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Genetic diversity and population structure of *Saccharomyces cerevisiae* strains isolated from traditional alcoholic beverages of Côte d'Ivoire

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Abstract

In order to assess the genetic diversity and population structure of indigenous *S. cerevisiae* from Côte d'Ivoire, a total of 170 strains were isolated from four traditional alcoholic beverages through nine regions. Microsatellite analysis performed at 12 loci revealed that strains of palm oil and raffia wine were genetically related, unlike those of tchapalo and ron wine which formed two distinct and homogeneous clusters. Furthermore, *S. cerevisiae* isolates of traditional beverages from Côte d'Ivoire appear to be differentiated according to the ecological niche. Indeed, a much higher heterozygosity was observed for isolates from ron wine and tchapalo beer, whereas isolates from palm oil wine and raffia wine were clearly inbred. In comparison with the European, North American, Asian and others West African populations, Ivorian population was well defined, although most of these strains were admixed. Among these strains, only isolates from raffia wine appeared to have alleles in common to all populations.

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Keywords: Microsatellite, population, *Saccharomyces cerevisiae*, diversity, traditional beverage, genotyping.

1 Introduction

Saccharomyces cerevisiae is one of the main actor involved in various biotechnology processes. These last decades, several studies have been focused on genetic diversity and biogeographical distribution of diverse *S. cerevisiae* strains. Molecular techniques used for this purpose among many others are pulsed field gel electrophoresis (PFGE) (Vezinhet *et al.,* 1990), restriction analysis of the mitochondrial DNA (mtDNA-RFLP) (Aigle *et al.,* 1984; Querol *et al.,* 1992), amplified fragment length polymorphism (AFLP) (Azumi and Goto-Yamamoto., 2001), amplification of interdelta regions by PCR (Ness *et al.,* 1993; Legras and Karst 2003) and microsatellite markers (Techera *et al.,* 2001; Legras *et al.,* 2005). Microsatellite analyses have offered significant advances in understanding distribution and dynamics of *S. cerevisiae* population from different terroirs. Thus, Viel *et al.* (2017) have detected specificities of *S. cerevisiae* at vineyard scale from the North-East of Italy, suggesting a geographic differentiation according to the winemaking region with a widespread dissemination of *S. cerevisiae* industrial strains that was very high in the areas where the native strains abundance was low. Likewise, microsatellite analyses on *S. cerevisiae* populations isolated from African traditional fermented foods allowed to highlight the presence of an African phylogenetic branch separate from European (bread, wine, beer, cheese) and Asian strains (sake) (Legras *et al.,* 2007; Ezeronye and Legras, 2009; Tapsoba *et al.,* 2015), which was confirmed with recent genomic data (Liti *et al.,* 2009; Peter *et al.,* 2018). This suggests that African traditional fermented foods could be a reservoir of genetically different strains of *S. cerevisiae* to be explored for applications such as food, bioethanol production, biopreservation, biocontrol, etc. Most of *S. cerevisiae* commonly used in biotechnology have been isolated from natural niches and have undergone various domestication events that have resulted in the selection of strains adapted to human activities (technological strains, etc.). Although several studies have been performed on *S. cerevisiae* isolated from some natural niches (Naumov *et al.,* 1998; Sniegowski *et al.,* 2002; Lorca *et al.,*

2018), it seems important to assess the structure, the genetic diversity and the distribution of native *S. cerevisiae* obtained from African, and Ivoirian niches particularly, which has been very little studied so far. The characterization of these unexplored ecological niches may provide new genotypes with beneficial properties for biotechnological applications. The aim of this work was firstly to assess the genetic diversity of *S. cerevisiae* strains present in traditional beverages from Côte d'Ivoire compared to other African beverages and then characterize the population structure of the isolates of this species. For this purpose, this study focused on the yeast populations isolated from four fermented beverages obtained with different vegetal material. These beverages were tchapalo beer produced from sorghum (*Sorghum bicolor*) and palm oil, raffia and ron wines respectively obtained from *Elaeis guineensis, Raphia hookeri and Borassus aethiopum* sap fermentation.

2 Material and Methods

2.1 Sample collection and yeast isolation

Samples of palm wine (raffia, palm oil and ron wines) were collected during ten sampling campaigns in different regions of Côte d'Ivoire. Sampling areas for raffia wine were located at Grand-Lahou, Alepé, Adzopé and Abengourou; those for palm oil wine were located at Bingerville, Bonoua, Grand-Lahou, Alépé and Attinguié while those for ron wine were located at Toumodi. Sorghum beer or tchapalo samples were collected during four sampling campaigns at Abidjan and Bingerville. These sampling areas were located in south-eastern and middle of Côte d'Ivoire as indicated by the sample map (Figure 1). Distance between the sampling areas and the city of Abidjan as well as their geographical coordinates are shown in the Table 1. Local producers in 26 different sites were contacted and sampling was carried out from September 2014 to January 2015 and from August 2016 to September 2016. Sampling was twice a day (morning and evening) done at weekly intervals, to collect samples of 9 to 15hr fermentation duration. Samples were collected into pre-sterilized 250 ml Plexiglas containers, and immediately immersed in an isothermal box containing dry ice, and brought to the laboratory within 3 h.

Total titratable acidity (TTA), pH, and total soluble sugars (TSS) for each sample were determined as previously described (Tra Bi *et al.,* 2016). For yeast isolation, each beverage sample (1 mL) was directly diluted in tenfold series in buffered peptoned water (BIO-RAD, France) and aliquots (0.1 mL) were plated in duplicate on Yeast Extract Peptone Dextrose (YPD) agar. After incubation at 30°C for 3-5 days, ten yeast colonies were randomly selected from each sample and purified by streaking two times on YPD agar. *Saccharomyces* yeasts

100 were presumptively isolated by cultivation of purified colonies on lysine agar medium
101 (Sigma-Aldrich, France). Thus, a total of 262 isolates were selected for molecular
102 identification and maintained at -80°C in YPD broth containing 20% (v/v) glycerol (Merck,
103 France).
104 Variance analysis (ANOVA) and Tukey HSD tests (Honestly Significant Difference) with R
105 statistical software v3.4.1 (R Core Team, 2017) were used to compare the physicochemical
106 and microbiological characteristics of each beverage taken from the different sampling areas.
107 Differences were considered significant for values of $P < 0.05$.
108
109 **2.2 Molecular identification of *Saccharomyces* species**
110 The species level identity of *Saccharomyces* strains was checked initially by non-transcribed
111 spacer PCR-Restriction Fragment Length Polymorphism (NTS2 PCR-RFLP) analysis using
112 *Alu*I and *Ban*I enzymes in comparison with reference strains. From strains showing *S.*
113 *cerevisiae* NTS2 PCR-RFLP profile (Nguyen and Gaillardin, 1997), thirty representative
114 strains were selected for D1/D2 region sequencing (Kurtzman and Robnett, 1998). The D1/D2
115 region of 26S rRNA gene was amplified with the primer pair NL-1 (5-
116 GCATATCAATAAGCGGAGGAAAAG-3) and NL-4 (5-GGTCCGTGTTTCAAGACGG-3)
117 (Kurtzman and Robnett, 1998). Reactions were performed in an automatic thermal cycler
118 (Gene Amp PCR System 2700, Thermo Fisher Scientific) under the following conditions:
119 initial denaturation at 94°C for 4 min followed by 30 cycles of denaturation at 94°C for 30 s,
120 primer annealing at 54°C for 40 s and DNA extension at 72°C for 1 min 30 s. A final
121 extension was completed at 72°C for 7 min. Amplicons were visualized on 0.8% agarose gel.
122 Finally, 20 µL of PCR products were sequenced on both strands with the primers used for the
123 PCR amplification (Eurofins, Germany). Assembly of both strands sequences was performed
124 using the phred/phrap/consed suite (Gordon *et al.*, 1998). Identification queries were fulfilled
125 by a BLAST search of the National Center for Biotechnology Information database (NCBI,
126 Bethesda, USA) and the YeastIP databases (http://genome.jouy.inra.fr/yeastip/).
127
128 **2.3 Microsatellite analysis**
129 The genomic DNA extraction of isolates was performed following the procedure described
130 by Hoffman and Winston (1987), based on a mechanical grinding of cells in presence of
131 detergent, followed by several steps of protein and RNA removal from a 24 hour yeast culture
132 in YPD broth. Microsatellite loci analysis was performed according to Legras *et al.* (2007).
133 Amplification was performed at 12 loci combined in two multiplex of 6 loci (Table S1 in the

