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Genetic diversity and differentiation of the frankincense tree (*Boswellia papyrifera* (Del.) Hochst) across Ethiopia and implications for its conservation

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ABSTRACT

Boswellia papyrifera is used to produce frankincense, a bark resin that has been a commodity of domestic and international trade since ancient times. It is harvested from natural forests. The tropical dry forest (Terminalia–Combretum) woodland ecosystems in which *B. papyrifera* is one of the dominant species, are facing anthropogenic threats. In Ethiopia *B. papyrifera* populations have decreased tremendously to smaller and isolated remnant patches, and many forests in the North-western and North-eastern parts of Ethiopia completely lack recruitment of saplings. This regeneration bottleneck, in combination with adult mortality, threatens the persistence of the species. Devising an effective strategy to conserve wild genetic resources needs information on the genetic diversity and the pattern of genetic differentiation across the species area. In the present study we analysed adult trees sampled in twelve populations across the growing area of the species in Ethiopia for genetic diversity and spatial genetic differentiation using 10 polymorphic microsatellite loci. The mean level of observed and expected heterozygosity were 0.669 and 0.681 respectively, and these levels were similar for trees from larger populations and those from degraded populations. A moderate level of among populations genetic differentiation (F_{ST} = 0.084) was detected. Genetic distance between populations was correlated with geographic distance (r = 0.663, p < 0.05). STRUCTURE analysis distinguished four distinct genetic clusters corresponding to regions with different environmental conditions. In the Western populations we detected recruitment of many seedlings and saplings, which is a significant novel finding as most of the other populations are completely devoid of saplings. We conclude that currently a high level of genetic variation is still maintained in *B. papyrifera* adult trees across the species' range in Ethiopia including the highly degraded remnant *B. papyrifera* population patches scattered on farm and pasture lands. An effective conservation strategy for the species has to take into account the geographic distribution of source populations.

1. Introduction

Widespread anthropogenic disturbance has resulted in the fragmentation and conversion of forest and woodland ecosystems into pasture and agricultural land (Lindenmayer and Fischer, 2006). Terminalia–Combretum woodlands in which *Boswellia papyrifera* is one of the dominant species (Vollesen, 1989) are no exception. These woodlands are threatened by various human-driven factors in a large part of its geographical distribution (Ogbazghi et al., 2006; Abtew et al., 2012; Abiyu et al., 2010; Eshete et al., 2011). As a result *B. papyrifera* populations have decreased tremendously to ever smaller and isolated remnant patches. In addition, a severe regeneration bottleneck is threatening the persistence of this species as many populations are devoid of seedlings and small recruiting individuals. This situation has been described in Eritrea (Ogbazghi et al., 2006), Sudan (Abtew et al., 2012) and North-western Ethiopia (Abiyu et al., 2010; Eshete et al., 2011). *B. papyrifera* is used for the production of frankincense, the dried bark resin that has been a commodity of domestic and international trade since ancient times (Groom, 1981; Gebrehiwot et al., 2003). In modern days, *B. papyrifera* is an important economic resource

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because of the growing demand of frankincense for diverse industrial uses, including food flavours, cosmetic ingredients (e.g. lotions, soaps, and ointment formulation), and perfumes (Tucker, 1986; Coppen, 2005). Frankincense is exclusively harvested from trees in natural forests.

In Ethiopia *B. papyrifera* is commonly distributed in the dry lowlands in the Western, Northern, North-western, and North-eastern part of the country including the river basins of the Blue Nile, Jamma, Tekeze, and Zarema. The only population in the central part of the country is located along the Jamma River Gorge, which is a tributary to the Blue Nile river. Most of the populations lack rejuvenation. Tolera et al. (2013) indicated that no recruitment has occurred for the last 50 years in the North-western (Metema) populations. Some of the populations in the North-east are so severely degraded that only few scattered remnant trees are left on farmlands and inaccessible hill tops (Addisalem, personal observation, 2011). A high adult mortality is leading to ever smaller populations (Abiyu et al., 2010; Groenendijk et al., 2012). The ongoing threats were predicted to lead to a 90% decline in number of trees in 50 years and this loss might lead to a 50% associated loss of frankincense yield in 15 years (Groenendijk et al., 2012). Groenendijk et al. (2012) and Lemenih et al. (2014) underscored the need of implementing intensive management to sustainably conserve *B. papyrifera* and the incense production from the species.

