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Genetic diversity and differentiation of two narrowly endemic Origanum Species in Morocco: implications for conservation

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Abstract

Oregano is a highly valued aromatic and medicinal herb in the Mediterranean, appreciated for its culinary, therapeutic, and ecological significance. Classification within the *Origanum* genus is complicated by extensive morphological variation and frequent interspecies hybridization. In this study, we assessed the genetic diversity and population structure of *O. elongatum* and *O. grosii* across their full distribution in Morocco, sampling 264 individuals from seven populations and analyzing them with 13 microsatellite (SSR) markers. Despite its restricted native range, *O. elongatum* displayed considerable genetic diversity (Ho = 0.455, He = 0.429), whereas *O. grosii* showed lower diversity (Ho = 0.285) but higher differentiation among populations. Bayesian clustering, Principal Coordinates Analysis, and STRUCTURE analysis revealed four main genetic groups largely corresponding to geographic origin and clearly separating the two species. These results indicate strong species divergence and the existence of distinct population structures, underscoring the need for species-specific conservation measures. We recommend integrating in-situ and ex-situ strategies, including habitat protection, genetic reinforcement, and germplasm preservation in gene banks. Overall, this study illustrates how genetic data can inform conservation planning for rare endemic plants in ecologically sensitive regions, providing a model applicable to other Mediterranean mountain ecosystems facing climate and land-use pressures.

Introduction

Morocco is a major biodiversity hotspot in the Mediterranean, hosting about 3,912 taxa and 1,298 subspecies, with roughly 22% endemism (Fennane and Ibn Tattou 2012). This exceptional endemic richness (878 taxa, nearly 600 at the species level) is mainly concentrated in the High Atlas, Middle Atlas, and Rif mountains. However, this diversity is increasingly threatened, as nearly 2,193 plant species are endangered, over half of which (53%) are highly vulnerable due to anthropogenic pressures and environmental change (Fennane and Ibn Tattou 1999). Endemics with narrow distributions are particularly at risk, as their limited range increases their vulnerability to stochastic events and environmental stressors (George et al. 2009; Lannuzel et al. 2021).

The genus *Origanum* L. (Lamiaceae), comprising about 18 hybrids and 43 species, is largely distributed around the Mediterranean region (Ietswaart, 1980). In Morocco, four taxa and one hybrid are recognized: *O. compactum* Benth. (also found in southern Spain), *O. vulgare* subsp. *virens*, and three endemic taxa : *O. elongatum*, *O. grosii*, and *O. × font-queri* (a spontaneously occurring hybrid resulting from *O. grosii* and *O. compactum*) (Bakha et al. 2017). The species *O. elongatum* has a geographic range that stretches mainly across northeastern Morocco, between the Middle Atlas and Rif Mountain systems, typically at high elevations (Bakha et al. 2020). *O. grosii* is among the rarest species, occurring in small, isolated populations in the humid western Rif at high altitudes (Bakha et al. 2017).

Taxonomic delimitation within Moroccan *Origanum* is controversial. Morphological similarities among *O. grosii*, *O. elongatum* and the hybrid *O. × font-queri* have led to confusion. Although certain databases (Euro + Med 2006; African Plant Database v3.4.0, Dobignard and Chatelain 2010–2013) classify *O. grosii* and the hybrid *O. × font-queri* under the same taxon as *O. elongatum* (Bakha et al. 2017), Ietswaart (1980) designated *O. grosii* as an independent species, based on morphological traits, including its shorter stems and leaves, as well as broader bracts in comparison to *O. elongatum*.

Populations of Moroccan *Origanum* species are highly fragmented and increasingly threatened by drought, climate change, habitat loss, overgrazing, and deforestation (Aboukhalid et al. 2017). Such fragmentation and population decline often result in enhanced genetic drift, reduced gene flow, and decline in genetic variability, all of which compromise the adaptive potential and long-term persistence of species (Holsinger and Gottlieb 1991).

Low levels of genetic variability and high levels of genetic divergence among populations are common characteristics of rare and geographically isolated species (Sullivan et al. 2019). Endemic medicinal plants like *Origanum* species are highly vulnerable to habitat degradation and climate change, emphasizing the need for conservation (Aboukhalid et al. 2017, 2023). Genetic diversity is crucial for adaptive capacity and long-term survival (Reed and Frankham 2003; Supple and Shapiro 2018), yet small populations often lose diversity through drift, bottlenecks, and founder effects (Banks et al. 2015), making its conservation a central goal (Frankham 1996). This study addresses the previously unexamined genetic diversity and population structure of Moroccan endemic *O. elongatum* and *O. grosii* using genomic and landscape approaches across their natural range. In particular, we investigate the following key questions: (i) What are the patterns of genetic polymorphism and population structure in *O. elongatum* and *O. grosii* across and within populations? (ii) Is there evidence of gene flow among these species? (iii) Do patterns of genetic differentiation support the current taxonomic distinctions proposed by Ietswaart (1980)? (iv) How might the genetic results contribute to the development of effective conservation and management plans for these rare and endangered endemics?

