genome by sequencing DNA fragments
82 flanking restriction sites. Some of the best-known reduced representation techniques include
83 RAD-sequencing (Baird *et al.*, 2008) and Genotyping by Sequencing or GBS (Elshire *et al.*,
84 2011). Their main interest is their relatively low cost and that they do not require any
85 reference genome (see Davey *et al.*, 2011 for details). The amount of SNPs ranges from
86 thousands to millions, which is most of the time enough to retrieve substantial information
87 about demography and sometimes selection (see Puritz *et al.*, 2014 for a detailed summary of
88 reduced-representation techniques).

89 Whole-genome resequencing requires a reference (at least at a draft stage) and is much more
90 expensive, especially for species with long and complex genomes. However, this approach
91 gives a complete overview of structural and coding variation, and allows some of the most
92 powerful methods currently available to track signatures of selection (see below). Pooled
93 sequencing (Futschik and Schlötterer, 2010) can be an option to reduce the costs, but restricts
94 the analysis to methods focusing on allele frequencies, losing most of the information
95 provided by variation in Linkage Disequilibrium (LD).

96 Shallow sequencing (1-5X per individual) may be a way to partly overpass this last issue for a
97 similar cost (Buerkle and Gompert, 2013), but should not be used for methods requiring
98 phasing and unbiased individual genotypes. Shallow shotgun sequencing also allows
99 retrieving complete plastomes, due to the representation bias of mitochondrial or chloroplast
100 sequences. Plastome sequences can provide insightful information about the evolutionary
101 history of populations or species. Recent work has successfully used shallow sequencing to
102 reconstruct mitochondrial or chloroplast sequences in plants (Malé *et al.*, 2014), animals
103 (Hahn *et al.*, 2013) or old and altered museum samples (Besnard *et al.*, 2016). Methods such
104 as MITObim (Hahn *et al.*, 2013) provide an automated and relatively user-friendly way to
105 reconstitute plastome sequences, which can then be analyzed as a single non-recombining
106 marker for phylogeny or population genetics.

107 **Obtain positional information for markers**

108 Whole-genome resequencing requires at least a draft genome, and reduced representations
109 methods can also benefit from a reference, either to order markers or retrieve information
110 about the nearest gene of a focal SNP. Methods inferring selection from haplotype extension
111 and patterns of LD (described further below) require that the relative order of markers on
112 genome sequence is known. A reference also allows analyzing separately sex chromosomes
113 (that can be haploid) and autosomes to correct for variation in ploidy between males and
114 females in gonochoric organisms. Obtaining a draft reference from deep Illumina sequencing
115 is now relatively common, but requires a good knowledge of assembly methods to choose the
116 tool adapted to the focal species. Initiatives such as Assemblathon (Bradnam *et al.*, 2013)
117 have provided valuable insights and advices from this regard. Once a draft is produced,
118 annotation of features is recommended since it allows linking variation at a locus to its
119 putative function. This requires either RNA-seq data to be mapped back on the reference or at
120 least that an annotation from a relatively close species is available.

121 It is possible to avoid these steps for species having a close relative already sequenced. Short-
122 reads alignment algorithms like BWA (Li and Durbin, 2009) generally assume relatively low
123 divergence between reads and reference. For species having less than 3% divergence, reads
124 may be directly mapped back onto the nearest genome. For more distantly related species, a
125 possible strategy would be using RAD-seq or GBS, build contigs for each locus with methods
126 like Stacks (Catchen *et al.*, 2011) or PyRAD (Eaton, 2014), and map those loci on the
127 reference with BLAT (Kent, 2002) or LASTZ (Schwartz *et al.*, 2003). Using a related
128 reference requires that synteny is conserved between species. While this assumption is
129 reasonable in, e.g., birds (Derjushcheva *et al.*, 2004), it becomes more doubtful in other clades,
130 like in plants (Molinari *et al.*, 2008; Soltis *et al.*, 2015). Before conducting a NGS study, it is
131 therefore important to know how genomes vary in their structure across related species. Some
132 methods do not even require any reference sequence to call SNPs from raw reads, like kSNP2
133 (Gardner and Hall, 2013). It is however advised to cautiously filter reads prior calling, since
134 the method does not distinguish between sequencing errors and actual variants.

135 Checking for the presence of large structural variants can be informative when performing
136 whole-genome resequencing. Structural variants include duplications and copy number
137 variation (CNV), deletions, inversions or translocations. Neglecting this variation can lead to
138 call spurious SNPs, for example in regions which are single copy in the reference but display
139 CNV in some individual. This can distort estimates of nucleotide diversity or homozygosity,
140 biasing analyses based on LD or allele frequencies. These variations can be partly masked by
141 filtering SNPs on the basis of Hardy-Weinberg equilibrium or sequencing depth. However,
142 more quantitative methods are available that allow to precisely characterize the nature and the
143 position of this type of variation, like Delly (Rausch *et al.*, 2012) or Lumpy (Layer *et al.*,
144 2014). Regions that display changes in genomic structure can then be excluded for analyses
145 requiring accurate estimates of diversity (e.g. Rasmussen *et al.*, 2014). On the other hand,
146 these variations can be used for studying association with traits of interest.

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148 **Assessing population history**

149 **Exploring population structure**

150 Checking for population structure is an essential step when performing analyses on genome-
151 level datasets. Neglecting it can bias demographic inferences (Chikhi *et al.*, 2010; Heller *et*

152 *al.*, 2013) or the detection of loci under selection (e.g. Nielsen *et al.*, 2007); thus, checking for
153 outlier individuals and assessing the global structure is required prior any more sophisticated
154 analysis. A simple approach that does not assume any *a priori* grouping is the Principal
155 Component Analysis (PCA), based on analyzing variance-covariance structure among
156 genotypes, which can be performed on both individual and pooled data. Methods such as
157 SMARTPCA (Patterson *et al.*, 2006) or EIGENSTRAT (Patterson *et al.*, 2006) emerged from
158 this framework. There are many software solutions and packages allowing to perform this
159 type of analysis, such as SNPRelate (Zheng *et al.*, 2012), implemented in Bioconductor
160 (Huber *et al.*, 2015), PLINK (Purcell *et al.*, 2007) or GenABEL (Aulchenko *et al.*, 2007). For
161 large whole-genome data or high-density RAD-seq, reducing SNP redundancy by
162 subsampling unlinked markers (having low LD or large physical distance between them) is a
163 way to reduce computation time while keeping the relevant information.

164 Taking into account the relatedness of individuals is recommended, for example to evaluate
165 the amount of inbreeding within a population. When each individual in a study is sampled
166 from a different location or environment, estimating relatedness also provides a way to assess
167 the genetic distance between them, in relation with geographical or ecological distance (e.g.
168 Fields *et al.*, 2015). VCFTools (Danecek *et al.*, 2011) provides two ways calculating
169 relatedness; unadjusted A_{jk} (Yang *et al.*, 2010) and a kinship coefficient also implemented in
170 KING (Manichaikul *et al.*, 2010). It also allows calculating Hardy-Weinberg equilibrium.
171 Population stratification and relatedness can also be explored in PLINK based on pairwise
172 identity-by-state (IBS) distance or identity by descent (IBD).

