

1 Demographic inference through 2 approximate-Bayesian-computation skyline 3 plots

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11 ABSTRACT

12 The skyline plot is a graphical representation of historical effective population sizes as a function of time.
13 Past population sizes for these plots are estimated from genetic data, without *a priori* assumptions on
14 the mathematical function defining the shape of the demographic trajectory. Because of this flexibility in
15 shape, skyline plots can, in principle, provide realistic descriptions of the complex demographic scenarios
16 that occur in natural populations. Currently, demographic estimates needed for skyline plots are estimated
17 using coalescent samplers or a composite likelihood approach. Here, we provide a way to estimate
18 historical effective population sizes using an Approximate Bayesian Computation (ABC) framework. We
19 assess its performance using simulated and actual microsatellite datasets. Our method correctly retrieves
20 the signal of contracting, constant and expanding populations, although the graphical shape of the plot is
21 not always an accurate representation of the true demographic trajectory, particularly for recent changes
22 in size and contracting populations. Because of the flexibility of ABC, similar approaches can be extended
23 to other types of data, to multiple populations, or to other parameters that can change through time, such
24 as the migration rate.

25 Inferring the historical demography of populations by means of genetic data is key to many studies
26 addressing the ecological and evolutionary dynamics of natural populations. Population genetics infer-
27 ence, with appropriate dating, can identify the likely factors (such as climatic events) determining the
28 demography of a species. With enough research resources, this can be done with outstanding detail (e.g.
29 in humans, reviewed in Nielsen et al., 2017). Demographic inference can also be used to generate null
30 models for the detection of loci under selection (as discussed in Hoban et al., 2016).

31 At present, most of the methods to estimate demography from genetic data are based on the coalescent.
32 The coalescent (see Wakeley, 2008, for a review) is a mathematical model that describes the rate at which
33 genetic lineages coalesce (i.e. join in a common ancestor) towards the past, forming the genealogy of the
34 sample. The coalescence probability depends on the effective population size at each time in the past, that
35 is, the demographic history of the population. Given a genealogy, the coalescent enables a calculation of
36 the likelihood of the demographic model. Demographic inference is obtained by calculating the likelihood
37 of the model given the data, which requires integrating over all possible genealogies for the data. This is
38 approximated by means of Monte Carlo algorithms known as coalescent samplers (see review by Kuhner,
39 2009).

40 Alternatively, the coalescent can be used to calculate the likelihood of the number of genetic differences
41 for a pair of gene copies under a given demographic model. The likelihood for all pairs in a sample
42 can be combined to obtain a composite-likelihood (which is not a true likelihood because pairs are not
43 independent and they are related by their genealogy). The composite-likelihood score can be used as
44 a criterion to estimate the parameters of the model with faster algorithms than the coalescent samplers
45 although with lower performance, particularly regarding confidence intervals (e.g. Navascués et al., 2009;
46 Nikolic and Chevalet, 2014).

47 Coalescent models can also be used in the likelihood-free framework known as Approximate Bayesian
48 Computation (ABC, Tavaré et al., 1997; Beaumont et al., 2002). In this approach, the likelihood is
49 substituted by the similarity between the observed data and simulated data generated from a given model.
50 Similarity is usually evaluated by means of a distance between observed and simulated summary statistics.
51 This distance allows one to select the simulations close to the observed data and reject those too far
52 away. Posterior probability distributions are estimated from the collection of parameter values used in the
53 selected simulations (see Beaumont, 2010, for a review on ABC).

54 A classical way to address the estimation of past population size changes by these methods is to
55 assume simple parametric models, such as exponential, logistic or instantaneous demographic change.
56 However, these are sometimes considered too simple to describe the dynamics of real populations. In the
57 skyline plot methods, the underlying demographic model is a piecewise constant population size model,
58 i.e. the demographic history consists of several periods of constant size, with instantaneous changes of
59 sizes between consecutive periods. The aim of this model is to provide a more flexible framework that
60 could capture the complex demography expected in natural populations. Skyline plots were introduced
61 by Pybus et al. (2000) who estimated the effective population size in the time intervals defined by the
62 coalescent events of a given genealogy (which was considered as known) from the expected waiting time
63 between coalescent events. The graphical representation of those estimates suggests the skyline of a city,
64 giving the name to the method. Such models have been implemented in a Markov chain Monte Carlo
65 coalescent sampler (BEAST software; Drummond et al., 2005; Minin et al., 2008; Heled and Drummond,
66 2008), and in an importance sampling coalescent sampler (Ait Kaci Azzou et al., 2015) for the analysis of
67 sequence data. The addition of microsatellite mutation models to BEAST (Wu and Drummond, 2011)
68 made it possible to infer skyline plots from this type of data (e.g. Allen et al., 2012; Molfetti et al., 2013;
69 Minhós et al., 2016). Also for microsatellite data, a composite-likelihood approach has been developed
70 (R package VarEff Nikolic and Chevalet, 2014).