Materials and Methods

Study Species

Origanum elongatum Emberger & Maire (Lamiaceae) is a perennial species native exclusively to Morocco. It is naturally distributed in Rif Mountain systems and the northern Middle Atlas, predominantly inhabiting schistose soils at elevations ranging from 681 to 1866 meters, with most populations occurring above 1300 m (Bakha et al. 2020). *O. elongatum* is a climate-tolerant species found from semi-arid to per-humid zones and

is valued in Morocco for its aromatic, culinary, melliferous, and ornamental uses. It is charachterized by tall purplish stems reaching up to 90 cm. Branches are largely composed of terminal spikes. Leaves are arranged in up to 30 opposite pairs per stem, becoming subsessile toward the apex, with petioles up to 5 mm in length (Ietswaart 1980). The flowering occurs from June to October. The leaves are densely encrusted with glandular trichomes that produce essential oils, resulting in a fragrant and flavored odor characteristically associated with this species (Simon et al. 1984).

Although specific pollination studies are lacking, flower morphology, strong scent, prominent stamens (up to 3.5 mm), and elongated styles (up to 8 mm), as well as the frequent visits by pollinating insects suggest a predominance of cross-pollination. This is consistent with the general reproductive patterns reported in the Lamiaceae family, which is typically insect-pollinated and predominantly outcrossing (Judd et al. 1999).

Origanum grosii Pau & Font Quer ex Ietswaart stands out as one of the scarcest and most localized species taxonomically assigned to the genus *Origanum*. This species is found only in the western Rif Mountains of northwestern Morocco. It grows mainly at high elevations under humid conditions, typically on calcareous substrates, at elevations ranging between 400 and 1900 m (Bakha et al. 2017).

Morphologically, *O. grosii* is quite distinctive. It has relatively short stems (up to 55 cm), which are lightly hairy, and long lateral branches stretching between 4 and 30 cm. Its bracts are notably larger (2–5 mm) than those of related species. The leaves are smaller than those of *O. elongatum*, usually between 5 and 19 mm long. The species has erected purplish-brown stems, with fine hairs (trichomes) concentrated at the base and continuing sparsely toward the top, ranging from 0.3 to 1.5 mm in length. Like *O. elongatum*, the flowering parts are arranged mostly in terminal spikes. Leaves are shortly petiolate at the base, becoming subsessile toward the apex, arranged in up to 25 pairs per stem. Floral characteristics include staminal filaments up to 4 mm and styles reaching 8 mm. *O. grosii* flowers mainly between June and July (Bakha et al. 2019).

Sampling and DNA Extraction

Overall, 210 individual plants of *Origanum elongatum* and 54 individuals of *O. grosii* were sampled from geographically distinct locations spanning the full known distribution range of each species in Morocco. Species identification was confirmed using the taxonomic keys provided by Ietswaart (1980). Specimen collection in the field was carried out during flowering season (June-July).

The sampling design aimed to capture maximum geographic and ecological variation. In most populations of *O. elongatum*, at least 25 individuals were sampled. However, in Boulmane region, only eight individuals could be located due to the low population density. Due to its extremely limited distribution, *O. grosii* is confined to a small area, which restricted the sampling to a single available population. *O. elongatum* and *O. grosii* samples were collected at distances ranging from 20 m to a few kilometers apart to minimize the risk of sampling clonal individuals. The sampled populations of *O. elongatum* cover its regional distribution across environmentally heterogeneous areas separated by major geographic barriers (deep valleys, Rif and Middle Atlas mountains) that may limit gene flow. Young leaves were collected and preserved at −80 °C, and genomic DNA was extracted from lyophilized tissue using the NucleoSpin® 96 Plant Kit following Aboukhalid et al. (2017). DNA quality was checked by 2.0% agarose gel electrophoresis, and purified DNA was stored at −20 °C for subsequent analyses.

SSR Amplification and Genotyping

Thirty SSR markers described by Ince et al. (2014) were screened on 56 individuals of *O. elongatum* and *O. grosii*, leading to the selection of 13 polymorphic markers with reliable amplification for genotyping. SSR genotyping employed M13-tailed primers following Schuelke (2000). Fluorescent labeling of PCR products was achieved by using M13 primers tagged with one of the four dyes: 6-FAM, VIC, PET, or NED (Eurofins Genomics, France). PCR amplification and genotyping procedures were conducted according to the protocol described by Aboukhalid et al. (2017). The resulting PCR products were analyzed with an ABI PRISM 3130 Genetic Analyzer, and GeneMapper software (Applied Biosystems) was used to analyze and determine fragments lengths.

Genetic diversity

Parameters including allele number (Na), observed (H_O) and expected heterozygosity (He), proportion of polymorphic loci (P%), Inbreeding coefficient (Fis) and genetic differentiation (Fst) were computed with GenAlEx 6.5 (Peakall and Smouse 2006). Allelic richness (Rs) and gene diversity (Hs) corrected for sample size (El Mousadik and Petit 1996) were calculated using FSTAT v. 2.9.3.2 (Goudet, 1995). The Hardy-Weinberg Equilibrium (HWE) analysis of the data was conducted using the Genepop v.1.2 (Raymond and Rousset 1995; Rousset 2008). Bottleneck effects were assessed using Bottleneck v.1.2.02 under infinite alleles model (IAM), stepwise mutation model (SMM), and two-phase model (TPM), applying Wilcoxon tests for heterozygosity excess and mode-shift analyses of allele frequency distributions.