173 Other approaches such as Structure (Pritchard *et al.*, 2000) and fastSTRUCTURE (Raj *et al.*,
174 2014) allow determining hierarchical population structure by grouping individuals in clusters
175 without any *a priori*. FastSTRUCTURE is computationally faster and more efficient with
176 large SNP datasets. These methods are also more efficient at detecting signatures of
177 admixture. Geneland (Guillot *et al.*, 2012), available as a R package, allows determining the
178 optimal number of population in a dataset by optimizing linkage and Hardy-Weinberg
179 equilibrium within clusters, and is also able to incorporate geographic coordinates in the
180 model to delineate their spatial organization. It can be useful to characterize the location and
181 shape of hybrid zones.

182 In order to properly test for the existence of hierarchical population structure, methods based
183 on differentiation measures (like F_{st}) can be used to build phylogenetic trees. POPTREE
184 (Takezaki *et al.*, 2010) allows to use various differentiation metrics to infer relationships

185 between populations. TreeMix (Pickrell and Pritchard, 2012) is a method building a
186 population tree based on the covariance matrix of population allele frequencies. It allows
187 tracking admixture events but requires the populations to be defined *a priori* (e.g. by a
188 Structure analysis). Other methods can use individual SNP data to reconstruct phylogenies,
189 like PhyML (Guindon *et al.*, 2010) or RAxML (Stamatakis, 2014). Splitstree (Huson and
190 Bryant, 2006) is a user-friendly software to compute phylogenies and networks on SNP
191 datasets and incorporate various methods for phylogeny reconstruction. Other pipelines, like
192 SNPhylo (Lee *et al.*, 2014), propose a complete framework from SNP filtering to tree
193 reconstruction that might help obtaining reliable topologies.

194 While useful to infer topologies, caution is advised when using branches lengths obtained
195 from SNP-only datasets, e.g. to calculate divergence times between different groups or
196 species (Leache *et al.*, 2015). For this purpose, it might therefore be easier to extract genes or
197 RAD contigs from the data and analyze them as DNA sequences in a software like BEAST2
198 (Drummond and Rambaut, 2007). In RAxML, a recent correction for bias on branch length
199 has been implemented that requires the number of monomorphic sites to be known (Leache *et al.*
200 *et al.*, 2015) when providing only SNP alignment. Dating species or population divergence and
201 changes in population sizes using SNP data is also possible in SNAPP (Bryant *et al.*, 2012),
202 although the method requires long computing times when many markers are included. For
203 dating purpose and resolution of individual and population/species trees, BEAST2 and
204 BEAST* can also be used on sequence data for moderate-sized datasets (Drummond and
205 Rambaut, 2007).

206 As a general word of caution, it is important to remind that RAD-sequencing and related
207 methods display specific properties that can bias genome-wide estimates of diversity, like
208 allelic dropout (Arnold *et al.*, 2013). However, this type of markers remains valuable for
209 phylogenetic estimation, even for distantly related species (Cariou *et al.*, 2013).

210 To assess how diversity is partitioned across the different groups inferred by the methods
211 described previously, it is advisable to perform an Analysis of Molecular Variance
212 (AMOVA). Arlequin (Excoffier and Lischer, 2010) is particularly suited for this task. More
213 generally, investigating patterns of nucleotide diversity, inbreeding, F_{st} or variation in LD
214 between populations and across the genome is useful to have a preliminary idea of the amount
215 of gene flow, admixture and variation in population sizes. These statistics can be easily
216 retrieved with VCFTools or PopGenome (Pfeifer *et al.*, 2014).

217 **Investigating population history with coalescent methods**

218 The coalescent has first emerged to provide population geneticists a way of modeling alleles
219 genealogy from a sample taken from a large population. Going backward in time, alleles
220 merge (coalesce) in a stochastic way until reaching their most recent common ancestor
221 (Kingman, 1982). A variety of methods used and enriched this theoretical framework to
222 resolve complex population histories and their associated demographic parameters, such as
223 divergence times, effective population sizes or gene flow. These parameters are usually scaled
224 by mutation rate per generation. Converting those parameters into demographic estimates
225 (e.g. time in years) requires that mutation rate and generation time be known or at least
226 reasonably well estimated, for example from other close species with similar life history.
227 Most well-known coalescent-based tools dedicated to population genetics include IMA (Hey
228 and Nielsen, 2007), Migrate-n (Beerli and Palczewski, 2010) or Lamarc (Kuhner, 2009).
229 Lamarc is the only one taking into account recombination in the model, the other ones
230 requiring non-recombining blocks of sequence or markers to be used. Although they are
231 powerful, these methods tend to be computationally slow (Excoffier *et al.*, 2013), since they
232 require a full evaluation of the likelihood function associated to the model, a procedure that
233 can be complex with hundreds or thousands of markers.

234 A way to bypass this issue has been the use of Approximate Bayesian Computation (ABC)
235 methods, which compare to the actual data a set of simulated data produced by coalescent
236 simulations under predefined scenarios. By measuring the distance between carefully chosen
237 summary statistics describing each simulation with those from the observed dataset, it is
238 possible to infer which scenario explains the data the best. DIYABC (Cornuet *et al.*, 2008) is
239 a popular and user-friendly software allowing to perform a full ABC analysis (from
240 simulations to model comparison), although it does not allow yet to model continuous gene
241 flow between populations. Another approach, which provides more control to the user,
242 consists in using coalescent simulators such as ms (Hudson, 2002) or fastsimcoal2 (Excoffier
243 and Foll, 2011). A pipeline allowing to perform all these steps is also available in
244 ABCtoolbox (Wegmann *et al.*, 2010). Fastsimcoal is a bit slower to simulate data, but is
245 more user-friendly than ms, and more effective when simulating recombination for sequence
246 data. Once simulations are done, one can compute summary statistics for the simulated
247 datasets (e.g. with Arlequin when using fastsimcoal2), then use packages like abc in R
248 (Csilléry *et al.*, 2012) to perform model choice, cross-validation, estimate model
249 misclassification and demographic parameters. More information on how to perform a proper

250 ABC analysis can be found in the work by Csilléry *et al.* (2010). The main advantage of ABC
251 is that it allows handling arbitrarily complex models, unlike methods like IMA where the
252 model is predefined. However, using summary statistics leads to the loss of potentially useful
253 information.

254 More recently, new methods based on the allele frequency spectrum (AFS) emerged to
255 facilitate and speed up the analysis of large SNP datasets. Different patterns of gene flow and
256 demographic events all shape the AFS in specific ways (e.g. more alleles are likely to be
257 found at similar frequencies in two recently diverged or highly connected populations). $\partial a \partial i$
258 (Gutenkunst *et al.*, 2009) does not rely on computationally intensive coalescent simulations
259 but rather on a diffusion approximation of alleles, and computes likelihoods for the alternative
260 models provided by the user. However, its current implementation does not handle more than
261 three populations. More recently, another likelihood-based approach has been implemented in
262 fastsimcoal2 (Excoffier *et al.*, 2013), that uses coalescent simulations and handles arbitrarily
263 complex scenarios while not being limited by the number of populations included. These two
264 methods assume that SNPs are under linkage equilibrium. Including SNPs in strong LD
265 should not particularly bias model comparison, but can be an issue when estimating
266 parameters (see fastsimcoal manual for more details). Note that the AFS can also be used as a
267 set of summary statistics for ABC inference. Using allele frequencies estimated from pooled
268 datasets should be feasible, although no study explored this possibility to my knowledge.

