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Conservation genetics and status of *Orania sylvicola* palms in Singapore – native relicts or historical re-introductions?

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ABSTRACT. *Orania sylvicola* (Griff.) H.E.Moore is a large, single-stemmed palm species widely distributed across Malesia. It is the only species of *Orania* present in Peninsular Malaysia and Singapore. In Singapore, the species is rare and critically endangered, although a full understanding of its distribution is hampered by sparse historical collections. However, conflicting records on the known localities in the literature, along with a recent discovery of a substantial population of *Orania sylvicola* within the Tyersall Learning Forest in Singapore Botanic Gardens, have led to questions on the origin of the extant populations of *O. sylvicola*. There are known instances of past introductions of non-native plant species in Singapore, which raised suspicions that some of the individuals were historical reintroductions from overseas. Genetic analyses, a comprehensive survey of existing literature and herbarium collections, and a topographical study of existing *Orania sylvicola* populations were carried out to determine the origin of these individuals in Singapore. Our results suggested that all *Orania sylvicola* individuals in Singapore are of native origin, with two populations present. The distribution of the two populations overlaps in the Singapore Botanic Gardens, which may indicate that some of the plants have been transferred to the Botanic Gardens from another location in Singapore. Preliminary results also revealed that sampled populations within Peninsular Malaysia and between Peninsular Malaysia and Singapore are highly differentiated from each other with very little gene flow either by pollen or seed dispersal. Our study highlights how the integration of genetic information, historical collections and topographical analyses can contribute to assessing the origin and conservation potential of endangered plant species in Singapore and provide insights into suitable conservation actions for *Orania sylvicola*.

Keywords. Arecaceae, Arecoideae, Bukit Timah Nature Reserve, ddRADseq, population genetics, Singapore Botanic Gardens

Introduction

Orania sylvicola (Griff.) H.E.Moore is a large, single-stemmed palm species which is distributed from South Thailand and across western Malesia, but is absent from the Philippines, Sabah and Sulawesi. The species can grow to a diameter of about 20 cm and a height of more than 20 m, which sometimes exceeds the height of the surrounding vegetation and makes it a conspicuous sight in the wild. Across its range it is found across a wide variety of habitats, from wet tropical rainforests to heath and coastal forests, with an altitudinal range from sea level to 600 m above sea level (Keim & Dransfield, 2012).

Orania sylvicola, like other members of its genus, is a monoecious species with both staminate and pistillate flowers borne on the same inflorescence. The staminate and pistillate flowers are arranged in groups of threes, or triads, at the base of the inflorescence rachis, while the apical portion of the inflorescence rachis may only be represented by paired or single staminate flowers. The fruits are dull green turning a greenish-yellow when mature, and are large, measuring c. 5 cm in diameter with a fibrous layer surrounding a large endocarp of 3–4 cm in diameter (Keim & Dransfield, 2012). The dispersal of the species is known to be mediated by animals, with observations of wild pigs in West Java consuming the fruits (Keim & Dransfield, 2012), and *Orania sylvicola* seeds have been found in elephant dung in Temengor Forest Reserve in Perak, Peninsular Malaysia (Tan et al., 2021). *Orania* species in Papua New Guinea have been recorded to be dispersed by wild pigs, cassowaries, and introduced mammals such as deer. The association of various *Orania* species near creeks and streams have also led to the hypothesis that water is also a means of dispersal for this genus (Keim & Dransfield, 2012).

In Peninsular Malaysia and Singapore, *Orania sylvicola* is the only *Orania* species present (Keim & Dransfield, 2012). *Orania sylvicola* is found across Peninsular Malaysia and on offshore islands such as Pulau Tioman and Pulau Tinggi (Henderson, 1930; Turner & Yong, 1998). Whitmore (1998) mentions that the species “could be found all over Peninsular Malaysia on hillsides in lowland rainforest. It is often seen along roadsides from a travelling car, especially on the main Titiwangsa range, with a large colony at the 44 ¾ mile on the north side of the Sungai Selangor along the Fraser’s Hill Road”. Kenneth Er (pers. obs.) has also observed large colonies of the palm on hillsides along Tapah Road towards Cameron Highlands from the 9th to 24th kilometre-mark, following after Sungei Batang on both sides of the valley. This is similar to that observed by Ridley in his account of *Orania sylvicola* sightings along the same road in the early 1900s (Ridley, 1909).

In Singapore, there have been conflicting records on the presence of the species, and sparse herbarium collections dating back to the 1890s and 1930s. Ridley, one of the former directors of the Singapore Botanic Gardens (SBG), wrote in *The Flora of Singapore* that *Orania sylvicola* was rare in Singapore (Ridley, 1900). Two specimens of the species were collected by Ridley in 1891—one from Chan Chu Kang (the current day Nee Soon Freshwater Swamp) and the other from Pulau Ubin. In a later publication, Ridley (1925) noted that the species was rare in Chan Chu Kang and more common in Pulau Ubin, and also present in Woodlands although this cannot be verified as the record is not accompanied by a herbarium specimen. However, there are no extant plants of *Orania sylvicola* at these three localities (K.Y. Chong, pers. comm.; K.B.H. Er, pers. obs.). The next herbarium record of *Orania sylvicola* was by Kiah bin Mohamed Salleh who managed Lawn Z of SBG in 1933. After this there were no collected specimens until more recently (the last five years). The lack of documentation has contributed to the poor understanding of the historical distribution of this palm in

Singapore. This could be attributed to the logistical difficulties of collecting specimens from large solitary palms (Dransfield, 1986). Consequently, the literature often cites these herbarium specimens as evidence of the presence of this species, but without further information on its present-day distribution.

In recent times, *Orania sylvicola* has been recorded from the Bukit Timah Nature Reserve (BTNR) (LaFrankie et al., 2005; Turner & Chua, 2011; Ho et al., 2019) and at SBG within the Rain Forest, Corner House Gate (Lawn Y), Marsh Gardens (Lawn Z) and Lawn F (Turner, 1990; Merklinger, 2016; Taylor, 2016). In 2017, the development and opening of the Tyersall Learning Forest of SBG led to the discovery of a substantial population of *Orania sylvicola* within it (Er et al., 2017). It is possible that there could still be individuals of *Orania sylvicola* to be discovered in Singapore, especially in the offshore islands that are less surveyed.