71 Similar piecewise models to infer historical population sizes through time have been proposed in the
72 context of population genomics (e.g. Li and Durbin, 2011; Terhorst et al., 2016). The methods discussed
73 above assume a set of independent (unlinked) genetic markers. However, if a large proportion of the
74 genome has been sequenced, the studied polymorphisms are in linkage disequilibrium. Methods such
75 as the Pairwise Sequentially Markovian Coalescent (PSMC, Li and Durbin, 2011) and its successors
76 profit from the additional information of linkage disequilibrium for the inference. We will not further
77 discuss this family of methods, as our focus here is on datasets of independent molecular markers, such as
78 microsatellites, which remain reliable markers for low-budget projects. Note, however, the PSMC-like
79 implementation on ABC by Boitard et al. (2016).

80 The use of the skyline plot in the ABC framework was first proposed in Burgarella et al. (2012). Here,
81 we provide a suite of R scripts (DIYABCskylineplot) to produce approximate-Bayesian-computation
82 skyline plots from microsatellite data and evaluate its performance on simulated pseudo-data. We show
83 the method to be useful for detecting population decline and expansion and discuss its limits. ABC skyline
84 plots are then built for four study cases (whale shark, leatherback turtle, Western black-and-white colobus
85 and Temminck's red colobus) and compared with the demographic inference obtained by an alternative
86 full likelihood method.

87 METHODS

88 ABC skyline plot

89 For a demographic skyline plot analysis within the ABC framework, our model consisted of a single
90 population with constant size that instantaneously changes to a new size n times through time. The
91 parameters (from present to past, as in the coalescent model) are the present scaled population size
92 $\theta_0 = 4N_0\mu$ (where N_0 is the effective population size in number of diploid individuals and μ is the
93 mutation rate per generation) which changes to θ_1 at time $\tau_1 = T_1\mu$ (where T is the time measured in
94 generations), remains at θ_1 and then it changes to θ_2 at τ_2 , and so on, until the last change to θ_n at τ_n .
95 Note that other models and parametrization could have been used for our purpose, as in the alternative
96 model that we present in the supplementary methods section S1.2.

97 The objective of a standard ABC analysis would be to estimate the posterior distribution for each
98 parameter of the model. In our case, the parameters $\{(\theta_i, \tau_i); i \in [0, n]\}$ have been treated as nuisance
99 parameters and we focused on inferring from them the trajectory of the scaled effective population size
100 along time, $\theta(t)$, as in Drummond et al. (2005). In order to approximate $\theta(t)$ we select m times of

101 interest, $\{t_j; j \in [1, m]\}$. Given a simulation k with parameters $\{(\theta_{k,i}, \tau_{k,i}); i \in [0, n_k]\}$, derived parameters
102 $\{\theta_k(t_j); j \in [1, m]\}$ are obtained as follows: $\theta_k(t_j) = \theta_{k,i}$ for i satisfying the condition $\tau_{k,i} \leq t_j < \tau_{k,i+1}$
103 (see Supplementary Figure S1 for some examples). For each t_j , inference of the derived parameters $\theta(t_j)$
104 were obtained following standard ABC procedures as described elsewhere (e.g. Beaumont et al., 2002).
105 Median and 95% highest posterior density (HPD) intervals of derived parameters $\theta(t_j)$ were used to draw
106 ABC skyline plots.

107 Simulations with different numbers of population size changes can be used for inference because of
108 the use of derived parameters $\theta(t_j)$, which are common to all models. We set the prior probability on
109 the number of constant size periods to be Poisson distributed with $\lambda = \ln(2)$ as in Heled and Drummond
110 (2008). This gives equal prior probability to stable populations (a single period of constant size) and
111 changing populations (two or more periods). Thus, posterior probability on the number of periods may
112 be used to discriminate between stable and changing demographics by estimating the Bayes factor of
113 one period (constant population size) *versus* several demographic periods (variable population size).
114 Posterior probabilities of contrasting models can be obtained by logistic regression as described elsewhere
115 (Beaumont, 2008).