134 supplemental material), and the size of fluorescent amplicons was measured on an ABI3100
135 (Applied Biosystems) capillary electrophoresis device, using Gene Scan HD400Rox size
136 standard (Applied Biosystems). These genotypes were compared to data obtained in previous
137 work on strains from other origins.
138
139 **2.4 Population analysis**
140 The evaluation of relationships between *S. cerevisiae* strains was carried out from the genetic
141 relative dissimilarity distance which reflects the number of alleles differing between two
142 individuals, and calculated with the library POPPR (v2.5.0) under R statistical software v3.4.1
143 (R Core Team, 2017). Trees were obtained from distance matrices with R library ape v5.0,
144 and drawn using R library ggtree v1.10.5 (Yu *et al.,* 2017). In order to assess genetic structure
145 in *S. cerevisiae* populations, two different approaches were used. The first one was Bayesian
146 clustering analysis implemented with InStruct 2.3.4 software (Gao *et al.,* 2007) to identify the
147 most likely number of genetic clusters or 'populations' (denoted by K) and assign individuals
148 to their most likely cluster. In order to deduce ancestry on that set of strains, 8 runs were
149 performed with Instruct for values from K = 2 to 17, and 8 additional runs for values from K
150 = 14 to 16. The most likely K value was identified as the value giving the lowest mean value
151 of Deviance Information Criterion (DIC) of the different runs. The results of the 16 runs of
152 the best value of K were combined with CLUMPAK (Kopelman *et al.,* 2015) according to the
153 LargeK Greedy method. The second was a multivariate method based on discriminant
154 analysis in principal components (DAPC) (Jombart *et al.*, 2010) and implemented with the R
155 Adegenet package v2.1.0 (Jombart, 2008) in R v3.4.1 (R Core Team, 2017). DAPC identified
156 clusters of genetically related organisms by partitioning genetic variability into clusters that
157 maximize between-group and minimize within-group differentiation. This approach was an
158 ideal clustering algorithm for datasets that does not followed Hardy–Weinberg equilibrium.
159 Analysis was performed taking into account alpha score function to choose the optimal
160 number of principal components for the analysis of dataset (López-Uribe *et al.,* 2016) due to
161 the changes of the individual membership probability according to the number of PCA axes
162 retained. Population analyses were performed using the hierfstat v0.04-22 R package (Goudet,
163 2005) for Nei (1973) estimates of effect of subpopulations compared to the total population
164 (Fst). Population differentiation according to sampling area was tested with OBSTRUCT
165 (Gayevskiy *et al.,* 2014). The inbreeding coefficient of an individual relative to the
166 subpopulation (Fis) was estimated with hierfstat. Average selfing rates were estimated as 1 –
167 o, where o the outcrossing rate is o = (1-Fis)/(1+Fis)

168

169 **3 Results**

170 **3.1 Characteristics of beverages**

171 The global characteristics of the four different beverages from which strains were isolated in
172 this study were presented in Table 2. It appeared from this investigation that these four
173 beverages had in general a similar acidic pH and a Total Titratable Acidity (TTA) varying
174 between 0.47 and 1.35. The soluble sugars content varied from one beverage to another with
175 the highest content in tchapalo. The yeast load observed in the Ivorian beverages sampled was
176 generally of the same order. Average values were 1.1×10^{8} CFU/mL in ron wine, 1.7×10^{7}
177 CFU/mL in tchapalo beer, 1.4×10^{7} CFU/mL in palm oil wine and 1.3×10^{7} CFU/mL in raffia
178 wine (Table 2).

179

180 **3.2 Assessment of *Saccharomyces cerevisiae* diversity**

181 A total of 170 isolates from Côte d'Ivoire traditional beverages (Table S2 in the supplemental
182 material) were identified as belonging to the species *S. cerevisiae* by using molecular
183 methods. Indeed, the RFLP of NTS2 PCR products showed 2 bands for *Alu*I (1500 and 200
184 pb) and 3 bands for *Ban*I (660, 450 and 220 pb) typical to *S. cerevisiae* strains (Nguyen and
185 Gaillardin, 1997). Then, the sequencing of the D1/D2 domains of the 26S rDNA confirmed
186 the identity of some strains tested as *S. cerevisiae* with a little sequence divergence of 1 to 4
187 bp for 16 strains and 100% identity for 14 strains (Table S3 in the supplemental material).
188 Sequence divergence of 3 to 4 bp was already observed from *S. cerevisiae* strains isolated
189 from tchapalo (N'Guessan *et al.,* 2011). The 170 isolates derived from four ecological niches
190 sampled at 26 different sites from 9 regions (south-eastern and middle) of Côte d'Ivoire.
191 Among them, 58 have been isolated from ron wine, 64 from raffia wine, 29 from palm oil
192 wine and 19 from tchapalo beer. These isolates were subjected to a microsatellite analysis
193 which allowed to generate genotypic data. In order to compare the populations between the
194 different type of beverages, Fst statistics between isolates from tchapalo beer, palm oil, raffia
195 and ron wines were calculated with these data. Population comparisons indicated significant
196 differences between all populations ($P< 0.01$). Fst data showed weak values ranging from 0-
197 0.107 (Table 3a), indicating a little genetic differentiation between the significantly different
198 beverage isolates. The smallest differentiation was observed between palm oil wine and raffia
199 wine strains, and in contrast, tchapalo was the most differentiated population from other
200 populations. Ron wine population presented a moderate differentiation from tchapalo and
201 raffia wine.