269 One drawback when using SNP data without considering monomorphic sites is that the
270 mutation rate per generation is not directly taken into account. For example, in DIYABC, it
271 does not matter when a mutation appears in the simulated genealogy, as long as it happens
272 only once before coalescence, a reasonable assumption for SNP markers. However, this
273 prevents any conversion of parameters into demographic estimates by using mutation rate.
274 Again, it is also possible to extract the complete DNA sequence for a set of randomly selected
275 markers and perform analyses on this dataset including monomorphic sites. Another
276 possibility consists in a calibration of parameter estimates by including in the analysis a fixed
277 parameter, such as population size or divergence time. This approach is also feasible when
278 estimating parameters from the allele frequency spectrum, like in $\partial a \partial i$ or fastsimcoal2.

279 When whole genome data are available, it is then possible to use methods such as those based
280 on Pairwise Sequentially Markovian Coalescent (PSMC), that require only a single diploid
281 genome (Li and Durbin, 2011). This method allows tracking changes in population size across
282 discrete time intervals. While powerful, PSMC is sensitive to confounding factors such as

283 population structure (Orozco-terWengel, 2016) that leads to false signatures of expansion or
284 bottleneck. It also does not allow studying recent demographic events. This is due to the fact
285 that coalescence events for only two alleles from a single individual in the recent past are
286 infrequent. However, extensions of the model allowing for several genomes have been
287 developed to precise population history in the recent past, like MSMC (Schiffels and Durbin,
288 2014) or diCal (Sheehan *et al.*, 2013). As these methods require that heterozygous positions
289 be properly called, it is required to correct for low depth of coverage (less than 8-10X) if
290 needed. Recently an ABC framework, implemented in PopSizeABC, has been proposed to
291 infer demographic variation from single genomes (Boistard *et al.*, 2016). The summary
292 statistics used describe variation in LD and the AFS, while being robust to sequencing errors.
293 This last method does not require phasing, which should limit the impact of phasing errors.

294 A recent extension of these methods takes into account population structure and aims at
295 identifying the number of islands contributing to a single genome, assuming it is sampled
296 from a Wright n-island meta-population (Mazet *et al.*, 2015). Such developments should help
297 increasing the amount of information retrieved from only a few genomes. However, it is
298 essential to keep in mind that natural populations are structured and connected in complex
299 ways, which can bias demographic inferences, even for popular markers such as
300 mitochondrial sequences (Heller *et al.*, 2013).

301 Reaching a high level of precision in demographic parameters estimation can be challenging
302 when perspective is lacking about the evolutionary history of the species considered. At larger
303 time-scales, the lack of fossil record can make difficult the calibration of molecular clocks.
304 Thus, for some species, only qualitative interpretation will be possible.

305

306 Screening for selection and association

307 **Selection and its impact on sequence variation**

308 The impact of selection on genetic variation has been extensively studied, but still remains a
309 central topic in evolutionary biology. Here I describe some features that are associated to
310 different types of selection.

311 Selection acts both on correlations i) between alleles and environment at selected loci and ii)
312 between alleles from different loci, either directly under selection or not. This is reflected

313 respectively by i) variation in polymorphism within and between populations and ii) linkage
314 disequilibrium between loci (Figure 2). A new mutation will see its frequency increase in a
315 population where it provides a selective advantage (hard sweep). When such an allele arose
316 recently, a large region around it can remain uniform, especially if selection is strong. As the
317 allele rises quickly in frequency, it has too little time to recombine with other ancestral
318 variants. This leads to an increase of linkage disequilibrium between variants associated to the
319 advantageous mutation, as well as a decrease in nucleotide diversity around the selected locus.
320 If selection occurs in one population but not others, it may be possible to observe a local
321 increase in differentiation, like higher F_{st} values. If selection acts on standing variation or
322 recurrent mutation, signature of selection can be less clear as several haplotypes surround the
323 mutation under positive selection (see however Messer and Petrov, 2013; Jensen, 2014 for a
324 discussion about the relative importance of soft selective sweeps).

325 Another type of selection is balancing selection, an umbrella term grouping all selective
326 processes that lead to the maintenance of genetic polymorphism at a locus and to an excess of
327 common alleles. Such processes include divergent selection (the same allele is under positive
328 selection in one population and selected against in another one), negative frequency-
329 dependent selection (a rare allele is preferably selected) or heterozygote advantage. In the
330 case of recent balancing selection, the signature of selection is similar to a partial selective
331 sweep, with the recently selected allele displaying reduced diversity and higher LD than the
332 ancestral one. In the case of long-term balancing selection, there is an accumulation of genetic
333 polymorphism around the selected loci, leading to the maintenance of haplotypes older and
334 more diverse than in the rest of the genome. This increase in diversity can be associated to
335 higher local estimates of effective population sizes and effective recombination rates. As
336 alleles are older, coalescence times tend to be higher and can sometimes predate speciation,
337 leading to trans-species polymorphism (see Charlesworth, 2006 for a detailed review). In
338 some cases, an allele under balancing selection is stabilized at a single equilibrium frequency
339 across populations, which can lead to a signature of lower differentiation compared to
340 genomic background. There is still a lack of methods aiming at detecting specifically
341 balancing selection compared to positive selection and recent hard sweeps (but see Fijarczyk
342 and Babik, 2015).

343 In the following parts I present tools that can be used to detect signatures of selection. The
344 methods that these tools implement fall into three main categories (partly reviewed in Vitti *et*
345 *al.*, 2013), corresponding to the signature they try to target: i) study of variation in allele

346 frequencies and polymorphism, ii) study of variation in linkage disequilibrium and iii)
347 reconstruction of allele genealogies using the coalescent. Most of these methods assume that
348 markers are ordered along a genome; although they can also be used to extract individual
349 markers under selection that can be then be aligned (except for most LD-based methods).

350 **Methods focusing on polymorphism**

351 While demographic forces such as drift and migration will affect the whole genome in a
352 similar way, local effects of selection should produce discrepancies with genome-wide
353 polymorphism (Lewontin and Krakauer, 1973). Selection affects allele frequencies and
354 polymorphism in predictable ways at the scale of single populations. Several statistics
355 summarize them, like π , the nucleotide diversity (Nei and Li, 1979), Tajima's D (Tajima,
356 1989) or Fay and Wu's H (Fay and Wu, 2000). They are sensitive to population demographic
357 history, that they allow characterizing as summary statistics in, e.g., ABC analyses. They have
358 nonetheless the potential to highlight genomic regions displaying clear signatures of selection,
359 or to confirm selection at candidate genes. For example, balancing selection should lead to an
360 excess of common polymorphisms, similar to a recent bottleneck, leading to high Tajima's D
361 and π values. Purifying selection leads to an opposite pattern, similar to a recent population
362 expansion, with an excess of rare variants and low diversity. More sophisticated methods
363 using allele frequency spectrum have been developed to detect positive selection, such as the
364 recent improvement of the composite likelihood ratio (CLR) test (Nielsen *et al.*, 2005)
365 performed in SweepFinder2 (Degiorgio *et al.*, 2016).