116 We implemented this approach in a suite of R scripts (R Core Team, 2017) that we named DIYABCsky-
117 lineplot (Navascués, 2017). For each simulation the number of population size changes is sampled using
118 the prior probabilities. Via command line version of DIYABC (v2.0, Cornuet et al., 2014), parameter val-
119 ues, $\{(\theta_{k,i}, \tau_{k,i}); i \in [0, n_k]\}$, are sampled from the prior distribution, coalescent simulations are performed
120 and summary statistics are calculated [mean across loci of the number of alleles, N_a ; heterozygosity, H_e ;
121 variance of allele size, V_a , and Garza and Williamson (2001) statistic, M]. In addition, the *Bottleneck*
122 statistic (ΔH ; Cornuet and Luikart, 1996), which compares the expected heterozygosity given the allele
123 frequencies with the expected heterozygosity given the observed number of alleles, is calculated in R
124 from the summary statistics provided by DIYABC. Derived parameter values, $\{\theta_k(t_j); j \in [1, m]\}$, are
125 calculated from the reference table (i.e. table of original parameters and summary statistics values for all
126 simulations) produced by DIYABC and their posterior probability distributions are estimated in R using
127 the *abc* package (Csilléry et al., 2012).

128 Simulations

129 The method described above was evaluated on simulated data (pseudo observed data-set, POD) of
130 contracting and expanding populations. Declining populations had a present effective size of $N_0 = 100$
131 diploid individuals that changed exponentially until time T , which had a value of 10, 50, 100 or 500
132 generations in the past, reaching an ancestral population sizes of N_A , which had a value of 1000, 10 000
133 or 100 000 individuals. Expanding populations had a present population size of N_0 with a value of 1000,
134 10 000 or 100 000 diploid individuals, which changed exponentially until reaching the size of the ancestral
135 population $N_A = 100$ at time T , which had a value of 10, 50, 100 or 500 generations in the past. For
136 times older than T , the population size is constant at N_A for all scenarios. In addition, we simulated
137 three constant population size scenarios with N taking a value of 1000, 10 000 or 100 000. Equivalent
138 scenarios were also evaluated in Girod et al. (2011) and Leblois et al. (2014). PODs were generated for
139 50 individuals genotyped at 30 microsatellite loci evolving under a generalised stepwise mutation model
140 (GSM, Slatkin, 1995). Additional PODs varying in number of loci (7, 15 or 60 loci) and sample size
141 (6, 12, 25 or 100 diploid individuals) were produced to evaluate the influence of the amount of data in
142 the detection of demographic change. Mutation rate was set to $\mu = 10^{-3}$ and P_{GSM} to 0, 0.22 or 0.74
143 (P_{GSM} is the parameter of a geometric distribution determining the mutation size in number of repeats).
144 One hundred replicates (i.e. PODs) were run for each scenario. Therefore, the mutation scaled parameter
145 values are for $\theta = 4N\mu$: 0.4, 4, 40 or 400 and for $\tau = T\mu$: 0.01, 0.05, 0.1 or 0.5. PODs were obtained
146 using the coalescent simulator fastsimcoal (Excoffier and Foll, 2011).

147 Every POD was analysed with the same set of prior probability distributions that largely includes
148 all parameter values of simulations. Scaled effective size parameters, θ_i , were taken from a log-uniform
149 distribution in the range $(10^{-3}, 10^4)$ and scaled times, τ_i , from a log-uniform distribution in the range
150 $(2.5 \times 10^{-4}, 4)$. A uniform prior in the range (0,1) was used for mutational parameter P_{GSM} . For each
151 replicate of each scenario, we obtained the skyline plot (median and 95%HPD intervals of the $\theta(t_j)$
152 posterior distributions) and estimated the Bayes factor between constant size and variable demography
153 by using logistic regression. Estimates of the mutational parameter P_{GSM} were also obtained for each
154 POD. For each scenario, mean absolute error, bias and proportion of times the true value falls outside the

155 credibility interval were estimated.