202 Impact of geographical origin on *S. cerevisiae* diversity was studied for isolates from palm oil
203 wine, raffia wine and tchapalo beer. Ron wine with only one sampling area was not
204 considered for this study. The Nei pairwise Fst distances matrix for the two sampling areas of
205 tchapalo beer (Abidjan and Bingerville) showed low Fst values and non-significant
206 differentiation. In contrast the four sampling areas of raffia wine (Grand-Lahou, Alépé,
207 Adzopé and Abengourou) and most of the five sampling areas of palm oil wine showed low
208 Fst values and significant differentiation between sites (Table 3). Furthermore, pairwise Fst
209 statistic values for all sampling areas demonstrated no correlation to geographical distance,
210 suggesting that diversity of *S. cerevisiae* was not related to geographical origin. Although
211 isolates of palm oil and raffia wines were genetically related, *S. cerevisiae* isolates from Côte
212 d'Ivoire traditional beverages appeared to be differentiated according to the ecological niche.
213 The Fis values evaluated for each population were 0.603 and 0.544, respectively, for
214 individuals from palm oil wine and raffia wine, 0.234 and 0.138 for individuals from ron wine
215 and tchapalo beer that contained strains not corresponding to one specific fermentation (Table
216 4). Such high values of Fis which indicate a low outcrossing rate (and a high mean inbreeding
217 rate) as well as the high observed homozygosity of strains isolated from palm oil wine and
218 raffia wine (26 and 25 % of observed sites are heterozygotes), suggest that palm oil wine and
219 raffia wine are inbred. In contrast, the low Fis values which indicate a high outcrossing rate
220 (and a low mean selfing rate) as well as the high observed heterozygosity (52 and 76 % of
221 observed sites are heterozygotes) for isolates from ron wine and tchapalo beer suggest more
222 outbred populations.
223
224 **3.3 Genetic relationship among strains**
225 The phylogenetic relationships among Ivorian strains of *S. cerevisiae* were highlighted by a
226 phylogenetic tree (Figure 2a). This dendrogram showed that strains of tchapalo (in green) and
227 ron wine (in blue) formed two distinct and homogeneous clusters, unlike those of palm oil (in
228 brown) and raffia wines (in red). Strains of the latter two groups appeared mixed in the
229 remaining clusters.
230 To further detect the specificities of these Ivorian *S. cerevisiae* populations in comparison to
231 populations from other African countries, and in comparison to well characterized populations
232 such as those from wine, sake, and bread, we built a tree including the 170 Ivorian strains and
233 214 strains from multiple African countries or other origins (Figure 2b) analysed in a previous
234 work (Tapsoba *et al.,* 2015). This consensus neighbour-joining tree was obtained from a
235 pairwise individual distance matrix calculated with relative dissimilarity distance. It showed

236 several clusters of different genetic origin closely related to geographic origin. Thus, there
237 was a cluster formed from European strains: wine and bread strains. Five clusters of strains
238 originated from African substrates, one of which consists of tchapalo and millet beer
239 (different shades of green) and the four others of different types of palm wine. The last two
240 clusters encompassed Asian and North American strains. Ivorian strains are mainly
241 encountered as sub groups inside the main clusters. In contrast to sorghum beer strains, ron
242 wine strains from Côte d'Ivoire are found in two main clusters, one close to Burkina Faso ron
243 wine strains, and in a second different cluster. A similar situation can be observed for raffia
244 wine strains: one group of strains is detected close to Nigerian raffia wine strains, whereas the
245 second group is different.
246
247 **3.4 Population structure**
248 Population structure was inferred with the Bayesian clustering software InStruct which does
249 not assume Hardy-Weinberg equilibrium, but instead assigns individuals proportional
250 membership coefficients in different populations based on inbreeding rates, which is well
251 suited for *S. cerevisiae* population analysis (Gao *et al.,* 2007; Martiniuk *et al.,* 2016).
252 Deviance information criterion indicated the most likely ancestral population number to be K
253 = 15.
254 Each population was labelled by the geographic location and/or substrate from which the
255 strains were isolated (Figure 3). The 15 populations included 11 African beverage populations
256 among them, five originated from Côte d'Ivoire, two from European wine and bread
257 populations, one from the Asian sake population and one from the North American oak
258 population. The InStruct inferences revealed that most strains were assigned to a single
259 ancestry cluster although admixtures were observed. About 8 out of 18 clusters were
260 composed of individuals with one ancestral cluster membership coefficients higher than 0.80.
261 The 10 other clusters (bread, sake from Japan, oak from North-America, laboratory, Bertram
262 palm from Malaysia, various origins from Africa, *Hyphaene thebaica* from Djibouti, raffia,
263 palm oil and ron wines from Côte d'Ivoire) appeared as admixtures. A small number of
264 individuals within ron (*Borassus aethiopum*) wine strains had alleles common with those of
265 ron strains from Burkina Faso, raffia and palm oil wines from Côte d'Ivoire. Strains of palm
266 oil wine have some alleles in common with strains from Djibouti, Ghana (bili), Burkina Faso
267 (dolo), other strains from Côte d'Ivoire (tchapalo and millet beer) and European wine strains.
268 Strains of raffia wine appeared to have alleles in common with all populations, but in small
269 proportion (Table S2 in the supplemental material).

In order to improve the detection of population structure of this study, a discriminant principal component analysis (Jombart *et al.,* 2010) based on 80 % of the global variation obtained from the 50 first PCA axis was conducted (Figure 4). The first two axes enabled differentiation of sorghum and millet beers strains (different shades of pink in the lower part of Figure 4a) from Burkina Faso ron wines strains (shade of brown), Côte d'Ivoire (coral) and miscellaneous African strains (magenta) in the upper part of Figure 4a. Genetic differentiation between African strains following some of their ecological niche was observed in the second axis: strains from the sap of *Arecaceae* family plants of the (palm oil palm, raffia palm, *Borassus* palm…) in the upper quadrant of Figure 4a were distinguished from those *Poaceae* family (millet and sorghum) in the lower part. The cluster strains corresponding to Ivorian tchapalo beer isolates were colocalized with Burkina Faso dolo beer isolates and Côte d'Ivoire millet beer strains. As for axes 3 and 4 (Figure 4b), they highlighted the differentiation between North-America populations and Ghana cocoa strains from that of the rest of the world, and the axis 4 differentiate Nigeria palm strains (and to a lower extent sake strains) from other strains. The two plots (Figure 4) showed that palm oil, raffia and ron wines from Côte d'Ivoire were also clustered but these clusters overlapped with clusters strains from Djibouti palm, Nigeria raffia wine, Burkina Faso ron wine and African miscellaneous origins. This clustering was also in agreement with the clustering obtained at K = 15 for InStruct.

Obstruct tests were performed to evaluate if the population structure revealed in Bayesian ancestry profiles obtained from Instruct was correlated with the geography and confirmed of Nei pairwise Fst distance. For this analyse, only palm oil and raffia wines isolates, which had more than two sampling areas were considered. Obstruct analysis and canonical discriminant analysis clearly showed that the differences between isolates for most sampling areas of palm oil wine (Attinguié, Grand-Lahou, Alépé, Bingerville and Bonoua) were significant (Figure 5, Table S4a in the supplemental material). Strains isolated from Bonoua and Grand-Lahou were very different from the others. For strains from sampling areas of raffia wine, the differences between populations were also significant with the isolates from Alépé and Grand-Lahou and very different from the others isolates (Figure 5, Table S4b in the supplemental material).