These recent records, which contrast with the collection localities recorded in the sparse specimen record, have raised questions on the origin of the present-day populations of *Orania sylvicola*. This is because the majority of the plants are at SBG, a site of long-standing botanical collection, plant cultivation and horticulture, with an abundance of non-native plant species and populations imported to its Living Collections. The second current day locality, BTNR is also known to have a history of plant introductions linked to plants collected from Peninsular Malaysia by E.J.H. Corner who worked at SBG from 1929 to 1945; many of these plants survive to date (Leong-Škorničková & Boyce, 2015; Niissalo et al., 2016; Niissalo & Choo, 2021; Choo et al., 2021). There are no historical records of introduction of *Orania sylvicola* from abroad. At SBG, most of the palm introductions have consisted of iconic, economic or ornamental palms such as *Elaeis guineensis* Jacq., *Licuala grandis* (T.Moore) H.Wendl. and *Lodoicea maldivica* (J.F.Gmel.) Pers. (Merklinger, 2016). Due to lack of evidence to the contrary, *Orania sylvicola* is currently considered native and Critically Endangered in Singapore (Lindsay et al., 2022). The native status of the species needs confirmation, and if the species is truly native, the population structure of the species can guide species recovery work, such as through the identification of the most genetically diverse individuals for propagation and ex-situ conservation (Griffith et al., 2021).

In this study, we sampled individuals from all existing *Orania sylvicola* populations in Singapore, with representatives across different life stages from seedlings to subadults to reproductively mature individuals. Given the possibility of the introduction of *Orania sylvicola*, we extended the sampling to a small sample of individuals from Johor, Kepong and Cameron Highlands in Peninsular Malaysia. This allowed us to check if the populations in Peninsular Malaysia formed a distinct population from the Singapore individuals. We studied the genetic diversity, population structure and parentage among the samples. We also examined the literature records comprehensively for all reports on *Orania sylvicola* in Singapore. A topographic and hydrological study of current day populations of *Orania sylvicola* species in Singapore was also done to see if samples were distributed along a certain landscape or hydrology type.

Methods

Field survey and collection

A total of 34 *Orania sylvicola* individuals were sampled. Of these, 25 were collected from Singapore, with six from BTNR (consisting of one sub-adult and one reproductively mature tree with four of its seedlings) and 19 from SBG including all individuals from across the Gardens (consisting of five from the Lawns, two in the SBG Rain Forest, and two cultivated seedlings in Palm Valley) except for the Tyersall population where a sample of 10 individuals of various sizes were sampled. The remaining nine samples were obtained from Peninsular Malaysia, with six samples from Cameron Highlands, two from the wild population at Forest Research Institute Malaysia at Kepong, Selangor, and one from a nursery in Muar, Johor. The sampled leaf material for each individual was dried in silica gel and stored at SBG. Herbarium specimens from selected individuals were deposited at the SING herbarium, and accession numbers of specimens from SBG were also listed. The heights of all sampled individuals from Singapore are also recorded as a proxy of their life stage. These details are listed in Table 1. A map of all individuals sampled from SBG is provided in Fig. 1.

Molecular work

Genomic DNA extraction was carried out using the CTAB method (Doyle & Doyle, 1978) and double-digest Restriction Associated DNA (ddRAD) sequencing libraries were prepared with the same protocol as in Choo et al. (2022), using the restriction enzymes ApeKI and PstI. These methods were based on the protocol first published in Peterson et al. (2012) and modified by Niissalo et al. (2018, 2020). The prepared libraries were pooled in equimolar amounts for sequencing, and the sequencing was done by NovogeneAIT Genomics (Singapore) using Illumina Novaseq.

Population genetics analysis

The sequencing data was demultiplexed using the “process_radtags” command in Stacks 1.37 (Catchen et al., 2013), following which the programme was also used for sequence assembly and single nucleotide polymorphism (SNP) calling using the function “denovomap” and “populations”. Loci with a minimum coverage of 15 were processed, with no missing data allowed and one SNP per locus reported using the option --write-random-snps.

For the dataset consisting of all individuals from Singapore and Peninsular Malaysia, 2,257 SNPs were recovered. A Neighbour-Net figure with uncorrelated p-distances was prepared using the Neighbour-Net function in SplitsTree v. 4.7.1 (Huson & Bryant, 2006). A STRUCTURE analysis (Pritchard et al., 2000) was also run to find out the optimal number of genetic clusters (K value) within the Singapore specimens. All 2,257 available SNPs were used, with a burn-in of 10,000 and 100,000 Markov Chain Monte Carlo (MCMC) repetitions, and values of K from one to eight, which is three more than the expected number of five populations as inferred from the Neighbour-Net figures (Evanno et al., 2005). STRUCTURE was run a total of 30 times for each value of K, and the optimal value of K was verified by calculating the mean

Fig. 1. Map of the *Orania sylvicola* specimens collected from the Singapore Botanic Gardens. Text and dot colours represent the assigned population of each individual. (Image: Google Earth)

log likelihood of K, L(K), and the standard deviation of L(K) for each value of K, and the ΔK for K=2 to K=8 using the R package pophelper (Francis, 2017). Fixation index (F_{ST}) values were also generated across the pairs of different populations to show the degree of genetic differentiation between the pairs of groups. F_{ST} is the probability of randomly drawing two alleles that are identical by descent from a sub-population and is used as an indication to measure the extent of inbreeding among sub-populations (Frankham et al., 2010).

For the dataset comprising only of the Singapore specimens, 1,467 loci were recovered. The same set of analyses as the previous paragraph was done to generate a Neighbour-Net figure and a STRUCTURE plot, and obtain the optimal value of ΔK, except that the maximum value of K for the STRUCTURE and ΔK analyses was K=5 as this was three more than the expected number of two populations. The F_{IS} value or inbreeding coefficient was calculated for all Singapore specimens as a whole, and then for each Singapore population to find out if there is an excess of homozygotes from self-pollination. The percentage of heterozygous loci in each individual was also assessed as a measure of genetic diversity. A scatterplot of the percentage of heterozygous loci against height, which is a proxy of the life stage of the individuals, was done to assess the genetic diversity of the palms across various life stages from seedlings to reproductively mature individuals.