156 Data sets

157 In addition to PODs, four real data-sets from the literature were re-analysed with the ABC skyline plot
158 described above. The first data-set comes from the whale shark (*Rhincodon typus*), the largest extant fish.
159 Whale sharks inhabit all tropical and warm temperate seas and are considered an endangered species with
160 a global population decline of more than 50% in the last three generations (Pierce and Norman, 2016).
161 We have re-analysed a data-set of 478 individuals genotyped at 14 microsatellite loci from Vignaud et al.
162 (2014b). The second example is the leatherback turtle (*Dermochelys coriacea*), the most widely distributed
163 sea turtle found from tropical to sub-polar waters. The global population has been reduced in about
164 40% in the last three generations. As species, the leatherback turtle is classified as vulnerable, mainly
165 because of the Northwest Atlantic population that shows an increase in number of nests. However, other
166 populations are critically endangered (Wallace et al., 2013). The data-set re-analysed (215 individuals
167 genotypes at 10 microsatellite loci; Molfetti et al., 2013) comes from the Northwest Atlantic population.
168 Last, we re-analysed the data from the populations of two colobus monkeys at the Cantanhez National
169 Park in Guinea Bissau (Minhós et al., 2016). The Western black-and-white colobus (*Colobus polykomos*,
170 22 individuals genotypes at 14 loci) and the Temminck's red colobus (*Procolobus badius* ssp. *temminckii*,
171 23 individuals genotyped at 13 loci) are two sympatric species from the Western African rainforest
172 considered to be vulnerable and endangered respectively (Oates et al., 2008; Galat-Luong et al., 2016).
173 Data were analysed with the same prior distributions as PODs except for the colobus monkeys datasets,
174 which consist of tetranucleotide markers. Previous evidence suggests that tetranucleotide microsatellite
175 mutations are mainly of only one repeat unit (e.g. Leopoldino and Pena, 2003; Sun et al., 2012). In order
176 to incorporate this prior knowledge, half of the simulations had $P_{GSM} = 0$ (i.e. a strict stepwise mutation
177 model, SMM) and the other half had the parameter sampled from a uniform distribution in the range (0,1).

178 For comparison, demographic history of the four real data sets was also explored using the MIGRAINE
179 software (Rousset and Leblois, 2016, <http://kimura.univ-montp2.fr/~rousset/Migraine.htm>) under the
180 model of a single panmictic population with an exponential change in population size. To infer model
181 parameters, MIGRAINE uses coalescence-based importance sampling algorithms under a maximum
182 likelihood framework (Leblois et al., 2014) using OnePopVarSize model. In this model, MIGRAINE
183 estimates present and ancestral scaled population sizes ($\theta_0 = 4N_0\mu$ and $\theta_A = 4N_A\mu$) and the scaled time of
184 occurrence of the past change in population size ($D = T/4N$, going backward from sampling time, when
185 the population size change began). The past change in population size is deterministic and modelled using
186 an exponential growth or decline that starts at time D . Before time D , scaled population size is stable and
187 equal to θ_A . MIGRAINE allows departure from the strict SMM by using a GSM with parameter P_{GSM} for
188 the geometric distribution of mutation sizes. Finally, detection of significant past change in population size
189 is based on the ratio of population size ($\theta_{ratio} = \theta_0/\theta_A$). $\theta_{ratio} > 1$ corresponds to a population expansion
190 and $\theta_{ratio} < 1$ to a bottleneck. If no significant demographic change is obtained, MIGRAINE is run again
191 under a model of stable demography (a single value of θ) for parameter estimation. For the whale shark
192 data set, MIGRAINE analysis was already done in Vignaud et al. (2014b). For the leatherback turtle,
193 MIGRAINE was run using 20 000 trees, 200 points at each iteration and a total of 16 iterations. For the
194 colobus monkeys, we considered 2 000 trees, 400 points at each iteration and a total of 8 iterations.

195 RESULTS

196 Simulations

197 The general behavior of the method can be described from three example scenarios (contraction with
198 $\theta_0 = 0.4$, $\theta_1 = 40$, $\tau = 0.1$, expansion with $\theta_0 = 40$, $\theta_1 = 0.4$, $\tau = 0.1$ and constant size with $\theta = 40$;
199 mutational model with $P_{GSM} = 0.22$). These examples correspond to intermediate parameter values.
200 Results for all simulations are available in the supplementary material.

201 The main output of the analysis is the graphical representation (i.e. the skyline plot) of the inferred
202 demographic trajectory. It consists of a plot with three curves, representing the point estimates (median)
203 and 95%HPD intervals of θ through time. Skyline plots obtained from PODs are congruent with the true
204 underlying demography simulated (Figure 1), except in the less favorable scenarios with very recent or
205 very small changes in population size (Supplementary Figures S2–S8). Although the trajectory of the
206 posterior median of θ and the true trajectory share the same trend (declining, increasing or constant), they
207 sometimes differ in magnitude or time-scale. This disparity is more prominent for bottleneck scenarios.