366 PopGenome (Pfeifer *et al.*, 2014) is a powerful R package that allows calculating AFS
367 statistics (including the CLR test) across many genomes, as well as a variety of statistics on
368 linkage disequilibrium and diversity. It also allows performing coalescent simulations to
369 contrast observed polymorphism to neutral expectations. It is probably one of the most
370 comprehensive tools to perform genome-wide analyses. Other possibilities include VCFTools,
371 POPBAM (Garrigan, 2013) or Biopython libraries. For pooled data, Popoolation (Kofler,
372 Orozco-terWengel, *et al.*, 2011; Kofler, Pandey, *et al.*, 2011) provides ways to calculate
373 Tajima's D and nucleotide diversity, as well as measures of differentiation between
374 populations.

375 Understanding the origin of genomic regions under selection highlights the evolutionary
376 history of adaptive alleles (e.g. Abi-Rached *et al.*, 2011). Advantageous alleles can migrate
377 from one population to another, or resist introgression from other populations (genomic

378 islands of speciation/adaptation). The relative importance of these islands resisting gene flow
379 after secondary contact has been recently discussed (Cruickshank and Hahn, 2014). Methods
380 aiming at characterizing heterogeneity in introgression rates are in this context useful and can
381 also refine the demographic history. A recent ABC framework has been developed to
382 characterize this heterogeneity (Roux *et al.*, 2014). Methods such as PCAdmix (Brisbin *et al.*,
383 2012) can also be used to estimate the relative contributions of putative sources to a given
384 sink population across the genome. A common test for introgression, available in
385 PopGenome, is the ABBA-BABA test, summarized by Patterson's D (Durand *et al.*, 2011).
386 Another possibility lies in the comparison of absolute and relative measures of divergence
387 (Cruickshank and Hahn, 2014), such as d_{xy} and F_{st} , which can be calculated in PopGenome.
388 Absolute measures of divergence are correlated to the time since coalescence. In the case of
389 local introgression, both statistics should be reduced. For balancing selection, the decline in
390 F_{st} is due to an excess of shared ancestral alleles, which should not impact d_{xy} , or should even
391 make it higher than genomic background. However, these methods do not prevent false
392 positives and results should (as usual) be interpreted with caution (Martin *et al.*, 2015).

393 When an allele is under positive selection in a population, its frequency tends to rise until
394 fixation, unless gene flow from other populations or strong drift prevents it. It is therefore
395 possible to contrast patterns of differentiation between populations adapted to their local
396 environment to detect loci under divergent selection (e.g. displaying a high F_{st}). However, it is
397 essential to control for population structure, as it may strongly affect the distribution of
398 differentiation measures and produce high rates of false positive. First attempts to take into
399 account population structure and variation in gene flow included FDIST2 (Beaumont and
400 Nichols, 1996), which modeled populations as islands and aimed at detecting loci under
401 selection by contrasting heterozygosity to F_{st} between populations. An extension of this
402 model, able to take into account predefined hierarchical population structure, is implemented
403 in Arlequin. More sophisticated methods are now available, dedicated to the detection of
404 outliers in large genomic datasets. Most of them correct for relatedness across samples, and
405 are reviewed extensively in the work by Francois *et al.* (2015). Some methods, like LFMM
406 (Frichot *et al.*, 2013), aim at detecting variants correlated to environmental factors.
407 Association methods may help targeting variants undergoing soft sweeps, weak selection or
408 involved in polygenic control of traits (Pritchard *et al.*, 2010), for which signatures of
409 selection are subtle and sometimes difficult to retrieve from allele frequencies data.

410 Other methods perform a “naïve scan” for outliers on the basis of differentiation, like
411 BAYESCAN (Foll and Gaggiotti, 2008) which considers all populations to drift at different
412 rates from a single ancestral pool. Most recent methods, like BAYENV (Günther and Coop,
413 2013) and its recent improvement, BAYPASS (Gautier, 2015), model demographic history by
414 computing a kinship matrix between populations. Contrasting allele frequencies for each
415 locus to the ones expected given this matrix allows testing deviation from neutrality. Those
416 two last methods also include Bayesian tools to test for association with environmental
417 features, facilitating further interpretation. BAYENV and BAYPASS also allow using pooled-
418 sequencing data, making these methods polyvalent and possibly useful to many research
419 teams. However, detecting association between environment and allele frequencies does not
420 necessarily imply a role for local adaptation. For example, in the case of secondary contact,
421 intrinsic genetic incompatibilities can lead to the formation of tension zones that may shift
422 until they reach an environmental barrier where they can be trapped (Bierne *et al.*, 2011).
423 Again, characterizing population history is required to conclude about the possible
424 involvement of a genomic region in adaptation to environment. Sampling strategy must take
425 into account the particular historical and demographic features of the species investigated to
426 gain power (Nielsen *et al.*, 2007). The sequencing strategy has also to be carefully picked.
427 Reduced representation methods do not cover all mutations in the genome and are thus more
428 likely to miss those actually under selection. Special care in the choice of the restriction
429 enzyme and determining the expected density of markers is needed to retrieve enough
430 mutations close to genes under selection.

431 The methods described above focus on allele frequencies at the population scale, but do not
432 allow characterizing properly association with a trait varying between individuals within
433 populations (e.g. resistance to a pathogen, symbiotic association, individual size or flowering
434 time). For this task, methods performing Genome-wide association analysis (GWAS) are
435 better suited. Methods such as GenABEL in R (Aulchenko *et al.*, 2007) or PLINK (Purcell *et*
436 *al.*, 2007) are powerful tool. Taking into account relatedness between samples and population
437 history (e.g. using EIGENSTRAT or PC-adjustment corrections in GenABEL or stratified
438 analyses in PLINK) is required to correct for false positives. This is especially recommended
439 for species that undergo episodes of selfing or strong bottlenecks, for which sampling
440 unrelated individuals may be unfeasible.

441 It is important to keep in mind that uncovering the genetic bases of complex, polygenic traits
442 remains challenging, even in model species (Pritchard and Di Rienzo, 2010; Rockman, 2012).

443 It may be unavoidable in a first step to focus only on traits that are under a relatively simple
444 genetic determinism. This can however lead to an overrepresentation of loci of major
445 phenotypic effect, a fact that should be acknowledged when discussing the impact of selection
446 on genome variation. The fact that loci of major effect are easier to target does not imply that
447 they are the main substrate of selection (Rockman, 2012).