4 Discussion

This study was centered on the genetic diversity and population structure of indigenous *S. cerevisiae* from Cote d'Ivoire, as well as the factors that may explain the structure of the resulting populations. For this purpose, four traditional alcoholic beverages (raffia wine, palm oil wine, ron wine and tchapalo beer) collected in nine regions of Côte d'Ivoire were studied.

304 The pH of these beverages, which fluctuated between 3 and 5, were similar to those reported
305 by several authors in palm wines after the first day of tapping (Amoa-Awua *et al.,* 2007;
306 Karamoko *et al.,* 2012) and in tchapalo beer produced by traditional brewers (Aka *et al.,*
307 2008). These ranges of pH could be a major advantage for the bio-preservation of these
308 beverages by protecting them from the development of some pathogenic flora (Aka *et al.,*
309 2008; Yao *et al.,* 2009). The Total Titratable Acidity (TTA) and Total Soluble Sugars (TSS)
310 contents were variable from one beverage to another, while the yeast average load was around
311 10^7 to 10^8 CFU /mL in the four beverages. The low TSS contents observed in beverages of
312 this study could be related to the high yeast load, but also to the rest of the microbiota of these
313 beverages. TTA rates observed in beverages could be due to the production of a variety of
314 organic acids by yeasts and bacteria (N'guessan *et al.,* 2008; Karamoko *et al.,* 2012). Yeast
315 loads found in this study could probably be due to the sum of various physical, chemical, and
316 biotic factors such as temperature, humidity, presence of nutrients, such as sugars (Santiago-
317 Urbina *et al.,* 2015). Similar yeast loads were reported in traditional fermented beverages
318 produced in Africa (Aka *et al.,* 2008; Stringini *et al.,* 2009; Karamoko *et al.,* 2012). Diversity
319 analysis indicated that ecological niche had a major influence on *S. cerevisiae* population's
320 native of Côte d'Ivoire. Fis values of palm oil and raffia wines strains (0.603 and 0.544)
321 revealed an excess of homozygote individuals which is very likely due to high inbreeding
322 within each population rather than to population differentiation (Albertin *et al.,* 2014).
323 Schuller and Casal (2007) also observed a significant excess of homozygotes in their study on
324 three vineyards located in the northwest of Portugal. These authors explained this fact by
325 asexual reproduction with some cycles of homothallic self-mating (genome renewal).
326 Moreover, these authors proposed as an alternative explanation that a mitotic recombination
327 or gene conversion during asexual reproduction may generate such a low of heterozygosity.
328 The high degree of homozygosity would point to the existence of genetically isolated clonal
329 subpopulations of *S. cerevisiae* strains with distinct genetic constitution. However, the
330 microsatellite loci analyzed here represent only a tiny fraction of the genome and provide an
331 indication of the mean heterozygosity. The heterozygosity detected at these microsatellite loci
332 was found in good agreement with genomic data (i.e. Legras *et al.*, 2007; 2018) but other loci
333 in the genome including those with technological relevance can present at different
334 heterozygosity status.
335 Evaluation of genetic diversity of Ivorian strains only revealed that isolates sharing the same
336 micro-environment would be genetically similar to each other, regardless of their
337 geographical origin, implying that ecological origin is the main factor responsible for the

338 close phylogenetic relationship between *S. cerevisiae* strains. Impact of geographical origin
339 on genetic diversity evaluated with Obstruct showed a difference between most of strains
340 population according to sampling area, which was in accordance with Nei Fst pairwise
341 distance. However these genetic distances remained low. Phylogenetic tree, Instruct and
342 Discriminant Analysis of Principal Components (DAPC), considering only African strains,
343 presented that subgroups appeared to be formed by differentiation according to ecological
344 origin. One of the possible explanations is that at a relative close geographical distance,
345 insects like bees, wasps, and fruit flies, as well as birds, which are known to be vectors for
346 yeasts, could have homogenized these yeast populations. Also, Human activities (sharing
347 seasonal staff and equipment during the harvest and fermentation periods) could influence the
348 yeast population structure and promote dispersal (Börlin *et al.,* 2016). Although strains of
349 palm oil wine, raffia wine and ron wine were isolated in Côte d'Ivoire, they have been
350 associated to the same cluster containing the strains of other African countries which derived
351 also of plant sap from the *Arecaceae* family (*Elaeis guineensis, Raphia hookeri, Borassus*
352 *aethiopum, Borassus akeassii* and *Hyphaene thebaica*). The clustering of strains from
353 different traditional beers, notably tchapalo in Côte d'Ivoire, dolo in Burkina Faso and bili bili
354 in Ghana in the same cluster would reflect the history of the inoculum used. In Côte d'Ivoire,
355 sorghum beer was initially produced by populations living in the North and North-East of the
356 country (Djè *et al.,* 2008; N'guessan *et al.,* 2010). Inocula found in Southern and Eastern Côte
357 d'Ivoire may have been introduced by traditional producers from North and North East of
358 Côte d'Ivoire and those of Burkina Faso, Mali and Ghana. An exception to this distribution of
359 strains according to their geographical origin was the genetic affiliation of strains from Asia
360 (Japanese sake, Malaysian palm wine), and from North America (oak) of some Ivorian
361 strains, a result in agreement with those of Liti et *al*. (2009). Strains from palm oil, raffia
362 wines and tchapalo beer had common alleles with those of European wine, suggesting that
363 these strains could have a hybrid origin as reported by Ezeronye and Legras (2009). This
364 origin could always be explained by the fact that the European colonization of Africa would
365 have led to the introduction of *S. cerevisiae* in the same way, as cachaça strains in Brazil
366 (Barbosa *et al.,* 2018). This particular ecological situation, where African and European wine
367 or bread species are present in the same environment, may have provided the opportunity for
368 cross-breeding (Legras *et al.,* 2007). Our results confirm that the beverage production areas
369 represent a reservoir of indigenous strains with a particular genotypic profile, selected by
370 interactions between yeasts and their environment, as suggested by Martínez *et al.* (2007).

The presence of few European wine and bread strains alleles, in low proportions among Ivorian strains would have provided them with some genes of interest, expressing phenotypic characteristics which could be interesting to exploit in fermentation processes. However, a thorough analysis of the biotechnological properties of these strains will be necessary to confirm this idea.