208 For a quantitative criterion to assert demographic change we explored the value of posterior proba-
209 bilities for constant and variable population size models, similar to the scheme proposed by Heled and
210 Drummond (2008). These probabilities (summarised as Bayes factors in Figure 2) proved to be useful
211 for distinguishing bottleneck and expansion scenarios from demographic stability, although with lower
212 performance for less favorable scenarios (Supplementary Figures S9–S15). Constant size scenarios show
213 no evidence for size change. The power to detect demographic change reduces with smaller sample size
214 and lower number of loci (Figure 2) because summary statistics are estimated with lower precision.

215 Changes in population size were co-estimated with the mutational model parameter P_{GSM} . Mean
216 absolute error, bias and proportion of replicates for which the true value was outside the 95%HPD interval
217 are reported in Table 1 for the three example scenarios and in Table S1 for all simulations. Estimates
218 from expanding and stable populations show a relatively low error and bias and a good coverage of the
219 credibility interval (except in the strict SMM case). However, estimates from declining populations show
220 higher error and bias.

221 Real Data

222 The ABC analyses show evidence of population expansion for the whale shark (BF=59.62) and the
223 leatherback turtle (BF=16.65); no evidence for population size changes in the black-and-white colobus
224 (BF=0.58) and some evidence for a bottleneck in the red colobus (BF=2.63). Respective skyline plots
225 reflect such trends (Figure 3). Results from MIGRAINE support the same trends, with θ_{ratio} significantly
226 larger than one for the whale shark and the leatherback turtle, significantly smaller than one for the
227 red colobus and not significantly different from one for the black-and-white colobus (Supplementary
228 Table S3). Scaled population size estimates through time are also in agreement, except for the leatherback
229 turtle, where the MIGRAINE result suggests a more ancestral expansion of much greater magnitude.

230 Regarding the mutational model, a large proportion of multi-step mutations seems to be present in
231 all datasets, with P_{GSM} estimates: $\hat{P}_{GSM} = 0.55$ (95%HPD=0.46–0.62) for the whale shark; $\hat{P}_{GSM} = 0.50$
232 (95%HPD=0.38–0.60) for the leatherback turtle; $\hat{P}_{GSM} = 0.43$ (95%HPD= 4.05×10^{-3} –0.53) for the black-
233 and-white colobus; and $\hat{P}_{GSM} = 0.18$ (95%HPD=0.02–0.75) for the red colobus (see also Supplementary
234 Figure S16). Although very small values of P_{GSM} are included in the credibility interval from the
235 colobus analyses, the GSM is favoured over the SMM when an ABC model choice analysis is performed
236 (BF=57.50 for the black-and-white colobus and BF=10.01 for the red colobus). These results are congruent
237 with estimates of P_{GSM} by MIGRAINE (Supplementary Table S3).

238 DISCUSSION

239 The ability of the ABC skyline plot to detect changes in population size varies largely across the different
240 scenarios evaluated. The evidence for demographic change was often strong (even very strong) in
241 declining and expanding populations. However, demographic changes of small magnitude and close
242 to the present were the hardest to detect. Recent or small magnitude events leave a weak signal in the
243 genetic data and are also hard to identify for alternative methods (see Girod et al., 2011; Leblois et al.,
244 2014; Nikolic and Chevalet, 2014). In any case, the method is conservative, since most analyses of stable
245 populations yielded negative or little evidence for demographic change.

246 The main appeal of skyline plots is to depict demographic trajectories not bounded by a mathematical
247 function; thus, potentially reflecting more realistically the demography of natural populations. However,
248 our results show that plotted trajectories only loosely reflect the true demography, particularly for
249 contracting populations. The match between the true and inferred demographic trajectory was good for
250 constant size populations and for some expanding populations. Ancestral and current population sizes
251 (the extremes of the skyline plot) were also retrieved accurately for favourable scenarios. Nevertheless,
252 the shape of the curve representing the transition between population sizes was a poor representation of
253 the true demographic trajectory in many cases. While this conclusion is specific for the implementation
254 presented in this work, it calls to caution for the interpretation of results from other methods yielding
255 smooth skyline plots (e.g. Heled and Drummond, 2008; Gill et al., 2013; Nikolic and Chevalet, 2014).
256 The key for a smooth skyline plot is the prior on the effective-size autocorrelation through time. The
257 demographic history consists of several demographic periods. Within each period the effective size at
258 consecutive generations is correlated through some mathematical function (often a constant). Between
259 consecutive periods, population size can be independent (our approach) or correlated by different sets of
260 priors. Drummond et al. (2005) proposed using an exponential prior for the effective size (θ_i) at period i

261 with mean equal to the previous period effective size (θ_{i-1}). In the Bayesian skyride and skygrid (Minin
262 et al., 2008; Gill et al., 2013) the correlation of effective size through time is modelled with a Gaussian
263 Markov random field that penalizes differences in effective size across periods in function of the temporal
264 distance among them. A superficial comparison with the VarEff method (Nikolic and Chevalet, 2014) and
265 the extended Bayesian skyline plot (Heled and Drummond, 2008) seems to indicate that their inferences
266 suffer from problems of performance (see Supplementary Figure S19).