448 **Detecting selection with methods focusing on LD**

449 LD is increased and diversity is decreased in the vicinity of a selected allele, especially after
450 recent selection. A class of methods aims at targeting those regions that display an excess of
451 long homozygous haplotypes, such as the extended haplotype homozygosity (EHH) test
452 (Sabeti *et al.*, 2002). It is also possible to compare haplotype extension across populations,
453 with the XP-EHH test (McCarroll *et al.*, 2007) or Rsb (Tang *et al.*, 2007). Individuals
454 included in the analysis should be as distantly related as possible to improve precision and
455 avoid an excess of false positives. These approaches are more powerful with a relatively high
456 density of markers, such as the ones obtained from whole-genome sequencing or high-density
457 RAD-seq. They also require data to be phased in order to reconstruct haplotypes. This
458 procedure can be performed with fastPhase (Scheet and Stephens, 2006), BEAGLE
459 (Browning and Browning, 2011) or SHAPEIT2 (O'Connell *et al.*, 2014). The R package rehh
460 (Gautier and Vitalis, 2012) allows calculating these statistics, as well as Sweep
461 (<http://www.broadinstitute.org/mpg/sweep/index.html>). Statistics dedicated to the detection of
462 soft sweeps are also available, like the H2/H1 statistics (Garud *et al.*, 2015), although further
463 studies are still needed to understand to what extent hard and soft sweeps can actually be
464 distinguished (Schrider *et al.*, 2015). This last statistics does not require data to be phased.

465 When the relative order of markers is not known, as it can be the case in RAD-seq studies
466 without a reference genome, LDna (Kempainen *et al.*, 2015) can be used to target sets of
467 markers displaying strong linkage disequilibrium. This approach can be useful not only to
468 detect selection but also structural variation such as large inversions.

469 Even hard selective sweeps can be challenging to detect with LD-based statistics (Jensen,
470 2014). It is advisable to combine several approaches to reach a better confidence when
471 pinpointing candidate genes for selection. Methods based on LD alone can sometimes miss
472 the actual variants under selection due to the impact of recombination on local polymorphism
473 that can mimic soft or ongoing hard sweeps (Schrider *et al.*, 2015).

474 **Detecting and characterizing selection with the coalescent**

475 When a candidate locus has been identified, it is possible to use coalescent simulations to
476 evaluate the strength of selection and estimate the age of alleles. A software such as msms
477 (Ewing and Hermisson, 2010), which is also available in PopGenome, can then be used. This
478 requires that the neutral history of population be known in order to properly control for, e.g.,
479 population structure and gene flow.

480 An advantage of full coalescent methods is that they provide a relatively complete picture of
481 individual loci history, by modeling coalescence, recombination and taking into account
482 variation in mutation rate. They are however computationally intensive, and thus difficult to
483 apply to whole genomes. However, recent computational improvements make this procedure
484 feasible, as illustrated by ARGWeaver (Rasmussen *et al.*, 2014). This method uses ancestral
485 recombination graphs to model the genealogy of each non-recombining block in the genome.
486 It allows extracting genealogies for these blocks and provides estimates for local
487 recombination rate, coalescence time and local effective population size for each block. This
488 approach is promising to characterize positive, purifying or balancing selection while taking
489 into account variation in recombination and mutation rate. However, the high stochasticity in
490 parameters estimation can limit resolution when targeting single genes.

491 Other methods use the theoretical framework of the coalescent to target sites under positive
492 selection. A recent method (SCCT) using conditional coalescent trees (Wang *et al.*, 2014)
493 claims to be faster and more precise in targeting selective sweeps. BALLET (DeGiorgio *et al.*,
494 2014) is a promising method to characterize ancient balancing selection. Most of those
495 methods are designed for medium-to-high depth whole-genome resequencing, and require that
496 individual genotypes be phased and well characterized.

497 **Variants annotation**

498 Characterizing the amount of synonymous or non-synonymous mutations is another way to
499 detect whether a specific gene undergoes purifying or positive selection. An excess of non-
500 synonymous mutations can signal positive or balancing selection, or a relaxation of selective
501 constraints on a given gene. This requires that an annotated genome is available. Annotation
502 of mutations can be done with SNPdat (Doran and Creevey, 2013), or directly in PopGenome,
503 which can also perform at the genome scale tests of selection such as the MK test (McDonald
504 and Kreitman, 1991). The MK test compares the amount of fixed and polymorphic mutations
505 relative to an outgroup, according to their synonymous/non-synonymous state. Another
506 popular test of selection is the comparison of non-synonymous and synonymous mutations

507 between orthologs from different species and can be performed in packages such as PAML
508 (Yang, 2007).

509 To recover information about the putative function of a gene or a genomic region, it may be
510 useful to perform a genome ontology (GO) enrichment analysis. BLAST2GO (Conesa *et al.*,
511 2005) allows annotating genes by using a database of related species. It also allows
512 performing GO enrichment analysis. These analyses must be carefully interpreted, depending
513 on the level of divergence from the closest annotated species. It is important not to jump to
514 the conclusion that orthologous genes must share the same function. When interpreting the
515 link between selection and genetic variation, a careful review of literature can fruitfully
516 complete the conclusions made using GO enrichment analyses.

517

518 Concluding remarks

519 In this contribution I highlighted different methods currently available to investigate how
520 history and selection shape diversity in natural populations. It is important to understand that
521 this dichotomy between selection and demography, while practical, remains artificial, and that
522 the study of one benefits from studying the other. With the decreasing cost of sequencing it
523 has been suggested that NGS should broaden quickly our perspective on complex
524 evolutionary processes, from biogeography (Lexer *et al.*, 2013) to genetic bases of traits
525 (Hohenlohe, 2014) or the maintenance of polymorphism (Hedrick, 2006). The study of DNA
526 sequence variation, while already challenging by itself, needs to be combined with other
527 disciplines such as ecology to be informative (Habel *et al.*, 2015). Although genome-scale
528 analyses can be insightful to this regard, it is necessary to be conscious of their limits and to
529 keep a biological perspective when interpreting their results. To do so, every analysis should
530 always begin with a proper understanding of the methods used, to avoid using them as black
531 boxes.

532 Before launching a project targeting thousands of markers in a species of interest, possibilities
533 and limits of the chosen protocol must be evaluated. Focusing on species history will not
534 necessarily require the same sampling strategy than focusing on local adaptation. While a
535 small number of markers and populations may be enough to recover global structure and infer
536 robustly demographic parameters, it will not provide enough resolution to target genes
537 involved in local adaptation. In many cases, a preliminary study focusing on a few markers

538 may already inform about the global species history and help to define an adapted design for
539 NGS.

540 There is a need for a more collaborative and open culture in biology, allowing the free access
541 to data and favoring good practices to allow repeatability of analyses (Nekrutenko and Taylor,
542 2012), although this cultural shift remains challenging (e.g. Mills *et al.*, 2015; Whitlock *et al.*,
543 2015). However, current challenges are not limited to data sharing, but also include dealing
544 with the inflation of bioinformatics tools that sometimes overlap. Instead of working
545 independently, researchers designing those tools could collaborate to propose free, robust and
546 unified pipelines (Prins *et al.*, 2015). Such initiatives, like Galaxy (Goecks *et al.*, 2010) or
547 Bioconductor (Huber *et al.*, 2015) are nonetheless emerging ; this should facilitate the
548 emergence of a unified framework to limit the time dedicated to data analysis and focus on
549 biological questions.

550

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904 Figures

905 Figure 1: Summary of methods assessing the demographic history of populations (middle
906 panel). References are provided in the main text.