Genetic Structure

The population genetic structure of *O. elongatum* and *O. grosii* was analyzed following the genotyping framework reported by Aboukhalid et al. (2017). Genetic variation within and among populations was assessed using Principal Coordinate Analysis (PCoA) using GenAlEx v6.4.1 (Peakall and Smouse 2006), while population structure was inferred through Bayesian clustering in STRUCTURE v2.3.4 (Pritchard et al. 2000). Model selection and visualization were performed using STRUCTURE HARVESTER (Evanno et al. 2005), CLUMPP (Jakobsson and Rosenberg 2007) and DISTRUCT (Rosenberg, 2003).

Results

Genetic Diversity

In total, 264 *O. elongatum* and *O. grosii* samples were examined using 13 highly polymorphic microsatellite markers. Out of these markers, a total of 92 alleles were identified, with an average of 7.1 alleles per locus. The average observed heterozygosity was HO = 0.439, slightly higher than the expected heterozygosity He = 0.415 for the entire set of samples (Table 1). This shows the high genetic diversity within the samples from the two *Origanum* species. However, four markers (LB004, LB035, LB039, and LB077) showed significant deviations from the Hardy-Weinberg equilibrium ($p < 0.05$) and high positive Fis values (Table 1). This could be due to heterozygote deficiencies, which could be the result of inbreeding, the presence of null alleles, or the Wahlund effect, i.e., the substructuring of the population. Nevertheless, the high number of alleles and the heterozygosity indicate the high genetic diversity in the two *Origanum* species, which could be the result of the ecological adaptability of these plants and potential for gene flow across populations.

Table 1 Summary of genetic diversity parameters estimated from 13 SSR loci in 264 individuals sampled from seven populations of *O. elongatum* and *O. grosii*.

Loci	Na	Ne	Ho	He	Fis	Fst	Nm	HWE
LB003	2.571	1.851	0.757	0.455	-0.664	0.049	4.681	ns
LB004	5.286	3.866	0.556	0.739	0.248	0.062	3.450	*
LB028	4.714	1.832	0.511	0.415	-0.230	0.156	1.535	ns
LB035	8.857	2.568	0.420	0.539	0.222	0.086	1.740	*
LB039	3.571	1.310	0.144	0.200	0.279	0.105	2.373	*
LB040	4.714	1.920	0.550	0.445	-0.236	0.052	4.364	ns
LB043	3.429	2.465	0.708	0.577	-0.228	0.218	1.230	ns
LB060	3.429	1.205	0.164	0.159	-0.034	0.053	4.278	ns
LB066	2.143	1.417	0.286	0.261	-0.097	0.284	0.560	ns
LB070	1.714	1.073	0.069	0.064	-0.071	0.036	6.634	ns
LB077	3.000	1.818	0.234	0.406	0.423	0.278	0.736	*
LB078	3.429	2.553	0.511	0.571	0.105	0.099	1.616	ns
LB081	3.000	2.057	0.687	0.494	-0.392	0.052	4.615	ns
Mean	1.863	2.022	0.439	0.415	-0.053	0.119	2.909	

Na: mean number of alleles, **Ne**: effective allele number, **Ho**: observed heterozygosity, **He**: expected heterozygosity, and **Fis**: inbreeding coefficient, computed as 1 minus Ho divided by He. **HWE**: the overall test for Hardy–Weinberg equilibrium deviations, with an asterisk (*) marking significant departures at the 0.05 level, and "ns" indicating non-significance.

The genetic diversity of six *O. elongatum* populations and one *O. grosii* population was assessed (Table 2). At the population scale, allele numbers (Na) per locus ranged between 2.538 in *O.e*1 (Boulmane region) and 4.692 in *O.e*5 (Taounate region). On average, the estimated effective number of alleles (Ne) reached 1.995. Genetic diversity varied substantially among populations. Observed heterozygosity (Ho) ranged from 0.285 in *O. grosii* to 0.505 in population *O.e*5, while expected heterozygosity (He) varied from 0.291 (*O. grosii*) to 0.495 (*O.e*5). The mean number of alleles per locus (Na) across 13 loci ranged between 2.538 (*O.e*1) and 4.692 (*O.e*5), and allelic richness (Rs) from 2.228 (*O. grosii*) to 2.989 (*O.e*5). Gene diversity (Hs) spanned 0.294 (*O. grosii*) to 0.500 (*O.e*5). A total of 26 private alleles were detected in 6 of 7 populations, with *O. grosii* containing the highest number (10). Overall, 94.51% of loci were polymorphic, with the lowest polymorphism in *O.e*1 (76.92%) and complete polymorphism (100%) in populations *O.e3*, *O.e*4, *O.e*5, and *O.e*6.

Table 2 Genetic diversity measures for populations of *O. elongatum* and *O. grosii*.