Two approaches were taken to assess the number of parent-offspring pairs in the samples set. An exclusion-based parentage analysis which counts the number of differences between the homozygous loci of two individuals was carried out to find support for the suspected parent-offspring pairs and to detect unknown pairs within the sequenced samples. If there were no mismatches (value of 0 or close to 0), a parent–child relationship is feasible. This pattern can be expected if the two samples are clones (the same genetic individual), or if they have a parent-child relationship (assuming a diploid, sexually reproducing organism). In our dataset, we consider pairs with a value of 0 to be clones or parent and child pairs, while those with 1 or 2 errors per 1000 SNPs (value of 0.001 to 0.002) can also be considered parent-offspring pairs; pairs with higher shared values could be siblings or more distantly related individuals. For each parent-offspring pair, the percentage of heterozygosity was compared to determine if the offspring were produced by selfing or outcrossing.

A maximum-likelihood parentage analysis was also carried out using the software Cervus 3.0.7 (Kalinowski et al., 2017), with 11 out of 25 individuals sampled with a height of 10 m or more arbitrarily recognised as candidate parents, since the shortest known fertile individual in the dataset was 12 m tall. The proportion of candidate parents sampled was set to 0.5, taking into account there were other potential parents not sampled in the Tyersall population, and the genotyping error rate was set at the default value of 1%. All parentage assignments with 95% or more confidence were reported in the Results section.

Historical collections and records of **Orania sylvicola** *in Singapore*

We consulted specimens collected from Singapore in K and SING (herbarium acronyms follow Thiers (continuously updated)), to understand the historical distribution and potential cultivation history of *Orania sylvicola* in Singapore. We also studied floristic accounts and books concerning the distribution of the species, especially from former SBG directors and staff in order to reconcile any mentions of the occurrence of the species in Singapore or in Peninsular Malaysia.

Mapping of species occurrence to topographic and hydrological characteristics

The GPS-mapped positions of the sampled wild *Orania sylvicola* specimens were overlaid against present day topographic contours and surface hydrological flows to visualise possible associations between their occurrence and topography. Surface flow analysis was performed using the 'Fill', 'Flow Direction' and 'Flow Accumulation' geoprocessing tools in ArcGIS Pro 2.8.2 on a Digital Elevation Model (DEM) with 1 m resolution using land survey data from the Singapore Land Authority in 2014 and contour lines from the National Parks Board in 2014 for localities in SBG and BTNR respectively. The resulting surface flow raster was converted to a polyline feature illustrating the direction of low to high water flow according to the topography. To produce contour lines, the DEM was resampled to a 5 m resolution raster and converted to contour lines at 10 m intervals. Both surface flow and contour line features were smoothened for visual representation.

Table 1. Details of the 34 samples in the population genetics analysis. SBG = Singapore Botanic Gardens; BTNR = Bukit Timah Nature Reserve.

Sample	**Country**	**Locality**	**Collector**	**Voucher**	**Accession**	**Height (m)**	**Notes**
OS001	Peninsular Malaysia	Cameron Highlands	Ang, W.F. et al.	Not vouchered	NA	Not recorded	
OS003	Peninsular Malaysia	Cameron Highlands	Ang, W.F. et al.	SING2022-607	NA	Not recorded	
OS004	Peninsular Malaysia	Cameron Highlands	Ang, W.F. et al.	Not vouchered	NA	Not recorded	
OS005	Peninsular Malaysia	Cameron Highlands	Ang, W.F. et al.	Not vouchered	NA	Not recorded	
OS006	Peninsular Malaysia	Cameron Highlands	Ang, W.F. et al.	Not vouchered	NA	Not recorded	
OS031	Peninsular Malaysia	Cameron Highlands	Ang, W.F. et al.	Not vouchered	NA	Not recorded	
OS042	Peninsular Malaysia	Kepong	Saw, L.G.	Not vouchered	NA	Not recorded	
OS043	Peninsular Malaysia	Kepong	Saw, L.G.	Not vouchered	NA	Not recorded	

Table 1 (continued).

Sample	Country	Locality	Collector	Voucher	Accession	Height (m)	Notes
OS044	Peninsular Malaysia	Johor	Zaki Jamil	Not vouchered	NA	Not recorded	Purchased from Chong Lee Nursery, Muar
OS007	Singapore	SBG Lawn Y	Choo, L.M. et al.	SING2022-683	LY 20160255*A	12	
OS008	Singapore	SBG Lawn Y	Choo, L.M. et al.	Not vouchered	LY. No accession.	6	Seedling of OS007
OS009	Singapore	SBG Lawn Y	Choo, L.M. et al.	Not vouchered	LY. No accession.	3	Seedling of OS007
OS011	Singapore	SBG Lawn Z	Choo, L.M. et al.	Not vouchered	LZ 00/9247*A	21	
OS012	Singapore	SBG Lawn G	Choo, L.M. et al.	SING2022-684	LG 00/6119*A	23	
OS013	Singapore	SBG Tyersall	Choo, L.M. et al.	Not vouchered	TY/FOR/A 20172640*V	15	
OS014	Singapore	SBG Tyersall	Choo, L.M. et al.	Not vouchered	TY/FOR/A 20172640*Y	15	

Table 1 (continued).

Sample	Country	Locality	Collector	Voucher	Accession	Height (m)	Notes
OS015	Singapore	SBG Tyersall	Choo, L.M. et al.	Not vouchered	TY/FOR/A 20172640*O	10	
OS016	Singapore	SBG Tyersall	Choo, L.M. et al.	Not vouchered	TY/FOR/A 20172640*AD	8	
OS017	Singapore	SBG Tyersall	Choo, L.M. et al.	Not vouchered	TY/FOR/A 20172640*E	6	
OS018	Singapore	SBG Tyersall	Choo, L.M. et al.	Not vouchered	TY/FOR/B 20172640*AZ	4.5	
OS019	Singapore	SBG Tyersall	Choo, L.M. et al.	Not vouchered	TY/FOR/A 20172640*CB	2.5	
OS020	Singapore	SBG Palm Valley	Choo, L.M. et al.	Not vouchered	Palm Valley A/ARECO 20211843*A	1.2	Cultivated
OS021	Singapore	SBG Palm Valley	Choo, L.M. et al.	Not vouchered	Palm Valley A/ARECO 20211843*B	1.2	Cultivated
OS022	Singapore	SBG Rain Forest	Choo, L.M. et al.	Not vouchered	FOREST/M4 20192775*A	15	

Table 1 (continued).