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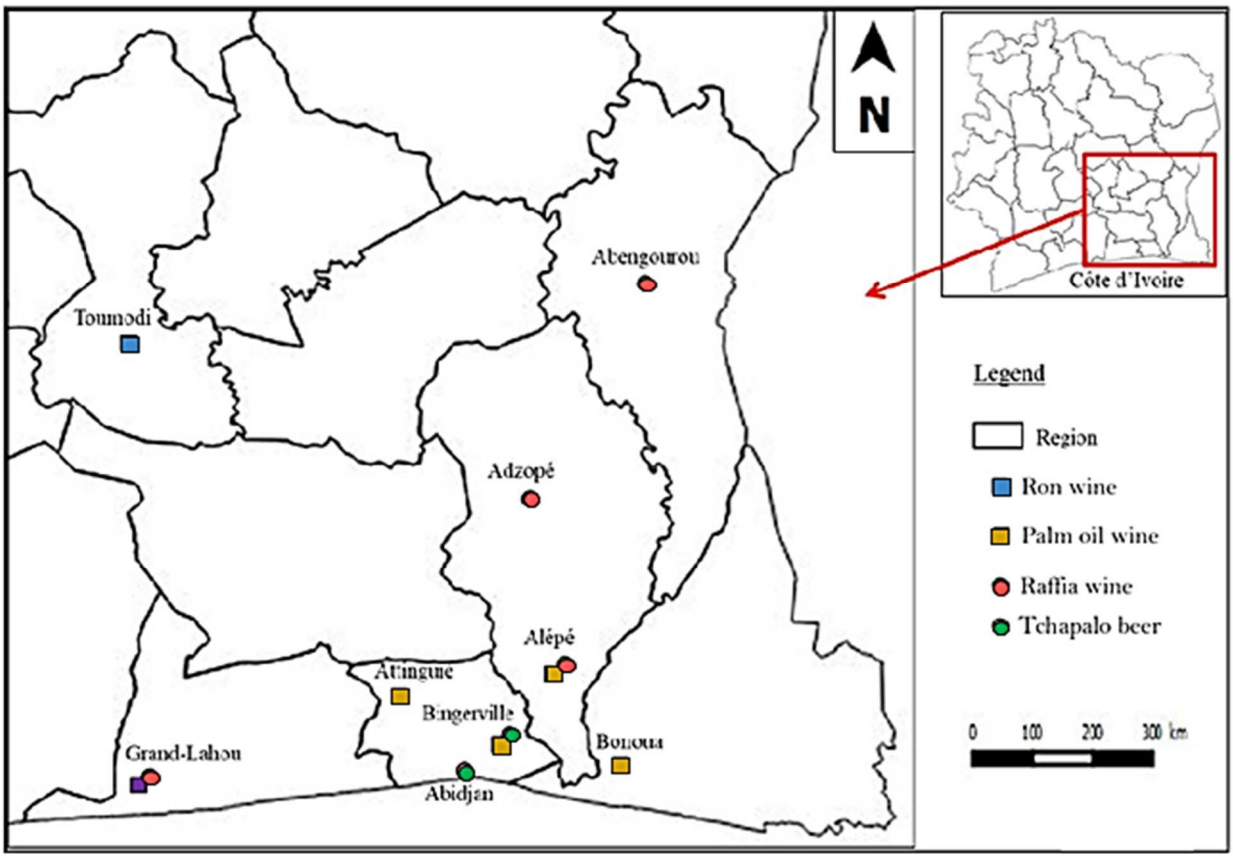


Figure 1: Map of Côte d'Ivoire showing the areas from where samples were collected.

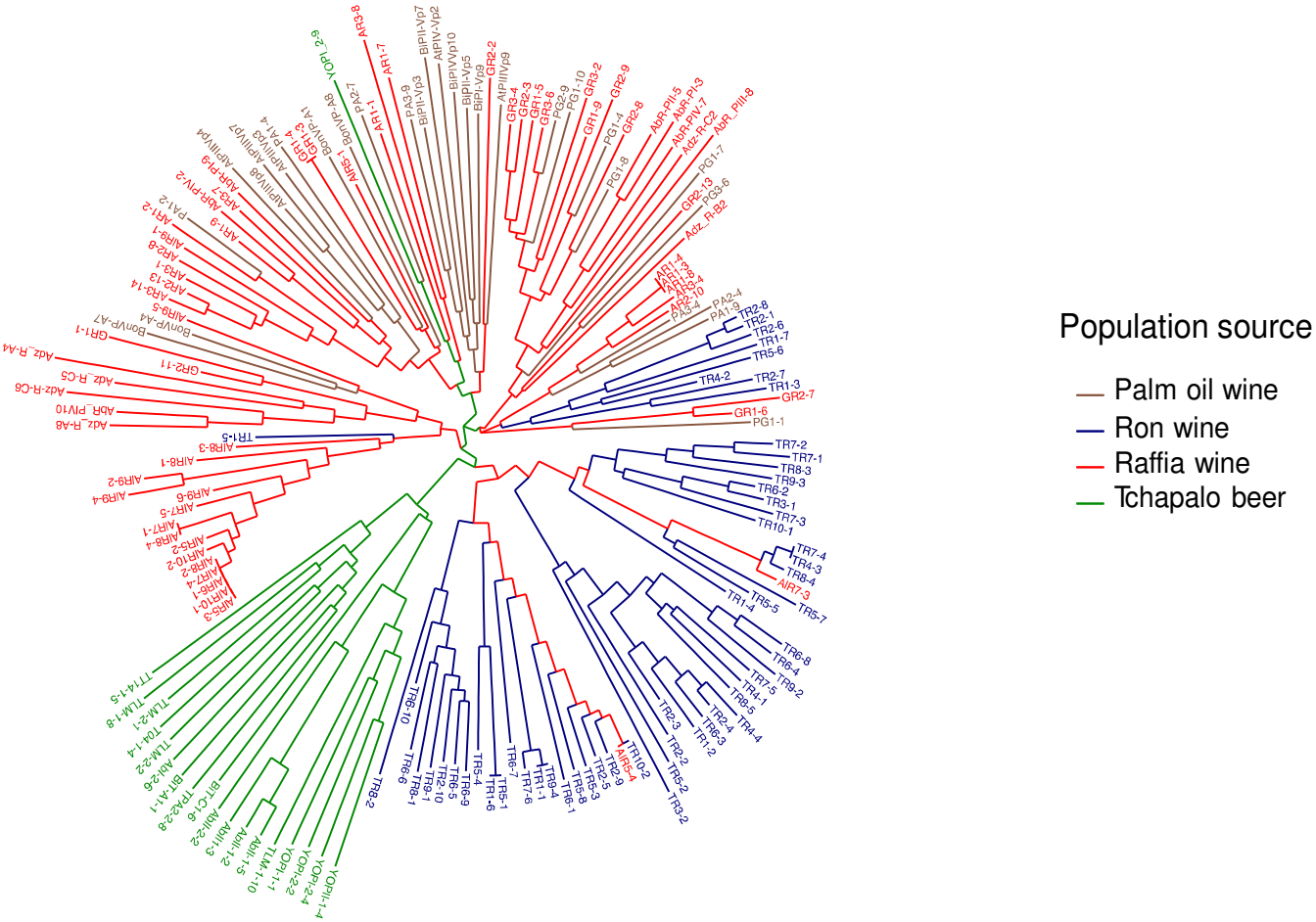


Figure 2a: Phylogenetic tree among Ivorian *S. cerevisiae* strains. The tree is drawn according to neighbour-joining method from a pairwise individual distance matrix calculated with relative dissimilarity distance between 170 strains and based on the polymorphism at 12 loci.