Species	Population code	Region	Sample size	Na	Rs	Hs	Np	(P)%	Ne	Ho	He	Fis	HWE
O. elongatum	*O.e*1	Boulmane	8	2.538	2.524	0.427	-	76.92	2.035	0.470	0.402		LB004***
O. elongatum	*O.e*2	Chefchaouen	25	3.154	2.468	0.410	1	92.31	1.879	0.462	0.403		LB077**
O. elongatum	*O.e*3	Targuist	65	4.615	2.836	0.399	6	100	2.149	0.374	0.395	***	LB004*** LB035*** LB039**
O. elongatum	*O.e*4	Al Hoceima	36	4.231	2.864	0.444	3	100	2.088	0.479	0.437	*	LB004** LB035*** LB077*
O. elongatum	*O.e*5	Taounate	44	4.692	2.989	0.500	5	100	2.182	0.505	0.495	**	LB004*** LB039*** LB077*
O. elongatum	*O.e*6	Taza	32	3.846	2.814	0.451	1	100	2.061	0.439	0.443	***	LB004*** LB035*** LB039*** LB077**
Mean				3.846	2.749	0.439	3.2	94.872	2.066	0.455	0.429		
O. grosii	*O.g*	Chefchaouen	54	3.769	2.228	0.294	10	92.31	1.572	0.285	0.291	***	LB003** LB035*** LB039**

N: number of individuals analyzed. **Na**: average allele per locus across the 13 SSR markers. **Rs**: allelic richness calculated following El Mousadik and Petit (1996) [24]. **Hs**: mean gene diversity over all loci. **Np**: number of private alleles identified. **P**: proportion of loci that are polymorphic. **Ho** and **He**: observed and expected heterozygosity, respectively. **FIS**: inbreeding coefficient, with significance levels for heterozygote deficits indicated by * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$) at the population scale. **HWE**: loci that significantly deviate from Hardy–Weinberg equilibrium according to a global test at the 5% threshold, applying a sequential Bonferroni correction; stars mark loci with significant heterozygote deficiencies at the indicated significance levels.

Population genetic structure based on microsatellite markers

Principal Coordinate Analysis (PCoA) revealed clear genetic differentiation between *O. elongatum* and *O. grosii* (Fig. 1), with each species forming distinct clusters and showing substantial interspecific divergence. While a few individuals displayed partial overlap, likely due to shared alleles or limited gene flow, most samples clustered according to species and geographic origin, indicating species-specific gene pools and restricted genetic exchange. The pronounced separation along PCoA axes reflects evolutionary and ecological processes, potentially driven by geographic isolation, ecological specialization, or reproductive barriers. These results confirm that *O. elongatum* and *O. grosii* are genetically distinct, supporting their taxonomic status and underscoring the need to conserve their unique genetic resources.

Bayesian Clustering and Genetic Structure

STRUCTURE analysis identified four genetic clusters (ΔK; Evanno et al., 2005), with *O. grosii* forming a distinct cluster separate from *O. elongatum*. Within *O. elongatum*, populations *O.e*2, *O.e*4, *O.e*5, and *O.e*6 shared a genetic background, indicating a common gene pool or ongoing gene flow among them. In contrast, the most geographically isolated populations, *O.e*1 and *O.e*3, exhibited unique genetic compositions that clearly distinguished them from the other *O. elongatum* populations, although a few individuals showed signatures of admixture (Fig. 2b; Fig. 3). *O. grosii*, a species with a limited distribution, was genetically uniform and structured into a single distinct cluster. It is found in a fragmented habitat and appears clearly differentiated from the broader genetic pool of *O. elongatum* (Fig. 3).

Pairwise genetic differentiation analysis corroborated the results and the distinctness of *O. grosii* with regard to *O. elongatum*. All the pairwise comparisons are significant even after correcting the results using the Bonferroni correction ($p < 0.002$). The genetic distances between the populations using Nei's genetic distance ranged from 0.017 to 0.234 (Table 3). The maximum genetic distance was observed between *O. grosii*

and *O. elongatum* from Taza (O.e6), while *O. elongatum* populations from Al Hoceima (*O.*e4) and Taza (*O.*e6) showed the lowest genetic distance. The genetic separation between *O. elongatum* and *O. grosii* and the effect of geographic continuity on the genetic similarity between *O. elongatum* populations are confirmed. The strong differentiation of *O. grosii* likely results from historical isolation, restricted gene flow, and species-specific evolutionary history. This strong genetic divergence supports its status as a distinct taxon and emphasizes the need for species-specific conservation strategies.

Table 3 Pairwise comparison matrix of Nei genetic distance for *O. elongatum* and *O. grosii* populations.

Populations	*O. e1*	*O. e2*	*O. e3*	*O. e4*	*O. e5*	*O. e6*	*O. g*
O.e1	****						
O.e2	0.178	****					
O.e3	0.096	0.197	****				
O.e4	0.100	0.036	0.114	****			
O.e5	0.108	0.055	0.141	0.036	****		
O.e6	0.099	0.052	0.096	0.017	0.024	****	
O.g	0.174	0.225	0.128	0.221	0.208	0.234	****

Mutation-Drift Equilibrium

The genetic signatures of the recent decline in the effective population size are usually characterized by reduced allelic richness and heterozygosity, with allelic richness being reduced at a faster rate, which could result in observed heterozygosity being higher than the mutation drift equilibrium (Cornuet and Luikart 1996). Bottleneck analysis was carried out using the IAM, SMM, and TPM models with Wilcoxon's signed-rank test for heterozygosity excess and the mode-shift test to identify changes in allele frequency distribution. Among the populations surveyed, *O.e*1 showed a change in allele frequency distribution and heterozygosity excess ($p < 0.05$) using all three models (Table 4). The bottleneck could be due to the reduced size of the effective population. Other populations showed the characteristic L-shaped curve, which is the expected curve in the absence of a bottleneck event. However, care must be taken while interpreting the results, and the presence of outbreeding depression, Hardy-Weinberg equilibrium, null alleles, and inbreeding could affect the results (Aboukhalid et al. 2017).