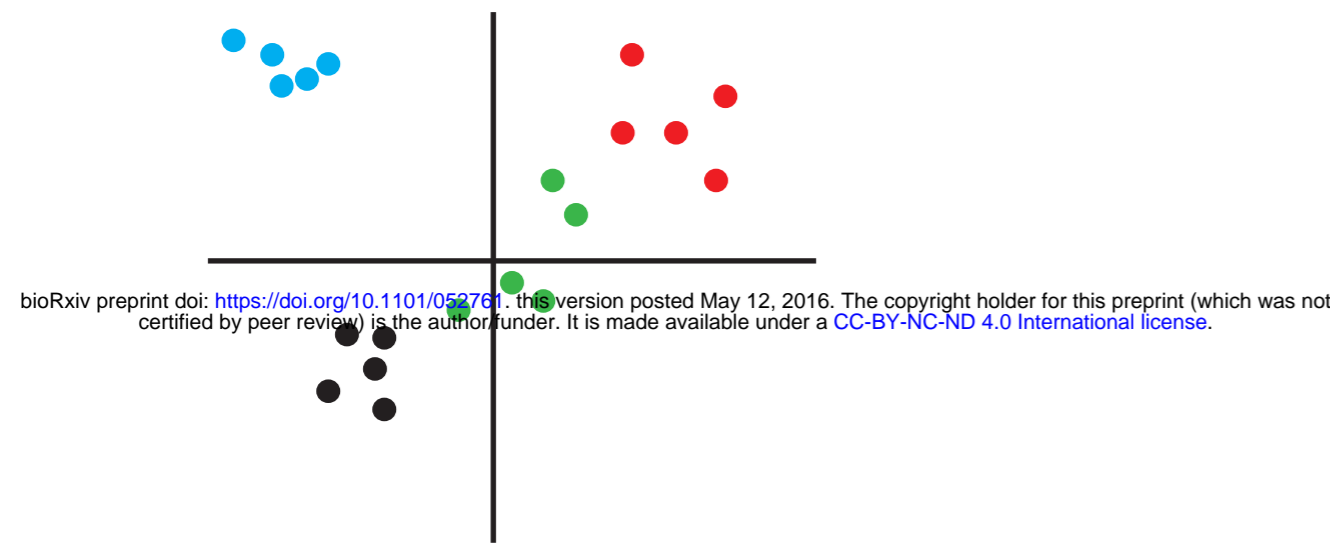
907 Figure 2: Summary of methods dedicated to the detection of various signatures of selection in
908 the genome. In this simple example, the mutation on the left is under positive selection
909 in one population (red) but not the other (black). The mutation on the right is under
910 ancient balancing selection in the two populations.

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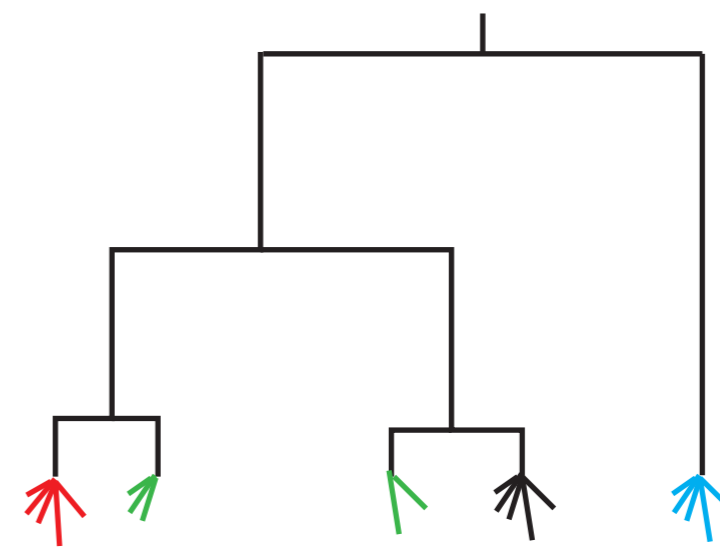
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Assessing population structure and family relationships

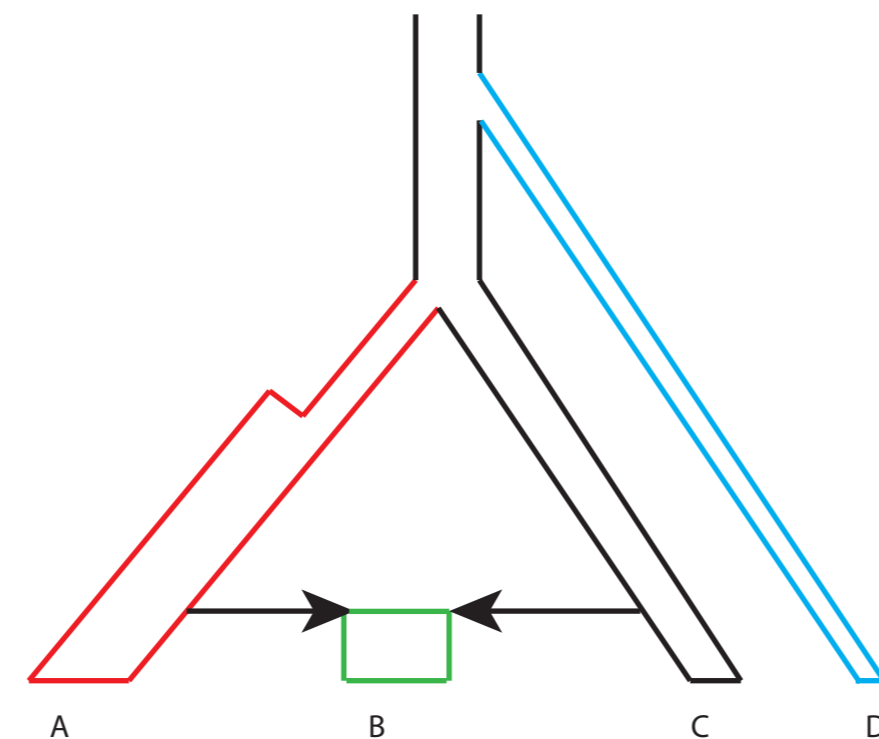


PCA (SNPRElate, GenABEL in R)
 STRUCTURE and fastSTRUCTURE
 GENELAND
 PLINK, VCFTOOLS, KING (relatedness)
 Arlequin (AMOVA)