Sample	Country	Locality	Collector	Voucher	Accession	Height (m)	Notes
OS023	Singapore	SBG Rain Forest	Choo, L.M. et al.	Not vouchered	FOREST/V3 20202598*A	10	
OS025	Singapore	SBG Tyersall	Choo, L.M. et al.	Not vouchered	TY/FOR/A 20172640*F	10	
OS026	Singapore	SBG Tyersall	Choo, L.M. et al.	Not vouchered	TY/FOR/A 20172640*AC	9	
OS027	Singapore	SBG Tyersall	Choo, L.M. et al.	Not vouchered	TY/FOR/B 20172640*AI	12	
OS029	Singapore	BTNR	Choo, L.M. et al.	SING2019-559	NA	6	
OS030	Singapore	BTNR	Choo, L.M. et al.	Not vouchered	NA	15	
OS035	Singapore	BTNR	Choo, L.M. et al.	Not vouchered	NA	0.8	Seedling of OS030
OS036	Singapore	BTNR	Choo, L.M. et al.	Not vouchered	NA	0.9	Seedling of OS030

Table 1 (continued).

Sample	**Country**	**Locality**	**Collector**	**Voucher**	**Accession**	**Height (m)**	**Notes**
OS037	Singapore	BTNR	Choo, L.M. et al.	Not vouchered	NA	1	Seedling of OS030
OS038	Singapore	BTNR	Choo, L.M. et al.	Not vouchered	NA	0.7	Seedling of OS030

Results

Population genetics analysis

Comparison of population genetics from Singapore and Peninsular Malaysia

The Neighbour-Net figure from SplitsTree (Fig. 2) showed that all individuals from Singapore formed a cluster separate from any of the Peninsular Malaysia individuals sampled, with a slight split of two populations among the Singapore specimens. Similarly, the STRUCTURE plots from K=2 to K=5 (Fig. 3) showed the hierarchical structure present in the data, firstly with a split at K=2 representing the samples from Peninsular Malaysia and Singapore, and then at K=4 which shows that the three Peninsular Malaysia populations are more different to each other than the populations within Singapore. Finally, at K=5, the substructure present in the Singapore dataset is revealed and each of the five known populations are resolved. The highest value of ΔK is obtained when K=2 (Fig. 4D) which shows that the strongest split in genetic clusters is found when the samples are split into two clusters. While it is not possible to calculate the ΔK value for K=1, we can see a clear split in the specimens from Singapore and Peninsular Malaysia, followed by finer-scale splits based on the localities within each country.

F_{ST} values (Table 2) of all Singapore-Peninsular Malaysia pairs and across Peninsular Malaysia population pairs showed values of 0.46 to 0.78, which suggests strong genetic differentiation. F_{ST} values range from 0 to 1, with 0 showing completely no differentiation whereas an F_{ST} value of 1 shows complete differentiation. However, this may be an artefact of small sample sizes, especially in Peninsular Malaysia; small sample size can lead to an overestimation of F_{ST} (Willing et al., 2012). Between the two Singapore populations, the F_{ST} value of 0.15 shows slight differentiation, and a further study of the population differentiation in Singapore was taken by focusing on the Singapore samples.

Patterns of population differentiation within Singapore

For the dataset of Singapore specimens alone, the Neighbour-Net figure (Fig. 5) split into two main populations. Within the two populations the samples were very similar to each other and showed little genetic differentiation as evidenced by the lack of extensive branching in the network. The STRUCTURE analysis of the 1,467 loci at K=2 (Fig. 6) also showed a similar split into two populations; although some individuals (OS022, 025, 029) seem slightly admixed, the lack of an admixture signal in the Neighbour-Net figure shows that this is rather an artefact of the high level of genetic similarity within each population, and any slight differentiation leading to a signal of it being assigned to the other population. To assess the optimal value of K, the following values, the mean likelihood of K (Fig. 7A), along with the first and second derivative of the mean likelihood (Figs. 7B, 7C) were calculated to derive the ΔK value. The highest value of ΔK is obtained when K=2 (Fig. 7D), which implies that the most likely number of populations in the dataset is two. It is not possible to calculate the ΔK value for K=1, but from the earlier Neighbour-Net plots (Figs. 2, 5) there is

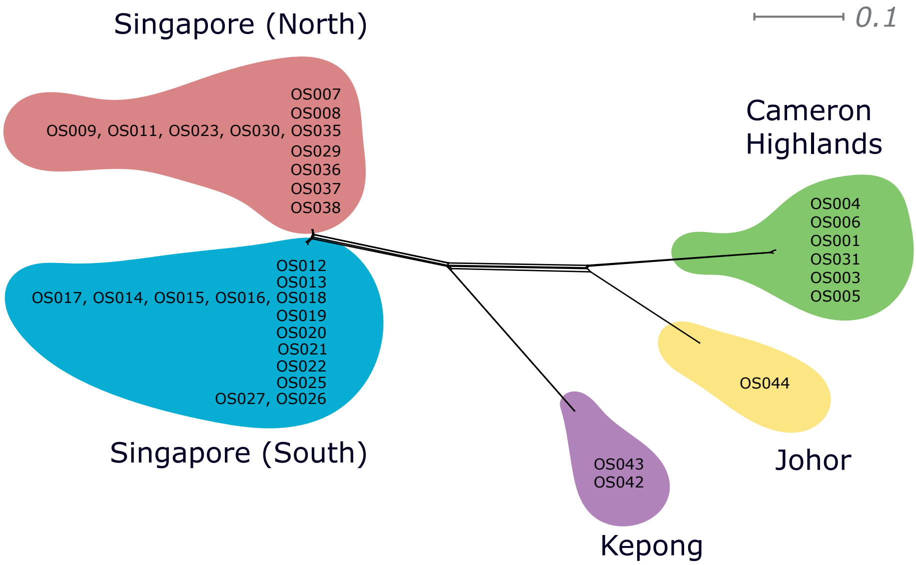


Fig. 2. Neighbour-Net figure showing population differentiation among *Orania sylvicola* samples from Singapore and Peninsular Malaysia.