267 Bottlenecked populations, which show the greatest discrepancy between the skyline plot and the
268 true demographic curve, are also the scenarios for which the mutational parameter P_{GSM} was inferred
269 with largest bias. Similar patterns of summary statistics are produced with large P_{GSM} values and with
270 a bottleneck (e.g. large allele size variance, see Supplementary Table S2), which make accurate joint
271 inference of demography and mutational models difficult. This difficulty of distinguishing between
272 scenarios with frequent multi-step mutations and contracting populations also explains the low power
273 to detect some bottleneck cases, such as those with large P_{GSM} values and strong declines in population
274 size (see Supplementary Figures S11). A negative effect on demographic inference due to mutational
275 model misspecification has been also reported for alternative methods (see Girod et al., 2011; Leblois
276 et al., 2014; Nikolic and Chevalet, 2014).

277 Globally, our results highlight the interest of using complementary data and inference methods. In the
278 four real-data populations, their demographies have been previously studied in the original publications.
279 In addition to the MIGRAINE analysis of microsatellite data, Vignaud et al. (2014b) inferred a population
280 expansion for the whale shark by using Bayesian skyline plot analysis on mitochondrial DNA sequence
281 data, corroborating the signal of expansion for this species. In the case of the leatherback turtle, the
282 previous analyses were less conclusive (Molfetti et al., 2013). An extended Bayesian skyline plot on
283 microsatellite data suggested an expansion, but it was not significant, and the skyline plot on mitochondrial
284 DNA data did not show any demographic change. In contrast, analysis of microsatellite data with
285 MSVAR (a coalescent sampler approach, Beaumont, 1999; Storz and Beaumont, 2002) suggested a strong
286 population decline. However, it must be noted that MSVAR assumes a strict SMM, which can lead to
287 biases in the demographic estimates when microsatellite mutations include a substantial proportion of
288 multi-step changes (Girod et al., 2011; Faurby and Pertoldi, 2012). Our estimates of the P_{GSM} parameter
289 and the two-phase model used in BEAST suggest a strong departure from the SMM and lead us to favour
290 the hypothesis of population expansion. Finally, the original analysis of the two colobus species found
291 significant evidence of population decline for both of them (Minhós et al., 2016). Again, this evidence
292 was obtained from MSVAR and the extended Bayesian skyline plot implemented in BEAST assuming a
293 SMM. Despite the prior results suggesting that tetranucleotide microsatellite mutations add or remove a
294 single repeat, our analyses (ABC skyline plot and MIGRAINE) rejected the SMM for the black-and-white
295 colobus. This explains the difference between their results and our demographic inference, which supports
296 a constant size for this population.

297 Results from demographic inferences have been reported in the form of the scaled parameters θ and τ
298 throughout this work. This is because rescaling to natural parameters (effective population size in number
299 of individuals and time in number of generations or years) requires independent knowledge of mutation
300 rates, which is unavailable for most species (including our four study cases). If such knowledge exists,
301 a prior can be used in DIYABC to incorporate this information in the analysis and make inferences on
302 natural scale parameters. Otherwise, we advocate reporting coalescent scaled parameters as results of the
303 analysis. This allows the discussion of the result considering different mutation rates and reinterpretation
304 of results if information on mutation rates is obtained in the future for the focal species.

305 A common problem for the inference of population size changes is the presence of population structure
306 or gene flow. Most methods aiming to detect population size change often assume the analysis of a single,
307 independent population, but violation of this assumption usually leads to false detection of bottlenecks
308 (e.g. Heller et al., 2013; Nikolic and Chevalet, 2014, for skyline plot approaches). We expect the same
309 effect in the implementation of the skyline plot analysis we present here. However, distinguishing between
310 population structure and population decline in the ABC framework is possible under some circumstances
311 with the appropriate summary statistics (Peter et al., 2010) that can be included in future implementations
312 of the ABC skyline plot.