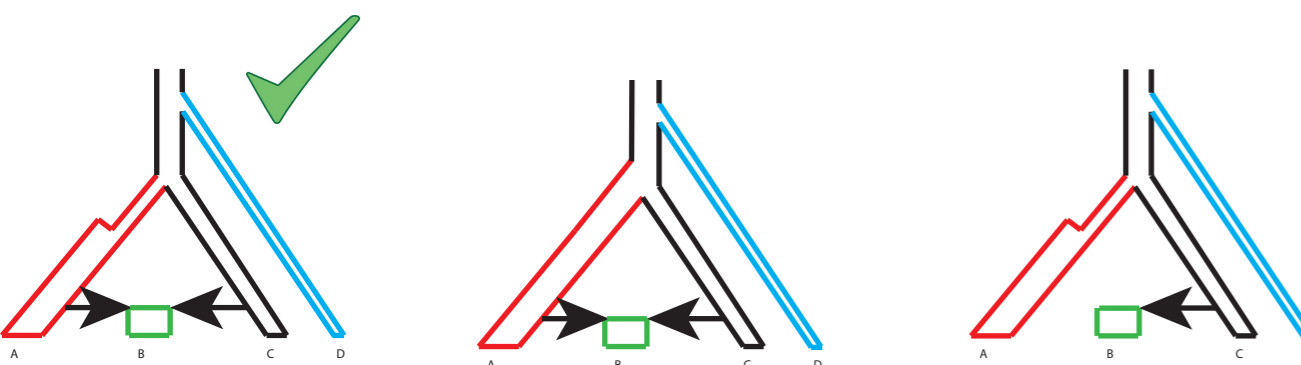
Phylogenetic relationships between individuals/populations



TREEMIX, fastSTRUCTURE (admixture)
 POPTREE, PopGenome, VCFTOOLS (differentiation)
 Splitstree, SNPhylo, RAxML, PhyML, BEAST2 (individual phylogeny)
 POPTREE, BEAST*, SNAPP (Species/Population trees)

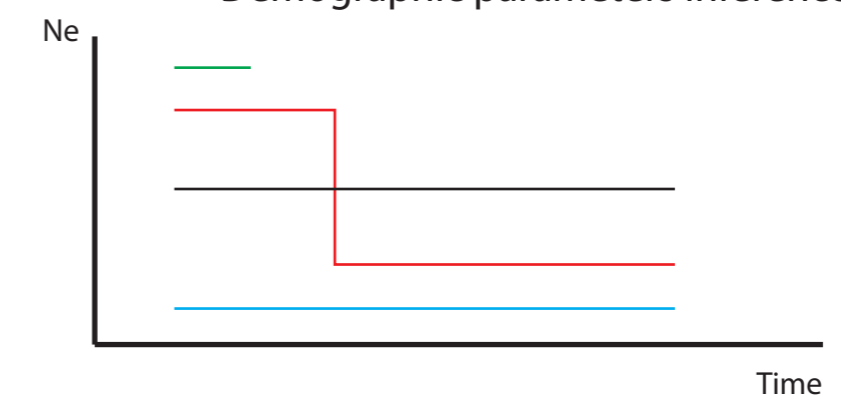


Model testing



Likelihood comparison: Migrate-n, IMA2 (for small datasets)
 ABC methods: DIYABC, ms, fastsimcoal2, ABCtoolbox, package abc in R.
 Likelihood comparison using AFS (fastsimcoal2, dadi)