Table 4 Summary of the Wilcoxon signed-rank test outcomes evaluating excess heterozygosity and allele frequency distribution shifts under the infinite alleles model (IAM), the stepwise mutation model (SMM), and the two-phase model (TPM). Significant deviations from mutation-drift equilibrium are indicated by *p*-values less than 0.05.

Populations	IAM model	TPM model	SMM model	Mode shift
O. e1	**0.00049**	**0.00244**	**0.01221**	Shifted mode
O.e2	0.16968	0.45483	0.66138	Normal L- shaped
O.e3	0.58032	0.90454	0.99573	Normal L- shaped
O.e4	0.47302	0.72913	0.90454	normal L- shaped
O.e5	0.29395	0.55371	0.79285	normal L- shaped
O.e6	0.29395	0.70605	0.96594	normal L- shaped
O.g	0.84937	0.95386	0.99329	normal L- shaped

Discussion

Patterns of Genetic Diversity

Small or narrowly distributed plant species are often susceptible to declines in genetic diversity, making their study essential for conservation planning. In this study, we assessed the genetic diversity of two Moroccan endemics, *O. elongatum* and *O. grosii*, using 13 microsatellite markers. Observed heterozygosity (Ho) ranged from 0.285 in *O. grosii* to 0.505 in *O. elongatum* (*O.*e5). Expected heterozygosity (He) was lower than Ho in four populations (*O.*e1, *O.*e2, *O.*e4, *O.*e5), suggesting heterozygote excess. On average, *O. elongatum* exhibited higher genetic diversity (Ho = 0.455; He = 0.429) than *O. compactum* from Morocco (Ho = 0.37; He = 0.35; Aboukhalid et al. 2017) and comparable values to *O. vulgare* subsp. *hirtum* from Bulgaria (He = 0.482; Ho = 0.448; Alekseeva et al. 2021).

In comparison with other species of the *Origanum* genus, *O. elongatum* showed exceptionally high levels of genetic diversity. In *O. compactum*, where the same SSR markers were used, the number of alleles per locus was 2.26, allelic richness was 1.72, and there were 14 private alleles (Aboukhalid et al. 2017). This study supported that low levels of genetic diversity are not universal among range-restricted species. Some range-restricted species may harbor levels of diversity comparable to or even higher than those of their common congeners (Gitzendanner and Soltis 2000). The high levels of genetic diversity found in *O. elongatum*, despite its range-restricted nature, may indicate ecological and genetic resilience that are likely linked to broad substrate adaptability and lack of recent founder events.

O. grosii showed lower levels of genetic diversity, especially regarding allelic richness compared to *O. elongatum*. This pattern is comparable to that found for other range-restricted endemic species (Gibson et al. 2008). Confined to a single locality, *O. grosii* illustrates this trend. Similar patterns of decreased genetic diversity have been recorded for other range-restricted species such as *Seseli farrenyi* (Garcia-Jacas et al. 2021). Although *O. grosii* is a perennial species, and thus not subject to bottlenecks due to short life cycles, its small effective population size increases susceptibility to genetic drift, leading to greater homozygosity (Gustafsson and Sjögren-Gulve 2002). Surveys revealed no additional populations of *O. grosii*, confirming its critical status and the need to prioritize its single population for conservation. Its heterozygote deficit may partly reflect null alleles, often overestimated in small or inbred populations (Van Oosterhout et al. 2005). Reduced genetic diversity in *O. grosii* and *O. elongatum* relative to widespread *O. vulgare* is consistent with effects of drift, inbreeding, and allelic loss in small populations (Spielman et al. 2004; Alekseeva et al. 2021). However, genetic diversity also depends on life history, breeding system, gene flow, dispersal, and historical biogeography (Wang et al. 2004). For *O. grosii*, low diversity is not explained by its perennial, allogamous nature; pollinator limitation or ecological specialization may contribute (Phillips et al. 2014). In *O. elongatum*, heterozygote deficits in O.e2 and O.e6 may reflect mating among relatives.

Genetic differentiation between *O. elongatum* and *O. grosii* is supported by species-specific alleles, with population assignment matching geographic origin even in sympatric areas like Chefchaouen. Admixed genotypes indicate past or ongoing gene flow, reflecting divergence with occasional interspecific exchange. The strong heterozygote deficit in O.e1 may result from null alleles (Arbeláez-Cortés et al. 2007), inbreeding or drift (Dodd et al. 2002), or a Wahlund effect from population structure (Dharmarajan et al. 2012).

Population Structure Analysis

The results of the Bayesian clustering analysis revealed the presence of four genetic clusters, (K = 4). The results of the STRUCTURE analysis revealed the presence of three lineages of *O. elongatum* and one lineage of *O. grosii*, with admixed populations showing evidence of historical or recent gene flow. The results were further confirmed by the Principal Coordinate Analysis (PCoA), which aligned with the inferred genetic structure and population assignments.