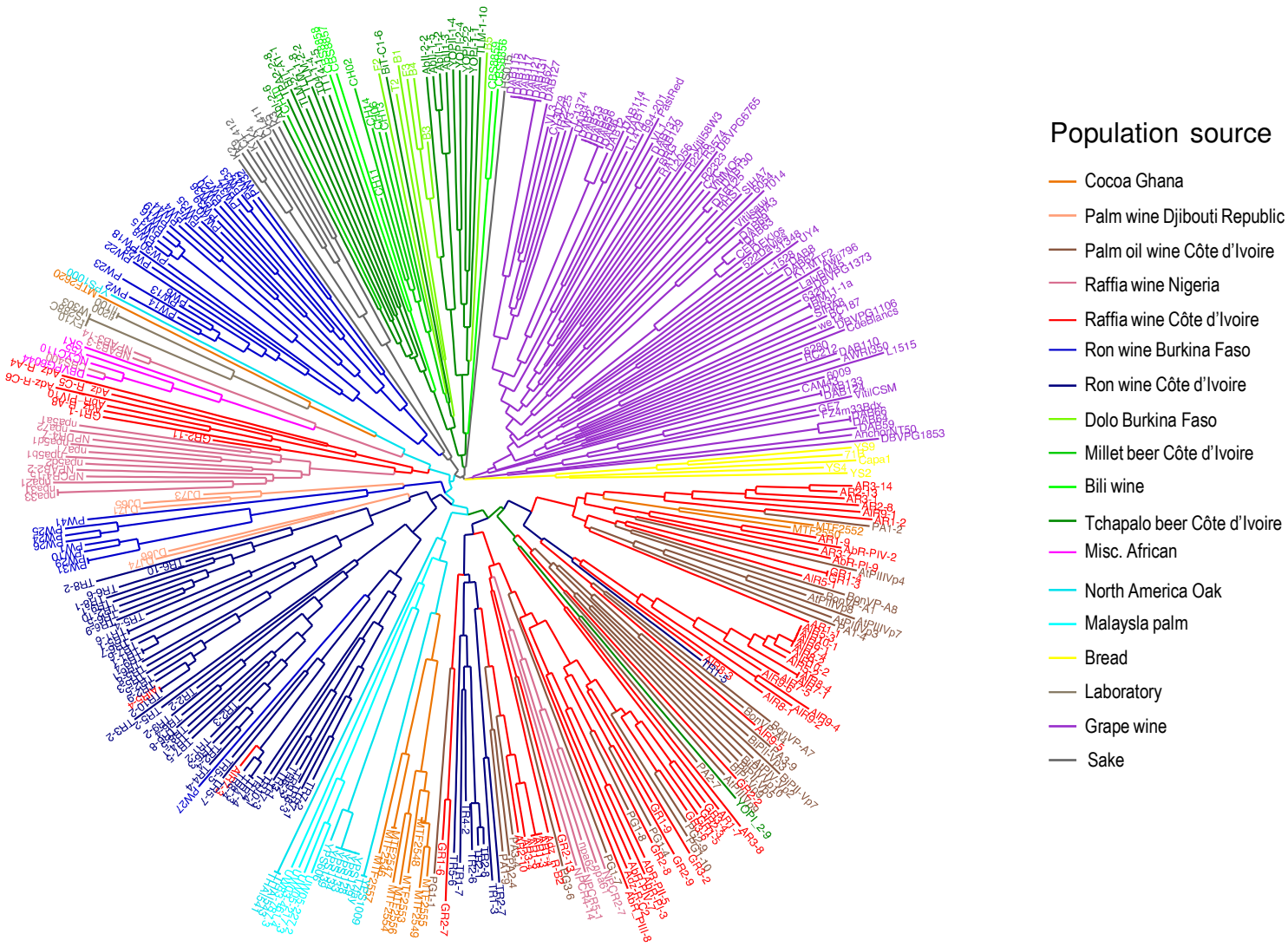


Figure 2b: Phylogenetic tree among *S. cerevisiae* strains of diverse geographical origins and substrates. The tree is drawn according to the neighbour-joining method from a pairwise individual distance matrix calculated with relative dissimilarity distance between the 284 strains (from 18 different ecological niches) and based on the polymorphism at 12 loci.

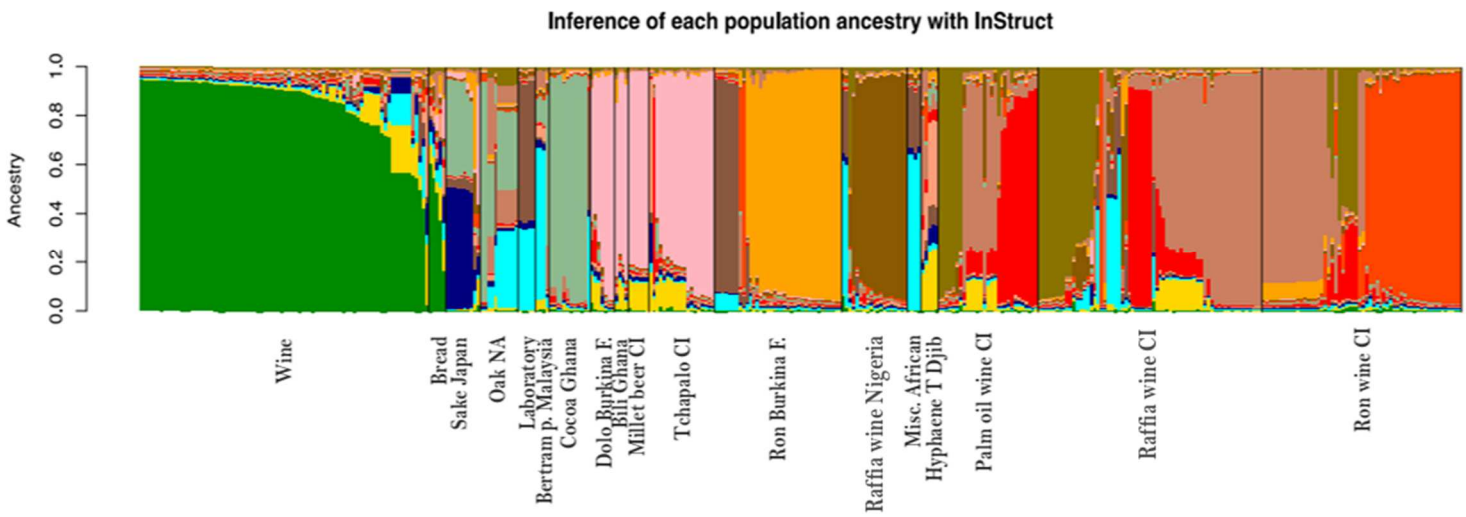
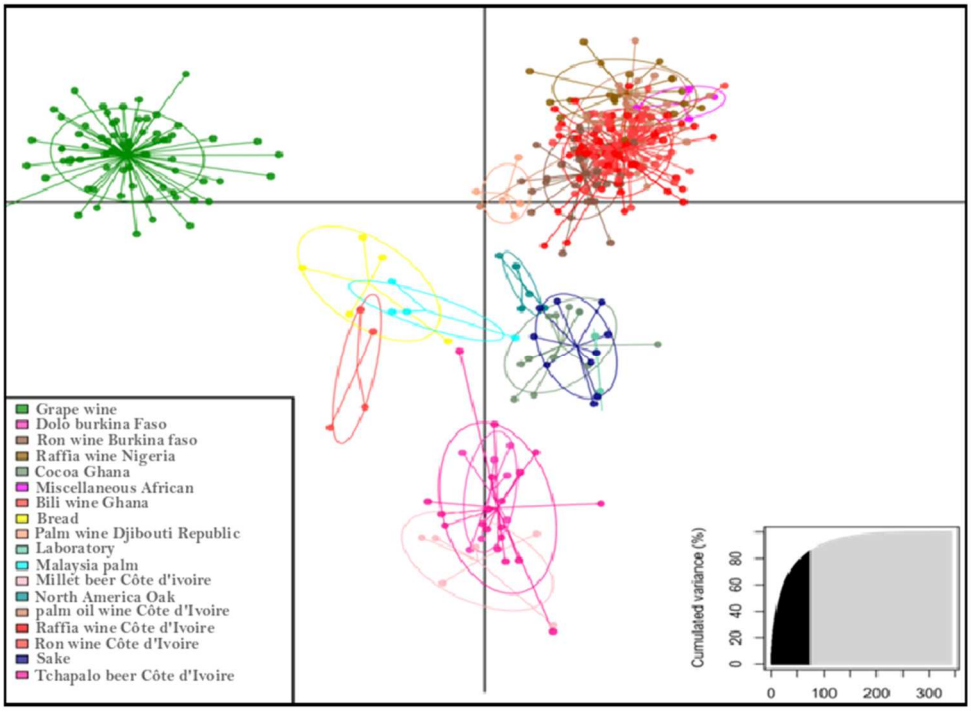