Demographic parameters inference

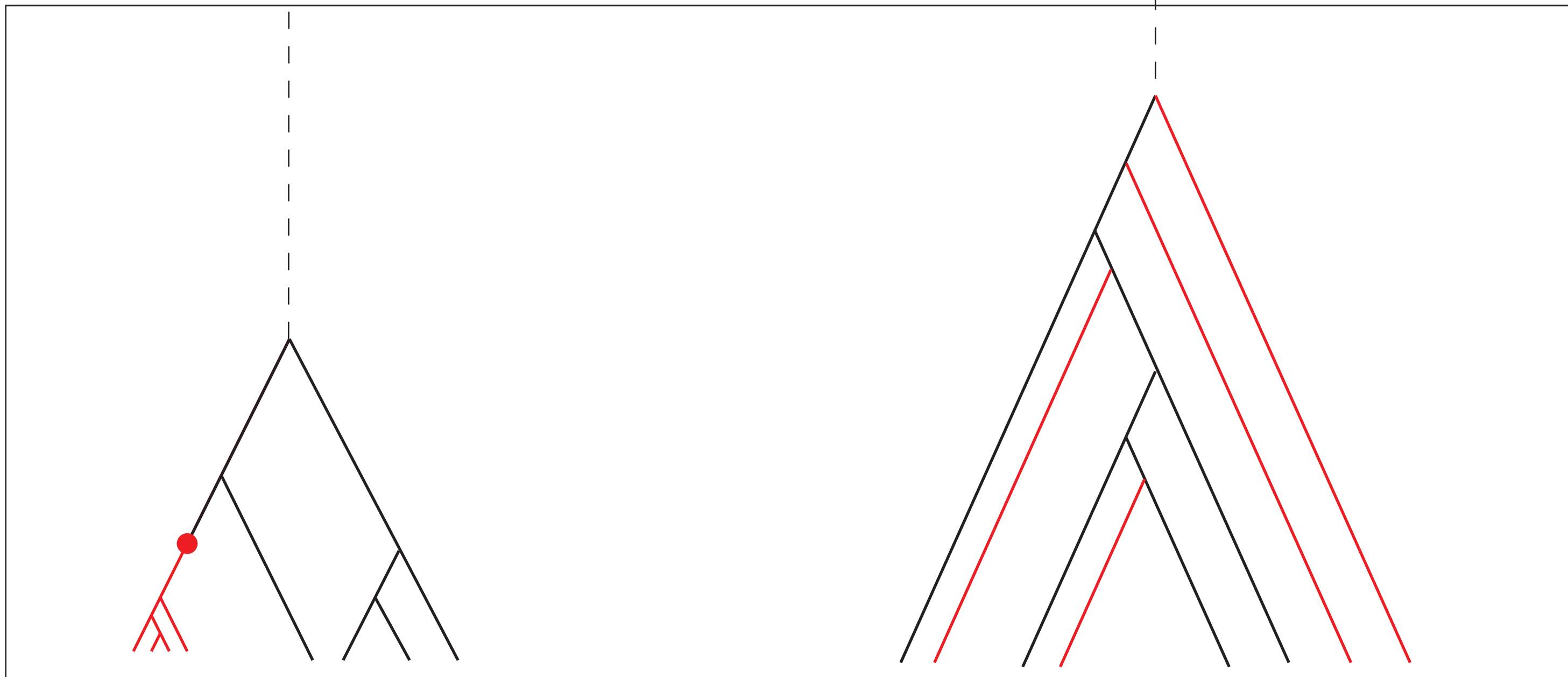
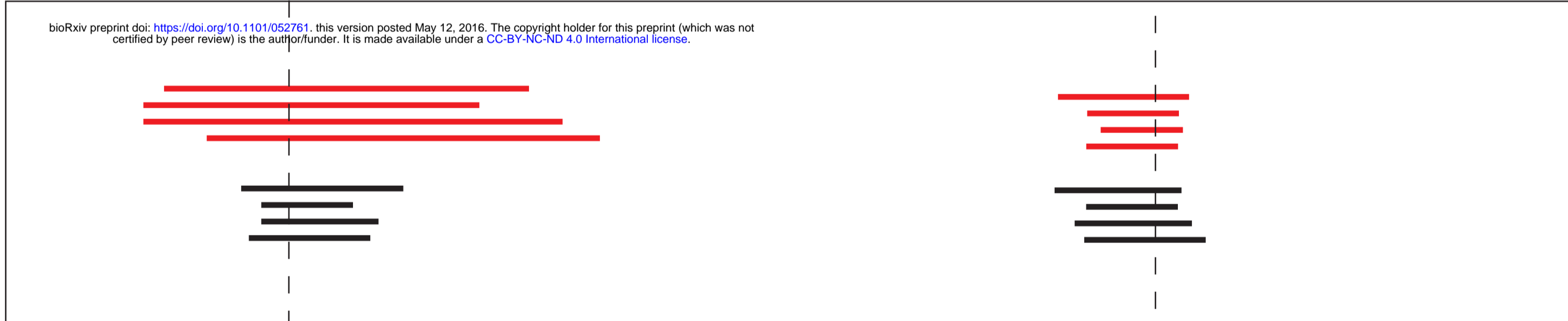
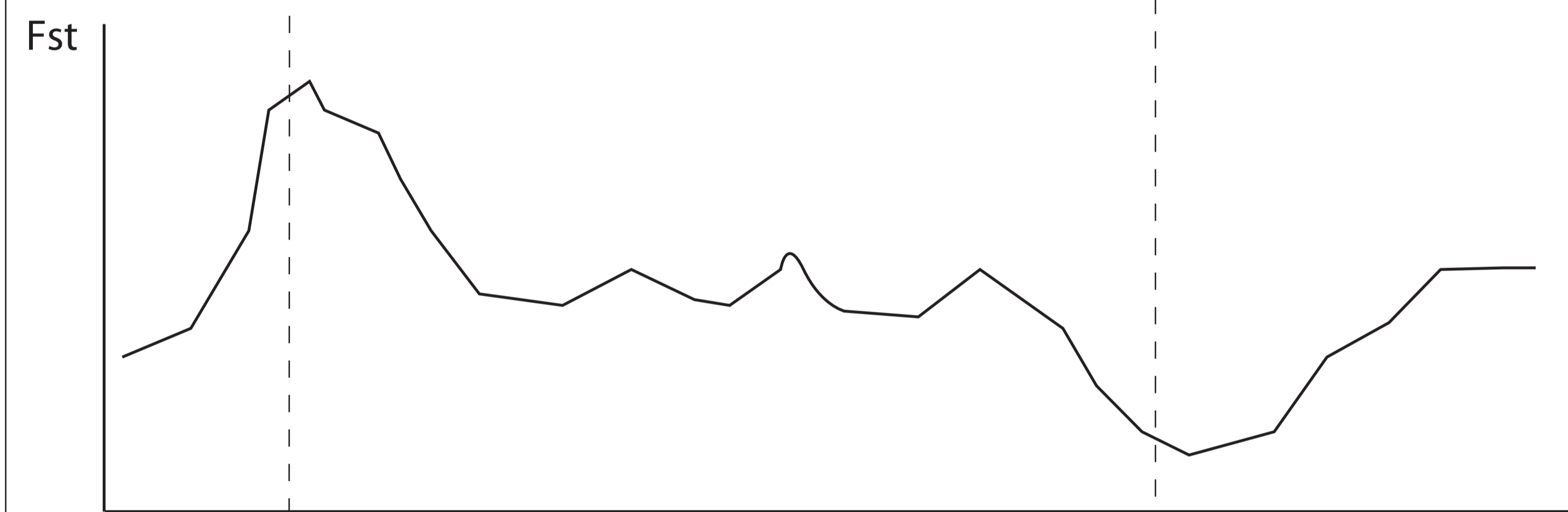
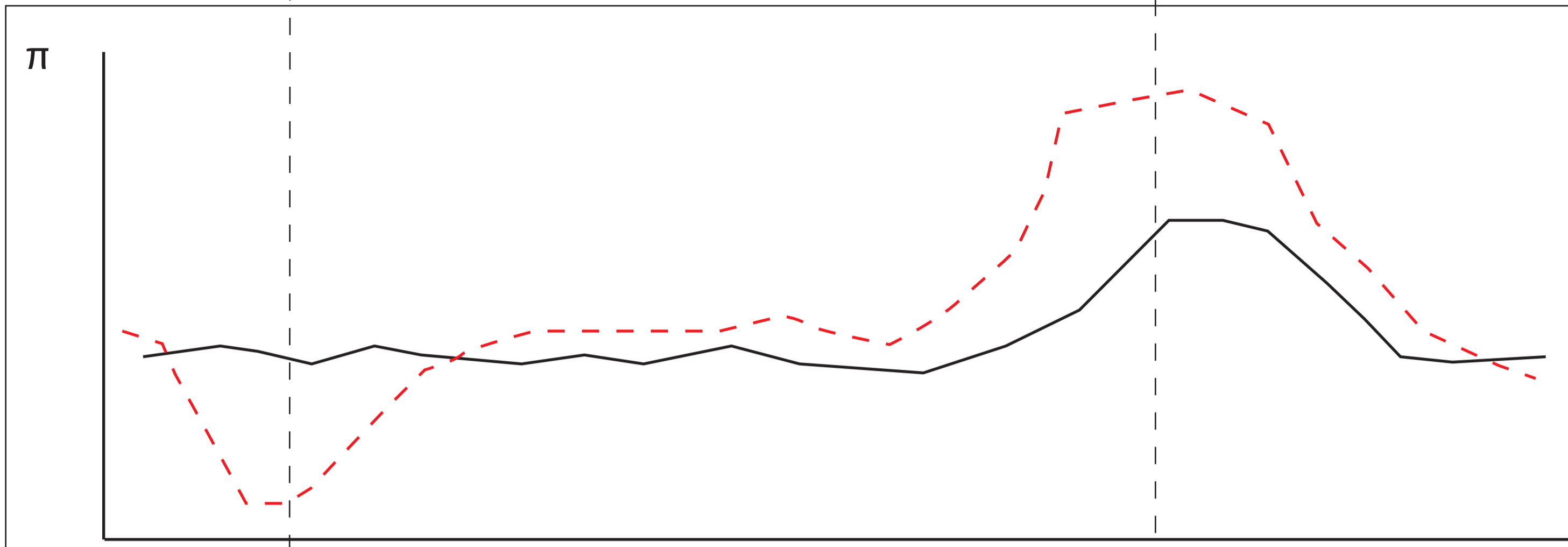


SNAPP, BEAST*, IMA, Migrate-n, LAMARC (for small datasets)
 ABC methods: DIYABC, ms, fastsimcoal2
 Inference from allele frequency spectrum (AFS): fastsimcoal2, dadi
 diCal, PSMC, MSMC (for whole genome resequencing)

Positive selection

Balancing selection

Methods and tools available



PopGenome, VCFTOOLS, POPBAM
 SweepFinder 2
 Biopython
 Popoolation (pooled data)

PopGenome, VCFTOOLS, Popoolation
 Fst outlier methods: BAYPASS, BAYENV
 Association with environment: BAYPASS, LFMM, BAYENV
 Association with phenotype: GENABEL, PLINK

Genome-wide LD: PLINK, VCFTOOLS
 EHH and Rsb tests: rehh package, Sweep
 LD clusters: LDna

MSMS, PopGenome
 ARGWeaver *
 BALLET *
 SCCT *
 * Better suited for whole-genome resequencing

Diversity

Differentiation/Association

Haplotype length and LD patterns

Coalescence and alleles history

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