In *O. elongatum*, the populations *O.e*2, *O.e*4, *O.e*5, and *O.e*6 were grouped together, showing evidence of evolutionary history or recurrent gene flow, which might be attributed to historical or recent gene flow, founder effects, and expansion events. In accordance with our findings, a previous study carried out by SSR markers on *O. compactum* from Morocco, revealed that founder effects and bottlenecks were responsible for the lower levels of genetic diversity (Aboukhalid et al. 2017).

Bottleneck analysis detected evidence of a recent demographic decline only in population *O.e*1, indicated by a mode-shifted allele frequency distribution and heterozygosity excess, although the small sample size (n = 8) may limit the robustness of this inference (Cornuet and Luikart 1996). The decline nevertheless appears plausible given the observed habitat degradation in this area.

Genetic structure mirrors ecological boundaries, likely driven by restricted gene flow (Epps and Keyghobadi, 2015). Admixture indicates past or ongoing gene flow, and *O. grosii* remains sufficiently related to *O. elongatum* for cross-species SSR amplification. Increasing habitat fragmentation from land-use change threatens gene flow and may accelerate genetic erosion in narrow endemics, highlighting the need to combine land-cover mapping and genetics to prioritize connectivity and conservation (Epps and Keyghobadi, 2015).

Conservation Implications

This study provides the first comprehensive genetic baseline for *O. grosii* and *O. elongatum*, demonstrating that conserving genetic diversity is essential for long-term persistence and identifying priority populations beyond morphological and geographic criteria. Genetically diverse and distinct populations (notably *O.e*4 and *O.e*5) should be prioritized for in situ conservation and integrated into ex situ programs, while the widespread presence of private alleles (except *O.e*1) enhances most populations' value for restoration and reintroduction (Frankham 2016).

Four genetic clusters were identified, supporting their management as separate Evolutionarily Significant Units (ESUs) and Management Units (MUs). While populations within clusters (e.g., *O.e*2, *O.e*4, *O.e*5, *O.e*6) may serve as mutual reinforcement sources, inter-cluster mixing should be avoided to reduce the risk of outbreeding depression (Waller 2015; Moritz 1994). Urgent genetic reinforcement and habitat restoration are needed for the genetically impoverished Boulemane population (O.e1). Ex situ conservation and use of local seed sources are advised to preserve local adaptation (Quilichini et al. 2004).

Major threats including overgrazing, agriculture, urbanization, and illegal harvesting highlight the need for legal protection, inclusion in Key Biodiversity Areas, and alignment with IUCN assessments. Although fragmented, *O. elongatum* maintains relatively high genetic diversity but

limited gene flow, calling for targeted management. Integrating population genetics with ecological and climate modeling is essential to conserve adaptive capacity and ensure long-term viability under climate change (Hoffmann and Sgrò 2021), providing a transferable framework for conserving other narrowly distributed endemic species.

Conclusion

This study provides the first comprehensive genetic assessment of *O. elongatum* and *O. grosii* across Morocco, revealing clear inter- and intraspecific differentiation with key conservation implications. Despite habitat fragmentation, *O. elongatum* maintains relatively high genetic diversity, though limited gene flow among populations calls for targeted actions. Populations in Taounate and Al Hoceima, with high diversity and unique genetic profiles, should be prioritized, while isolated, genetically poor populations like Boulemane need urgent reinforcement and habitat restoration. Four genetic clusters support their designation as separate management units for restoration programs. The findings underscore the value of integrating molecular data with conservation planning, considering geographic isolation, ecological gradients, and land-use pressures, to guide strategies that preserve evolutionary potential and adaptive resilience in Morocco's endemic flora and other threatened species in fragile regions.

Declarations

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Author contribution K.A., N.M., A.L. and C.A.F. designed and conceptualized the study. K.A. performed formal analyses and wrote the original draft. K.A., M.B. and A.K. conducted fieldwork, sample collection and identified plant material. J.L. conceived the laboratory analyses and participated in DNA extraction, PCR amplification, and SSR genotyping. M.N., A.M. and G.K.G. performed statistical analyses, prepared some figures and contributed to manuscript review and editing. N.M., C.A.F. and A.L. supervised the project. N.M. and C.A.F. acquired funding and administered the project. All authors gave final approval for publication.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest The authors confirm that there are no financial conflicts or personal connections that might have influenced the research presented in this article.