Figure 3: Inferred population structure using InStruct program on the 384 *S. cerevisiae* strains. Deviance information criterion indicated the most likely population number to be K = 15. *S. cerevisiae* populations are delimited vertically according to their ecological origin. Colours represent the different alleles that constitute each population.

(a)



(b)

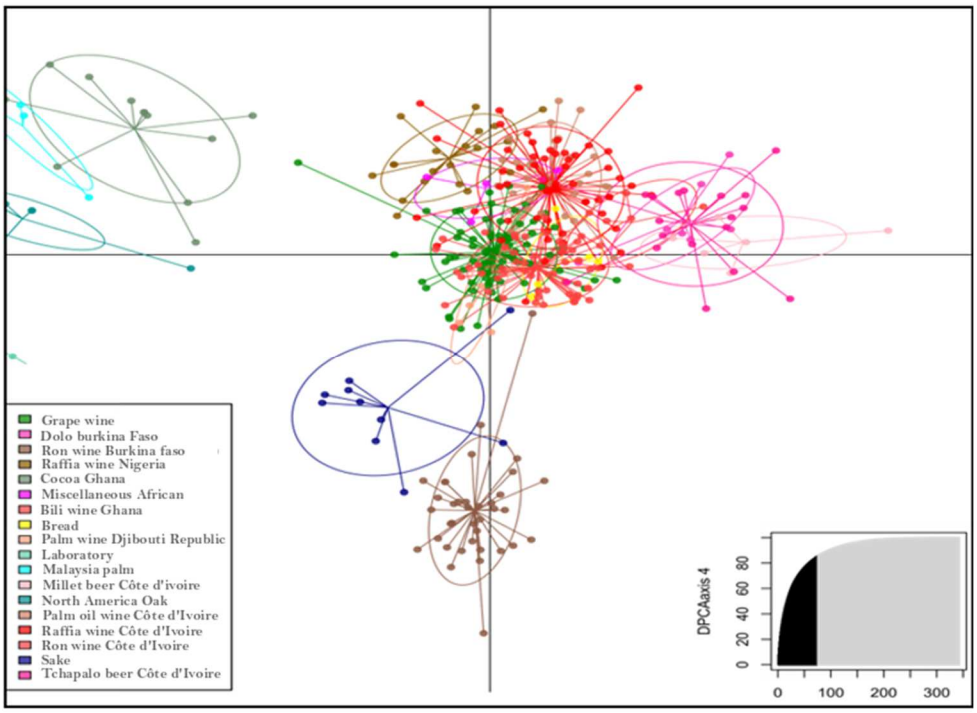
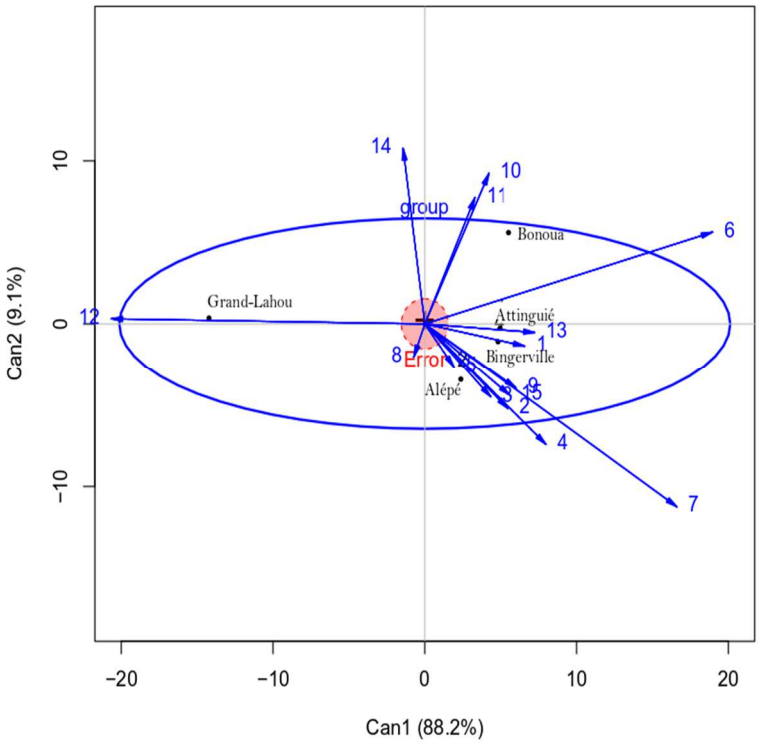


Figure 4: Discriminant principal component analysis (DPCA) (a): Plot showing representation of the different clusters according to axes 1 and 2; (b) plot showing representation of the different clusters according to axes 3 and 4. Clusters are shown by different colours and inertia ellipses, while dots represent individuals.