313 Indeed, the ease of incorporating new summary statistics and models is of prime interest for imple-
314 menting the skyline plot in the ABC framework. Multiple samples of the same population at different
315 times (as in experimental or monitored populations and ancient DNA studies) can easily be simulated

316 allowing for better estimates of the effective population size (Waples, 1989; Navascués et al., 2010).
317 Models with multiple populations can also be simulated and skyline plots for each of the populations
318 estimated. Extensions to other molecular markers will be straightforward to develop and already exist for
319 genomic data (e.g. Boitard et al., 2016). Finally, other demographic parameters, such as the migration
320 rate (Pool and Nielsen, 2009), could be subject to variation with time and they, too, could be inferred with
321 a similar scheme. To sum up, there is potential to develop this approach in different directions, to address
322 new questions in future research.

323 In this work we presented a detailed description of how to compute an approximate-Bayesian-
324 computation skyline plot and assessed its performance on stable and changing simulated populations
325 characterized with microsatellite markers. Its power to detect the signal of demographic change is similar
326 to alternative methods. However, its potential ability to depict the demography of natural populations
327 more realistically must not be overrated. Still it offers an analysis complementary to other methods and
328 there is great potential to develop it to cover other models and types of genetic data.

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461 DATA ACCESSIBILITY

462 Code for DIYABCskylineplot is available on ZENODO (doi:10.5281/zenodo.267182) and on GitHub
463 (<http://github.com/mnavascues/DIYABCskylineplot>) including code to automatically simulate pseudo-
464 data. Data from whale sharks is available at DRYAD database (Vignaud et al., 2014a).

465 AUTHOR CONTRIBUTIONS

466 MN and CB conceived and designed the work. MN developed the code. MN, RL and CB analysed the
467 data and discussed the results. MN and RL wrote the article. All authors read and approved the final
468 manuscript.

469 TABLES AND FIGURES

Table 1. Estimation of mutational parameter P_{GSM}

model	θ_0	θ_1	τ	P_{GSM}	MAE	bias	out of CI
contraction	0.4	40	0.1	0.22	0.14	0.13	0.01
expansion	40	0.4	0.1	0.22	0.05	-0.04	0.05
constant size	40			0.22	0.06	-0.03	0.00

MAE: mean absolute error; out of CI: proportion outside credibility interval (95%HPD). Estimates from 100 replicates.

Figure 1. ABC Skyline plots: simulations. Superimposed skyline plots (median in black, and 95%HPD interval in grey) of the posterior probability distribution for $\theta(t)$ from 100 replicates for example (A) contraction ($\theta_0 = 0.4$, $\theta_1 = 40$, $\tau = 0.1$), (B) expansion ($\theta_0 = 40$, $\theta_1 = 0.4$, $\tau = 0.1$) and (C) constant size ($\theta = 40$) scenarios with mutational model $P_{GSM} = 0.22$. Simulation of 30 loci sampled at 50 diploid individuals. True demography is shown in orange. Note that present is at $\tau = 0$ (left).