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Figures

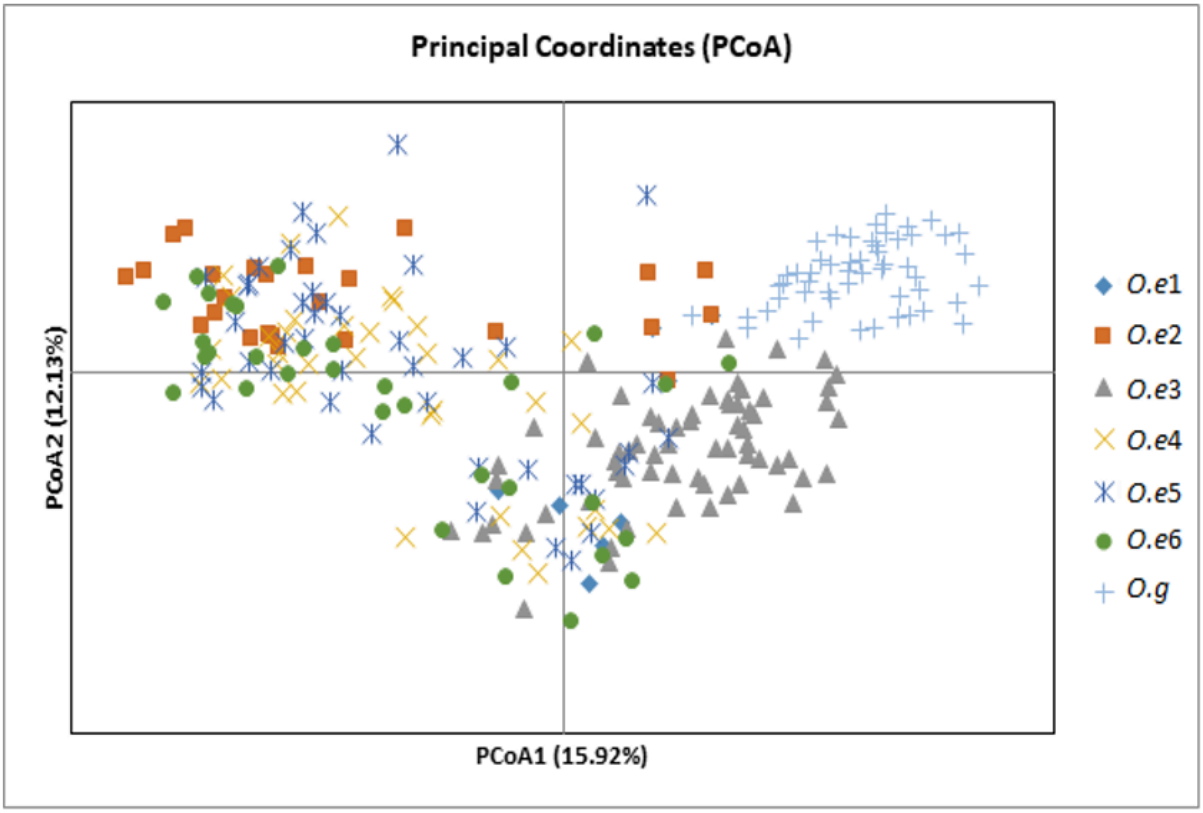


Figure 1

Principal Coordinates Analysis (PCoA) illustrating genetic relationships among individuals. The first and second axes account for 15.92% and 12.13% of the total genetic variation, respectively.