(a)



(b)

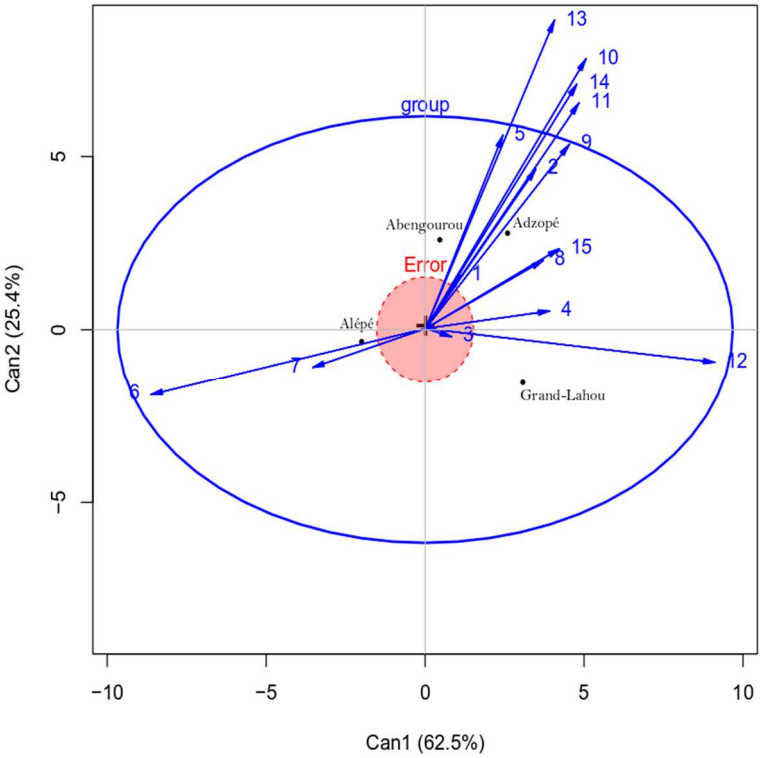


Figure 5: Canonical discriminant analysis of the ancestry obtained for palm oil wine (a) and raffia wine (b) isolates from Côte d'Ivoire with OBSTRUCT, after removal of strains from other countries. The outer blue ellipsoid labelled group reflects the variation of the group means around the grand mean while the red circle reflects the pooled within-group dispersion and covariation. The black points indicate predefined populations while numbers at the arrows indicate inferred populations.

Table 1: Geographical information of the sampling areas and sample codes

Sampling areas	Geographical coordinates	Distance between the sampling area and the city of Abidjan (Km)	Beverage collected	Code
Abidjan	l : 5°18.5796′ N L : 4°0.7596′ W	0	Tchapalo beer	TLM/ Ab/ TT14/ TPA/
Bingerville	l : 5°21.3486′ N L : 3°53.1222′ W	18	Palm oil wine Tchapalo beer	BingerVP Binger-T
Attinguié	l : 5°29′40″ N L : 4°03′06″ W	22	Palm oil wine	AttgiéP-Vp
Alépé	l : 5°29′46″ N L : 3°39′49″ W	45	Palm oil wine Raffia wine	PA AR
Bonoua	l : 5°16.3482′ N L : 3°35.775′ W	59	Palm oil wine	Bon Vp
Adzopé	l : 6°06′24″ N L : 3°51′42″ W	104	Raffia wine	Adz-R
Grand-Lahou	l : 5° 8' 7 N L : 5° 1' 26 ′ W	152	Palm oil wine Raffia wine	PG GR
Toumodi	l : 6°33.4794′ N L : 5°1.0614′ W	198	Ron wine	TR
Abengourou	l : 6°43′46″ N L : 3°29′47″ W	210	Raffia wine	Abeng R

Table 2: Physico-chemical characteristics and yeast count of alcoholic beverages of the study

Beverages		pH	Total titratable acidity (%)	Total soluble sugars (°Brix)	Yeast count (CFU/mL)
Palm oil wine	Average	3.73 ± 0.09*	0.80 ± 0.05*	5.41 ± 0.77*	$1.4 \pm 0.1\times10^{7*}$
	Minimum	3.45 ± 0.04	0.75 ± 0.07	3.72 ± 1.13	$9.7 \pm 0.5\times10^{6}$
	Maximum	4.30 ± 0.19	0.87 ± 0.03	6.65 ± 1.41	$1.7 \pm 0.2\times10^{7}$
Raffia wine	Average	3.80 ± 0.12*	0.81 ± 0.10*	4.35 ± 0.79†	$1.3 \pm 0.2\times10^{7*}$
	Minimum	3.21 ± 0.26	0.47 ± 0.04	3.00 ± 0.30	$9.5 \pm 2.3\times10^{6}$
	Maximum	4.35 ± 0.01	1.35 ± 0.07	7.00 ± 0.40	$4.8 \pm 1.8\times10^{7}$
Ron wine	Average	3.72 ± 0.12*	0.68 ± 0.09†	4.88 ± 0.40†	$1.1 \pm 0.3\times10^{8\dagger}$
	Minimum	3.54 ± 0.08	0.48 ± 0.11	4.03 ± 0.25	$1.9 \pm 0.7\times10^{7}$
	Maximum	3.91 ± 0.21	1.00 ± 0.14	5.80 ± 0.30	$2.6 \pm 0.6\times10^{8}$
Tchapalo beer	Average	3.46 ± 0.13*	0.93 ± 0.03‡	7.70 ± 1.04‡	$1.7 \pm 0.1\times10^{7*}$
	Minimum	3.38 ± 0.1	0.92 ± 0.02	5.81 ± 0.57	$1.2 \pm 0.1\times10^{7}$
	Maximum	3.5 ± 0.14	0.94 ± 0.04	10.87 ± 2.0	$2.4 \pm 0.1\times10^{7}$

The values expressed are the average of three measurements. On the same column, mean values with the same symbol are not significantly different (Tukey HSD test range at $P < 0.05$).

Table 3a: Pairwise Fst distance matrix between populations isolated from different beverages. Fst is calculated according to Nei (1973). Fst values are given in the lower matrix and upper matrix indicates P-value estimated from 1000 permutations of the genotypes dataset.

	Palm oil wine	Raffia wine	Ron wine	Tchapalo beer
Palm oil wine	0.000	0.008	0.001	0.001
Raffia wine	0.021	0.000	0.001	0.001
Ron wine	0.061	0.069	0.000	0.001
Tchapalo beer	0.107	0.074	0.073	0.000

Table 3b: Pairwise Fst distance matrix between sampling areas. Fst is calculated according to Nei (1973). Fst values are given in the lower matrix and upper matrix indicates P-value estimated from 1000 permutations of the genotypes dataset.

Palm oil wine					
	Attingué	Grand-Lahou	Alépé	Bingerville	Bonoua
Number of individuals	6	7	7	5	4
Attingué	0.000	0.022	0.035	0.077	0.002
Grand-Lahou	0.066	0.000	0.001	0.033	0.001
Alépé	0.045	0.070	0.000	0.008	0.001
Bingerville	0.093	0.092	0.084	0.000	0.001
Bonoua	0.070	0.064	0.073	0.118	0.000

Raffia wine				
	Grand-Lahou	Alépé	Adzopé	Abengourou
Number of individuals	16	35	6	7
Grand-Lahou	0.000	0.003	0.008	0.044
Alépé	0.011	0.000	0.019	0.031
Adzopé	0.049	0.022	0.000	0.051
Abengourou	0.054	0.026	0.061	0.000

Tchapalo beer

	Abidjan	Bingerville
Number of individuals	17	2
Abidjan	0.000	0.198
Bingerville	0.022	0.000

Values are significantly different for P-value < 0.05.

Table 4: Fis value per population and corresponding estimate of selfing rate

	Ecological niche			
	Raffia wine	Palm oil wine	Tchapalo beer	Ron wine
Number of individuals	64	29	19	58
Fis	0.603	0.544	0.138	0.214
Outcrossing estimate (o=(1-Fis)/(1+Fis))	0.25	0.30	0.76	0.65
Selfing estimate (=1-o = 2Fis/(1+Fis))	0.75	0.70	0.24	0.35