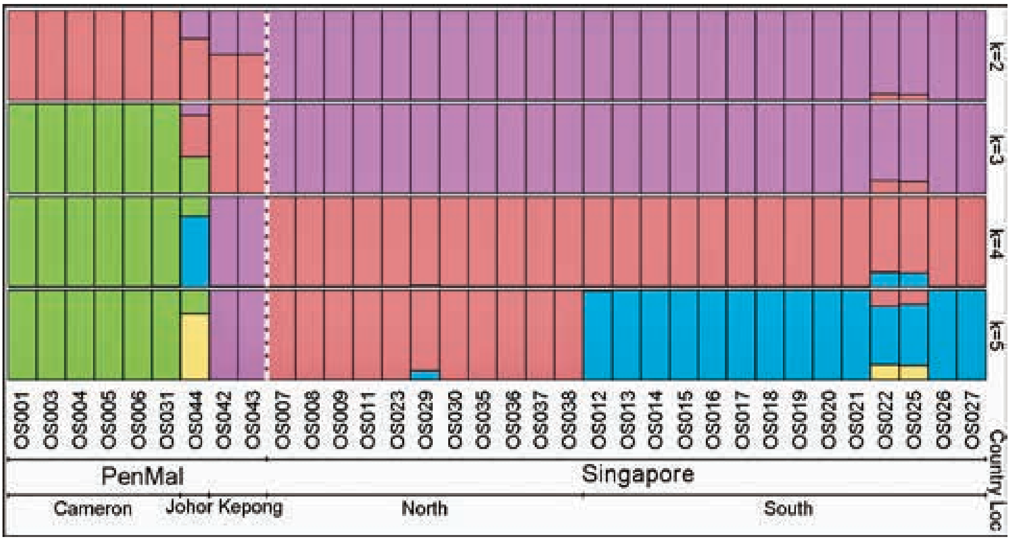


Fig. 3. STRUCTURE output showing the population structure of all *Orania sylvicola* samples from Peninsular Malaysia and Singapore, with values of K from 2 to 5. The country “Country” and locality “Loc” information are indicated below the sample names.

a clear split of two main populations within the Singapore specimens, hence we can conclude that K=2 would best explain the patterns seen in the data.

In essence, there are two slightly differentiated populations in Singapore that are detectable in both the Neighbour-Net and STRUCTURE results; these populations are here called the “North” and “South” populations.

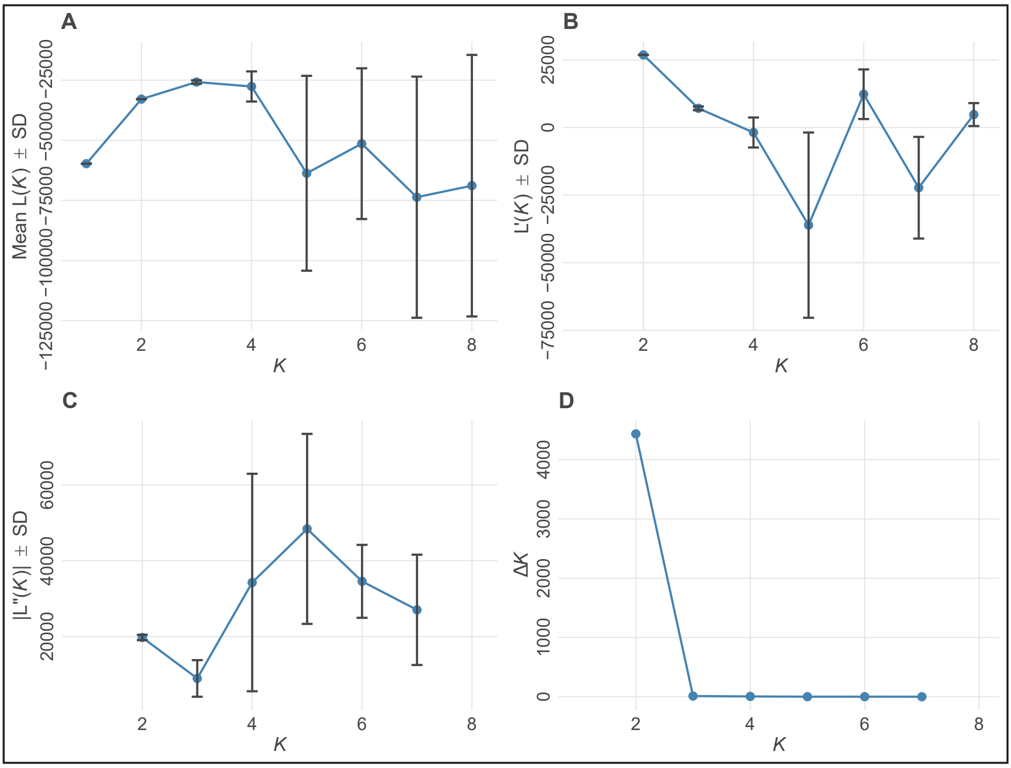


Fig. 4. Series of plots resulting from the Evanno method on the 30 iterations of STRUCTURE conducted on all (Singapore and Peninsular Malaysia) specimens, for K=2 to K=8. **A.** Mean log likelihood of the data at each K value with standard deviation. **B.** The first derivative or rate of change of the mean log likelihood at each value of K. **C.** The second derivative of the mean log likelihood at each value of K. **D.** ΔK value for the values of K=2–7. The highest value of ΔK at K=2 shows that 2 is the optimal value of K for the STRUCTURE analysis.