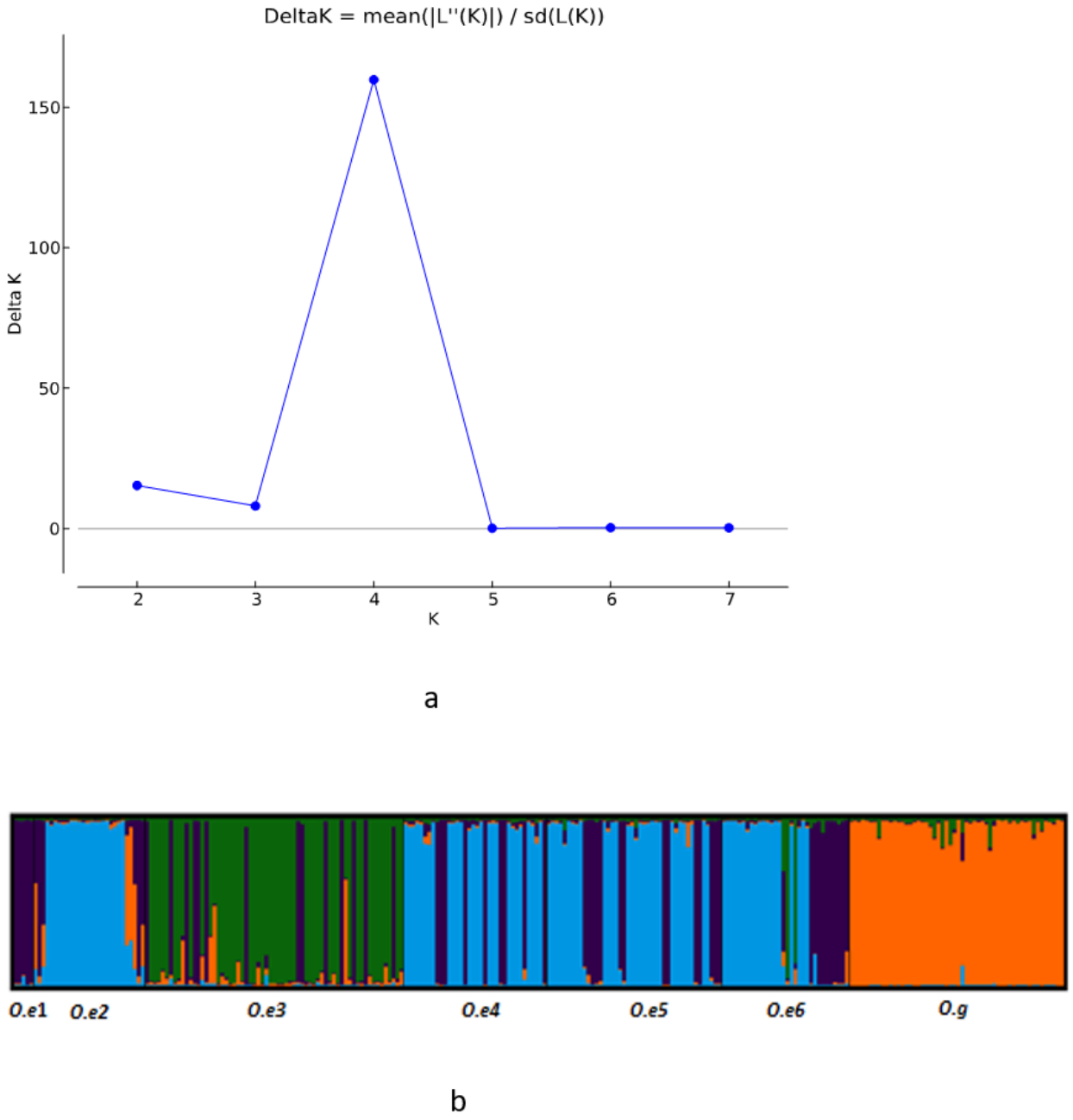


Figure 2

a Bayesian clustering results used to estimate the most likely number of genetic groups, following the method of Evanno et al. (2005) [29]. The ΔK value, representing the second-order rate of change in log-likelihood between successive K values, was calculated to identify the optimal K. Both ln(K) and ΔK were analyzed using STRUCTURE HARVESTER [32] to determine the best-supported number of clusters.

b Bayesian clustering analysis performed using the STRUCTURE software [28]. The bar plots illustrate the inferred genetic structure of *O. elongatum* and *O. grosii*, assuming four clusters (K = 4). Each bar on the Y-axis corresponds to an individual, with its genetic ancestry depicted by color-coded segments indicating proportional membership in the inferred clusters. Thin vertical lines separate individuals within populations, while bold lines distinguish different populations.

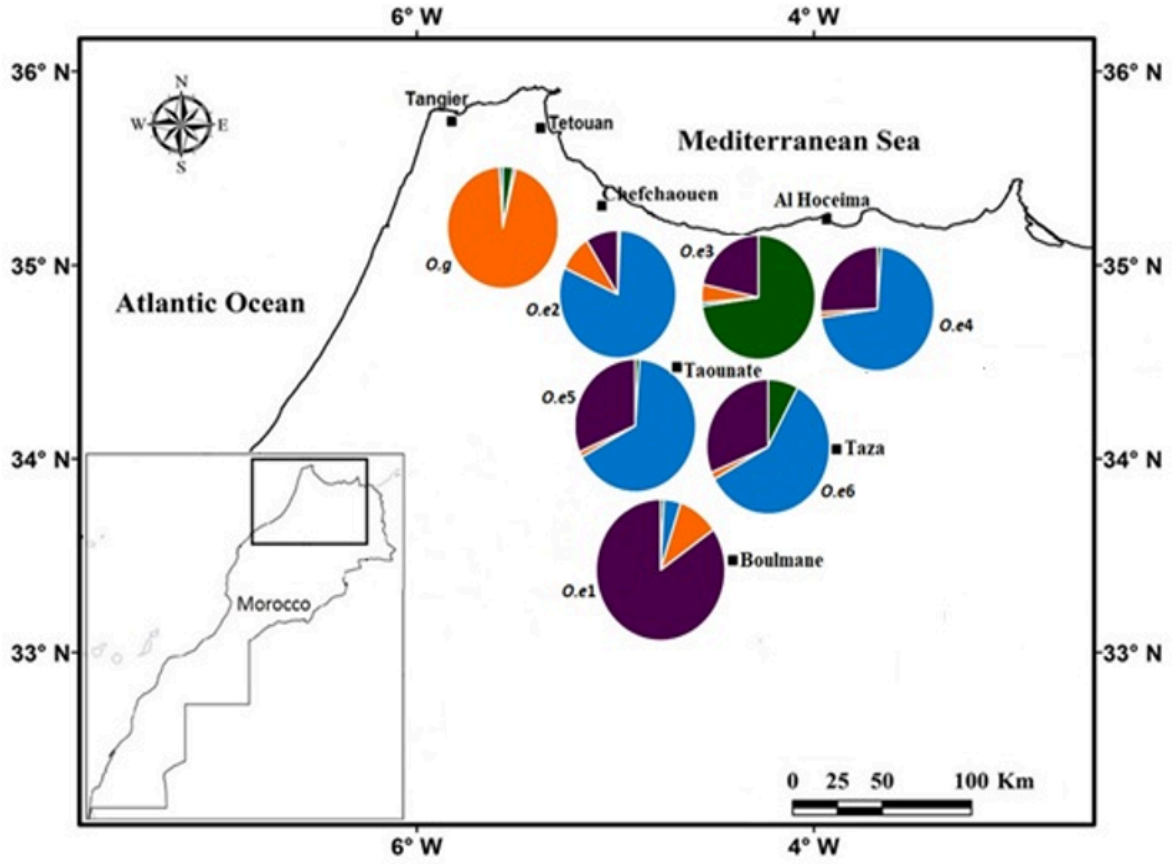


Figure 3

Spatial distribution and inferred genetic structure of *O. elongatum* and *O. grosii* across seven populations. Pie charts indicate the average cluster membership proportions for each population, based on the Bayesian clustering results at K = 4, as determined by STRUCTURE (corresponding to Fig. 2b).