Devising an effective strategy to conserve wild plant genetic resources requires basic information on the genetic diversity, differentiation among populations, and understanding the underlying processes that play role in determining the level and distribution of the genetic variation (Booy et al., 2000). Large populations of naturally outbreeding species usually have extensive genetic diversity (Frankham, 1995). In long-lived outcrossing plants such as trees, the majority of that genetic diversity occurs within populations. Pairs of populations farther away from each other will be genetically differentiated because of limited gene flow explained as movement of individuals, their seeds, or pollen over longer distances (Wright, 1943; Schaal et al., 1998). Geographic distance alone, however, may not explain all of the genetic differentiation because patterns of variation may also be impacted by environmental variables that are important for growth or survival of the species (Ledig and Fryer, 1972; Duminil et al., 2013). In addition, anthropogenic interference may affect the genetic variation and structure of a population (Frankham, 1995; Arens et al., 2007). Changes in population demography as a result of a rapid decline in population size may result in substantial loss of genetic diversity and may lead to increased differentiation across populations as a result of genetic drift if it continues for more than one or two generations (Hamrick et al., 1992; Frankham, 1995; Hamrick and Godt, 1996).

In this study we determined the genetic diversity and population structure of Frankincense tree (*B. papyrifera*) populations across the species range in Ethiopia. We hypothesized that (i) the genetic differentiation among populations is low since *B. papyrifera* is a long-lived and widespread species and (ii) populations farther away from each other are genetically more distant than populations close to each other due to the limited gene dispersal caused by spatial distance. We used the resulting information to identify to-be-conserved populations representative of the genetic diversity of the species.

2. Material and methods

2.1. Study species

B. papyrifera is one of the twenty species in the genus *Boswellia* (*Burseraceae* family; Vollesen, 1989). The species is a deciduous monoecious tree with sweet scented flowers that are frequently visited by honeybees for pollen and nectar (Fichtl and Admasu, 1994). Fruits are about 2 cm long, usually containing three tapered seeds (Vollesen, 1989). Next to the stem being the source of frankincense, the flowers are the source of honey, the leaves provide animal fodder and various parts of the tree are used as traditional medicines.

2.2. Field sampling

Adult trees were sampled in twelve populations across the species range in Ethiopia (Supplementary .kmz file). Population were sampled from natural forests, or fragmented and degraded landscapes in areas where natural forests were not available. The geographical location and altitude of the populations were recorded using a handheld Garmin Dakota 20 GPS. Characteristics of the sampled populations (altitude, temperature, rainfall and soil) are listed in Table 1.

A total of 344 genotypes were sampled (25–43 individuals per population except for one population (AF-BIR) where sampling was possible only for 8 individuals because of inaccessibility of the area). As mechanisms of pollination and seed dispersal are not well known, a minimum of 25 m distance was kept between sampled individuals to reduce the risk of sampling genetically related individuals. Young leaves were sampled from growing shoots of the plants and dried and stored on silica gel until DNA extraction.

2.3. DNA extraction, PCR amplification and genotyping

Total DNA was isolated from dried young leaves following the cetyl trimethylammonium bromide (CTAB) protocol of Fulton et al. (1995) modified with 2% pvp-40 in the extraction buffer and 1% mercaptoethanol in the microprep buffer, added immediately before use, and followed by purification using DNeasy (Qiagen, Venlo, The Netherlands) according to Smulders et al. (2010). DNA yield and quality were visually assessed on a 1% agarose gel. Ten polymorphic microsatellite markers: Bp02, Bp11, Bp17, Bp18, Bp20, Bp21, Bp22, Bp23, Bp29 and Bp39 (Addisalem et al., 2015) were used for genotyping. PCR amplification was in a 10 μl reaction mix containing 4 μl 2 ng/μl DNA, 5 μl MP mix from Qiagen kit, 0.8 μl (2 μM) universal fluorescently labelled primer and 0.2 μl mix of the forward and reverse primers. For fluorescently labelling the forward primers were extended with a universal M13 sequence (AACAGGTATGACCATGA) at the 5′ end (Schuelke, 2000) while the reverse primers were tailed with GTTT at their 5′ end according to Brownstein et al. (1996) to reduce stutter bands. The PCR cycling profile consisted of 15 min denaturation at 95 °C, followed by 30 cycles of 30 s denaturation at 94 °C, 45 s annealing at 56 °C and 45 s extension at 72 °C. This was followed by 8 cycles at 53 °C annealing temperature to facilitate the annealing of the fluorescent dye-labeled M13 primer, and a final extension step of 10 min at 72 °C. After amplification 10 μl water was added for dilution and increase the volume of the product. Fluorescently labelled amplicons were resolved on a 4200 or 4300 Licor DNA analyzer. Bands were scored manually.

2.4. Data analyses

Based on the microsatellite data, the total number of alleles, effective number of alleles, mean values of expected and observed heterozygosity, and inbreeding coefficient were estimated and mean values over all loci and populations computed using GenAlEx 6.5 (Peakall and Smouse, 2006, 2012). The genetic differentiation based on allelic frequency (F_{ST}), AMOVA, and pair-wise Nei's unbiased genetic distance were also analysed using this program.