Genetic diversity

The F_{IS} value or inbreeding coefficient calculated for the entire Singapore population was 0.0323, while the values for the "North" population was -0.0018, and that of the "South" population was -0.0309. F_{IS} ranges from -1 to 1, with -1 indicating an excess of heterozygosity, and 1 indicating an excess of homozygosity due to inbreeding. All calculated values of F_{IS} were very close to 0, which suggests that heterozygosity was near expected levels for both populations. The percentage of heterozygous loci across all Singapore specimens varied from 7% to 48%, with a general trend that heterozygosity was higher in tall individuals than in seedlings 5 m or less high (Fig. 8). The South population has more individuals and higher average heterozygosity than the less numerous North population.

Table 2. Table of pairwise F_{ST} values between the five populations from Singapore and Peninsular Malaysia.

	Cameron Highlands	**Singapore (North)**	**Singapore (South)**	**Kepong**	**Johor**
Cameron Highlands	-	0.697	0.551	0.784	0.635
Singapore (North)	-	-	0.148	0.624	0.601
Singapore (South)	-	-	-	0.461	0.461
Kepong	-	-	-	-	0.675

Fig. 5. Neighbour-Net figure showing population differentiation among *Orania sylvicola* samples from Singapore, with names and locality of samples belonging to the North and South populations respectively.

Parentage analysis

The exclusion-based parentage analysis (Fig. 9) supports our initial suspected parent-offspring pairs: OS007 is the parent of OS008 and OS009 (all specimens from SBG Lawn Y); and OS030 is the parent of four seedlings collected near it (OS035–038) (all specimens from BTNR except for OS029). There are also two other clusters that can be identified from the results. The first is a cluster of two individuals, OS011 (from SBG Lawn Z) and OS023 (from SBG Rain Forest) (OS011 is larger of the two and

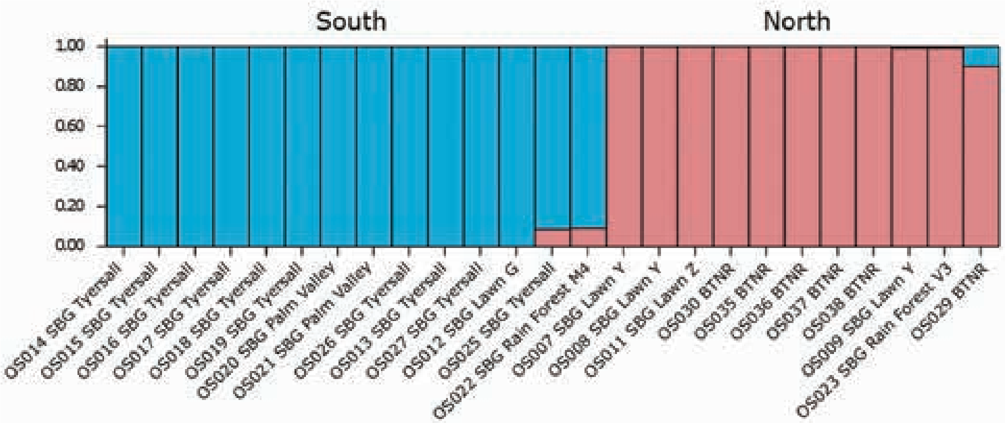


Fig. 6. STRUCTURE output showing the population structure of *Orania sylvicola* samples from Singapore, at K=2.

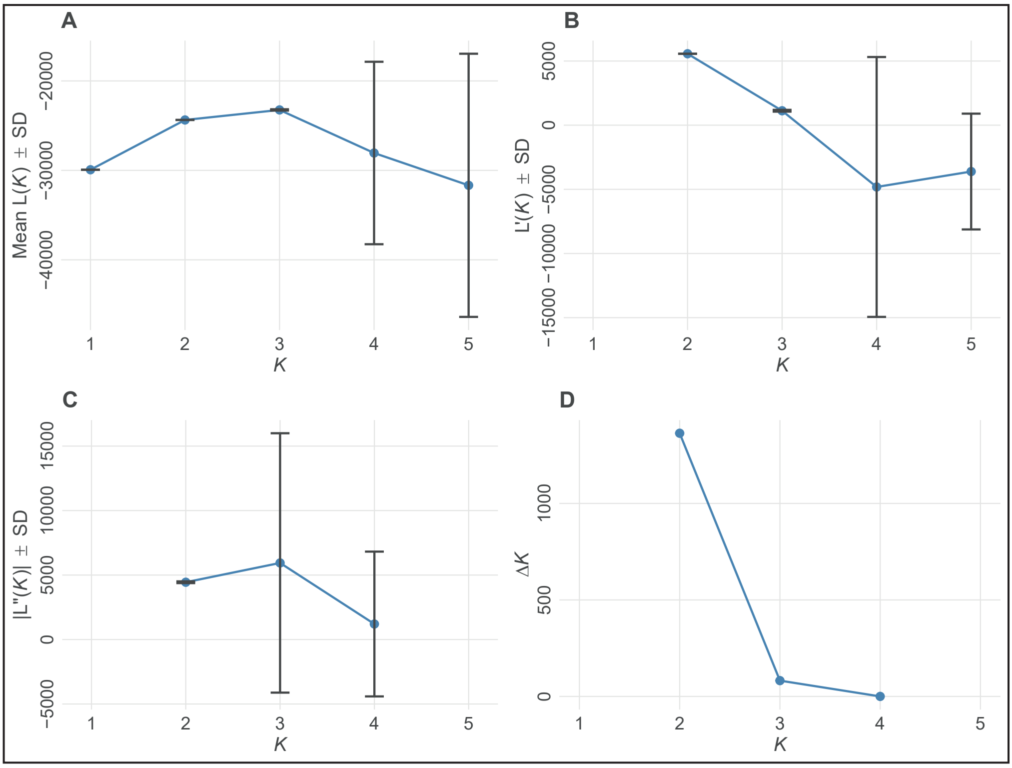


Fig. 7. Series of plots resulting from the Evanno method on the 30 iterations of STRUCTURE conducted on all 25 Singapore specimens, for K=2 to K=5. **A.** Mean log likelihood of the data at each K value with standard deviation. **B.** The first derivative or rate of change of the mean log likelihood at each value of K. **C.** The second derivative of the mean log likelihood at each value of K. **D.** ΔK value for the values of K=2–7. The highest value of ΔK at K=2 shows that 2 is the optimal value of K for the STRUCTURE analysis.