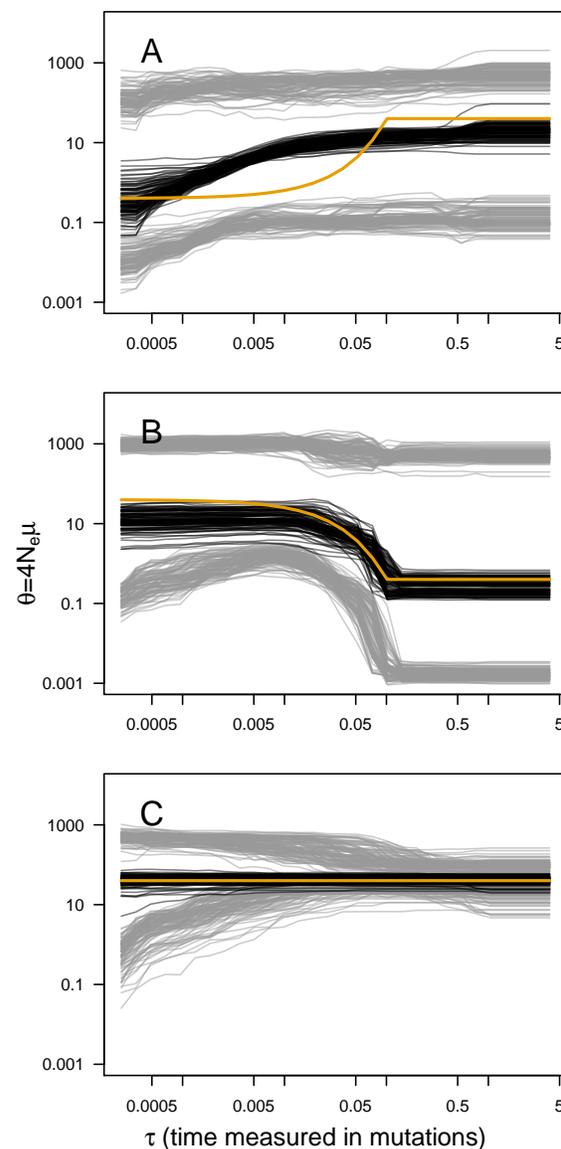


Figure 2. Evidence for variable population size. Distribution of Bayes factor values (boxplot) from 100 replicates for example (A,D) contraction ($\theta_0 = 0.4$, $\theta_1 = 40$, $\tau = 0.1$), (B,E) expansion ($\theta_0 = 40$, $\theta_1 = 0.4$, $\tau = 0.1$) and (C,F) constant size ($\theta = 40$) scenarios with mutational model $P_{GSM} = 0.22$. Different sized data sets (number of individuals and loci) are presented, with simulation of 30 loci (A,B,C) and simulation with 50 diploid individuals (D,E,F). For reference, Jeffreys (1961) scale is given for the evidence against constant size.

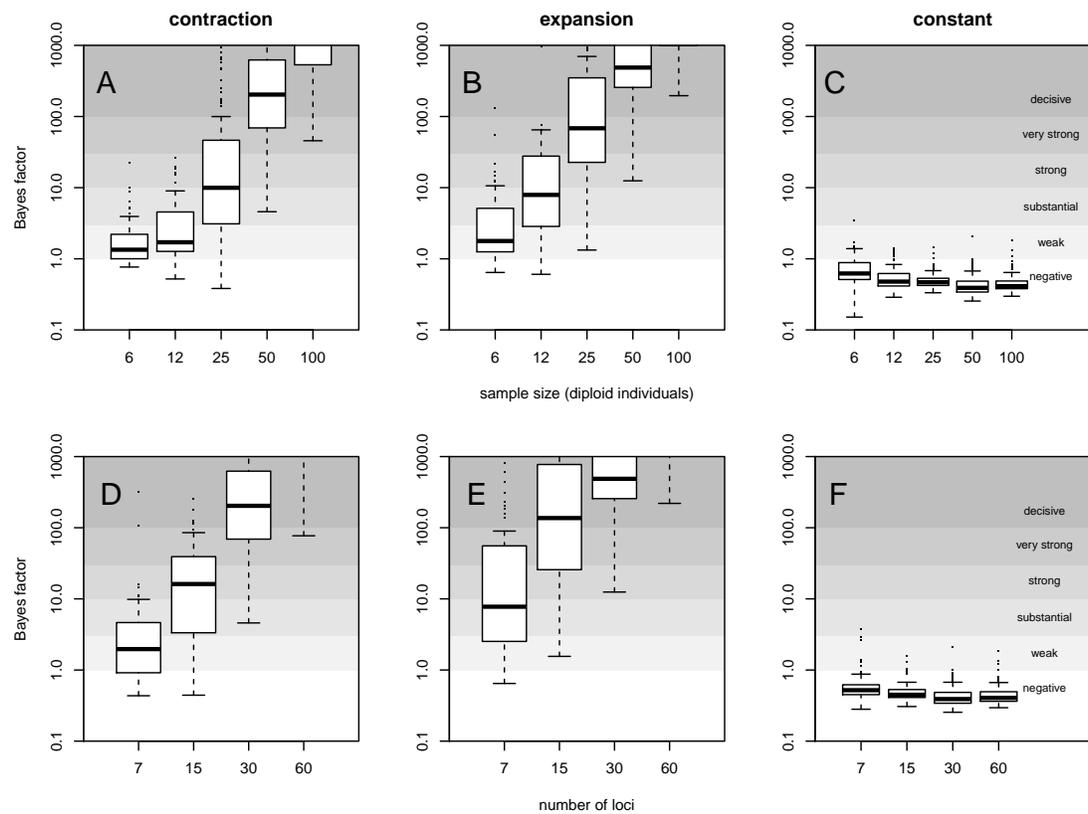


Figure 3. ABC Skyline plots: real data. Skyline plots (median in black, and 95%HPD interval in grey of the posterior probability distribution for $\theta(t)$) for whale shark (A), leatherback turtle (B), Western black-and-white colobus (C) and Temminck's red colobus (D). Bayes Factors (BF) are reported for the variable *versus* constant size model. Demographic trajectories based on parameters point estimates from MIGRAINE analysis are shown with a green line for reference. Note that present is $\tau = 0$ (left).