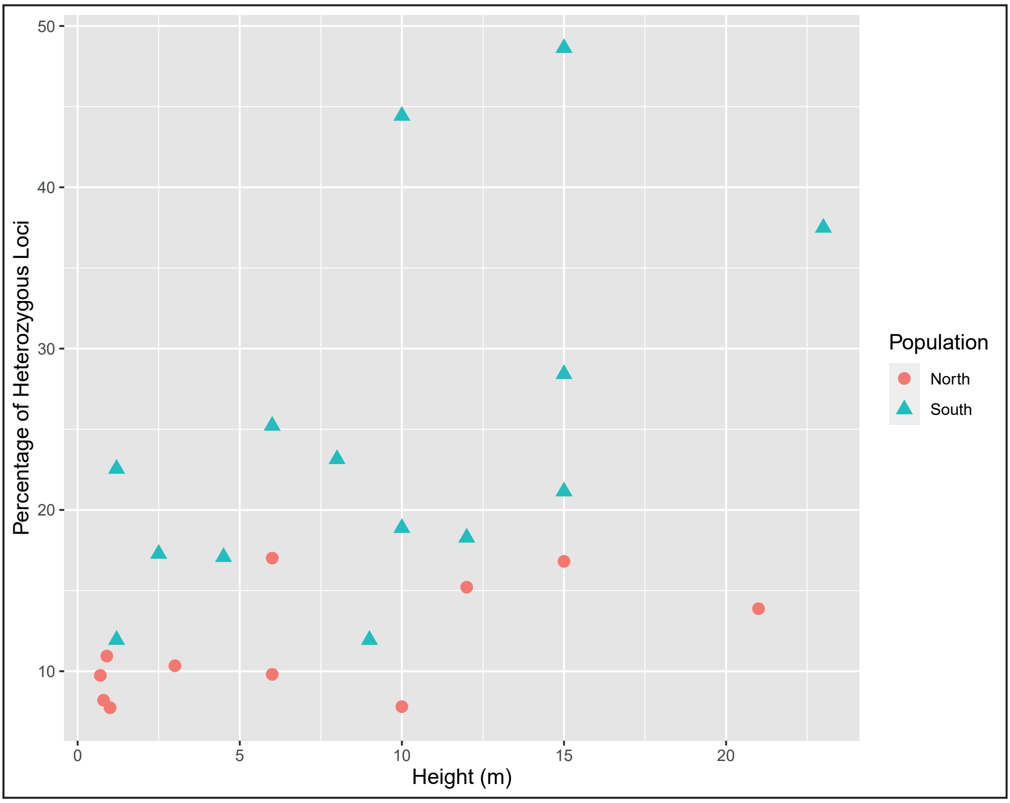
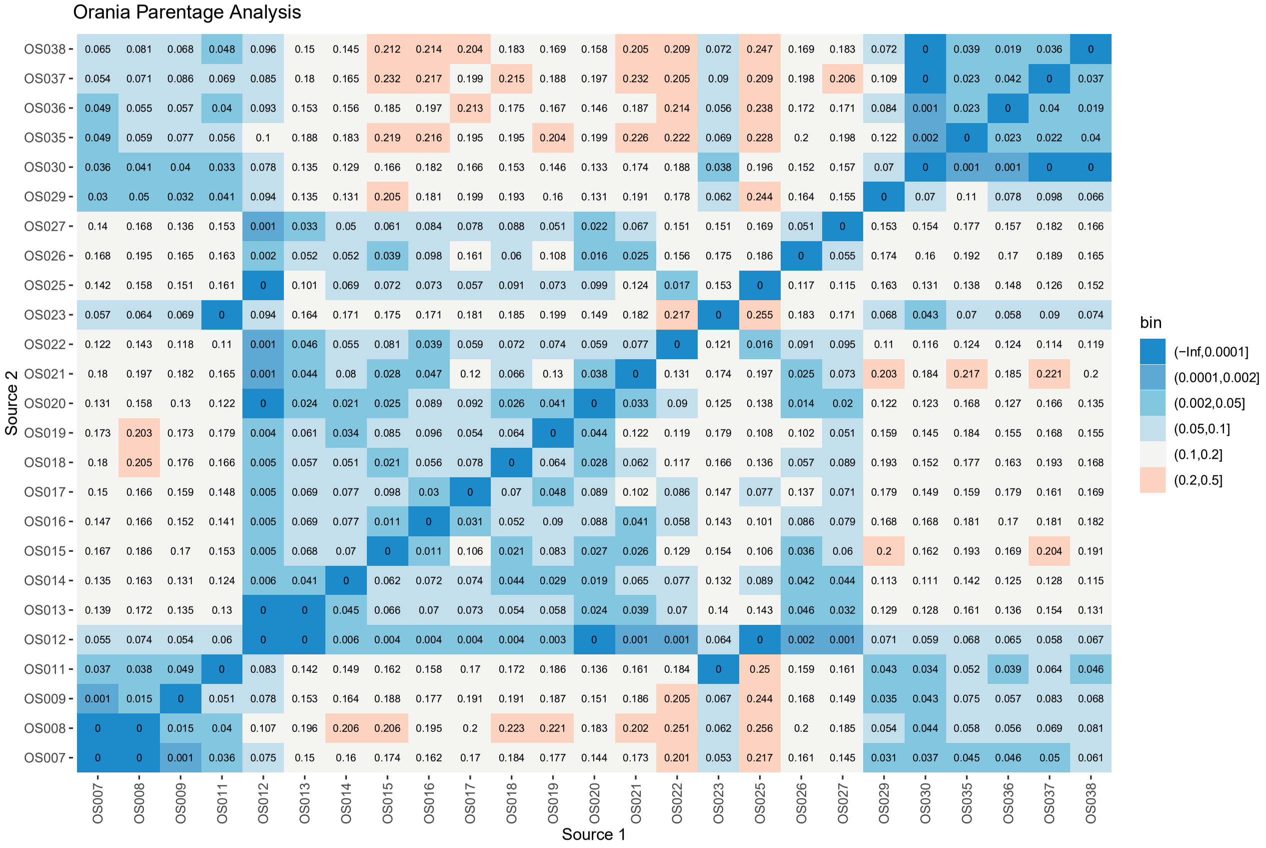


Fig. 8. Scatter plot showing the relationship between the percentage of heterozygous loci and height for all Singapore samples, with individuals from the "North" and "South" populations indicated by the shape and colour of the point.

more likely to be a parent), while the second is a cluster of 8 individuals, comprising OS012 (from SBG Lawn G) (a large plant and a likely parent) with several smaller palms: 013, 020, 021, 022, 025, 026, and 027 (from SBG Tyersall and the SBG Rain Forest).

The parentage analysis using CERVUS 3.0.7 highlighted the same parent-offspring pairs with additional suggested parent-offspring pairs. Table 3 details the offspring with their putative parent pairs identified by the program, along with their percentage of heterozygous loci (also known as "heterozygosity"), comparison of the results with the exclusion-based method, and whether they were selfed or outcrossed by looking at the percentage of heterozygous loci. Out of the 14 offspring identified in both methods, eight of them had the same results for both the direct exclusion-based method and CERVUS. For the remaining six, only one parent was identified with direct exclusion, but two parents were identified using CERVUS. As CERVUS did not take into account the life stage of the samples, there were some biologically irrelevant results where seedlings (e.g., OS020, OS021) were assigned as parents; and where a shorter individual (OS025) was listed as a parent of a taller individual (OS022). These exceptions have been noted in Table 3.



	OS007	OS008	OS009	OS011	OS012	OS013	OS014	OS015	OS016	OS017	OS018	OS019	OS020	OS021	OS022	OS023	OS025	OS026	OS027	OS029	OS030	OS035	OS036	OS037	OS038
OS038	0.065	0.081	0.068	0.048	0.096	0.15	0.145	0.212	0.214	0.204	0.183	0.169	0.158	0.205	0.209	0.072	0.247	0.169	0.183	0.072	0	0.039	0.019	0.036	0
OS037	0.054	0.071	0.086	0.069	0.085	0.18	0.165	0.232	0.217	0.199	0.215	0.188	0.197	0.232	0.205	0.09	0.209	0.198	0.206	0.109	0	0.023	0.042	0	0.037
OS036	0.049	0.055	0.057	0.04	0.093	0.153	0.156	0.185	0.197	0.213	0.175	0.167	0.146	0.187	0.214	0.056	0.238	0.172	0.171	0.084	0.001	0.023	0	0.04	0.019
OS035	0.049	0.059	0.077	0.056	0.1	0.188	0.183	0.219	0.216	0.195	0.195	0.204	0.199	0.226	0.222	0.069	0.228	0.2	0.198	0.122	0.002	0	0.023	0.022	0.04
OS030	0.036	0.041	0.04	0.033	0.078	0.135	0.129	0.166	0.182	0.166	0.153	0.146	0.133	0.174	0.188	0.038	0.196	0.152	0.157	0.07	0	0.001	0.001	0	0
OS029	0.03	0.05	0.032	0.041	0.094	0.135	0.131	0.205	0.181	0.199	0.193	0.16	0.131	0.191	0.178	0.062	0.244	0.164	0.155	0	0.07	0.11	0.078	0.098	0.066
OS027	0.14	0.168	0.136	0.153	0.001	0.033	0.05	0.061	0.084	0.078	0.088	0.051	0.022	0.067	0.151	0.151	0.169	0.051	0	0.153	0.154	0.177	0.157	0.182	0.166
OS026	0.168	0.195	0.165	0.163	0.002	0.052	0.052	0.039	0.098	0.161	0.06	0.108	0.016	0.025	0.156	0.175	0.186	0	0.055	0.174	0.16	0.192	0.17	0.189	0.165
OS025	0.142	0.158	0.151	0.161	0	0.101	0.069	0.072	0.073	0.057	0.091	0.073	0.099	0.124	0.017	0.153	0	0.117	0.115	0.163	0.131	0.138	0.148	0.126	0.152
OS023	0.057	0.064	0.069	0	0.094	0.164	0.171	0.175	0.171	0.181	0.185	0.199	0.149	0.182	0.217	0	0.255	0.183	0.171	0.068	0.043	0.07	0.058	0.09	0.074
OS022	0.122	0.143	0.118	0.11	0.001	0.046	0.055	0.081	0.039	0.059	0.072	0.074	0.059	0.077	0	0.121	0.016	0.091	0.095	0.11	0.116	0.124	0.124	0.114	0.119
OS021	0.18	0.197	0.182	0.165	0.001	0.044	0.08	0.028	0.047	0.12	0.066	0.13	0.038	0	0.131	0.174	0.197	0.025	0.073	0.203	0.184	0.217	0.185	0.221	0.2
OS020	0.131	0.158	0.13	0.122	0	0.024	0.021	0.025	0.089	0.092	0.026	0.041	0	0.033	0.09	0.125	0.138	0.014	0.02	0.122	0.123	0.168	0.127	0.166	0.135
OS019	0.173	0.203	0.173	0.179	0.004	0.061	0.034	0.085	0.096	0.054	0.064	0	0.044	0.122	0.119	0.179	0.108	0.102	0.051	0.159	0.145	0.184	0.155	0.168	0.155
OS018	0.18	0.205	0.176	0.166	0.005	0.057	0.051	0.021	0.056	0.078	0	0.064	0.028	0.062	0.117	0.166	0.136	0.057	0.089	0.193	0.152	0.177	0.163	0.193	0.168
OS017	0.15	0.166	0.159	0.148	0.005	0.069	0.077	0.098	0.03	0	0.07	0.048	0.089	0.102	0.086	0.147	0.077	0.137	0.071	0.179	0.149	0.159	0.179	0.161	0.169
OS016	0.147	0.166	0.152	0.141	0.005	0.069	0.077	0.011	0	0.031	0.052	0.09	0.088	0.041	0.058	0.143	0.101	0.086	0.079	0.168	0.168	0.181	0.17	0.181	0.182
OS015	0.167	0.186	0.17	0.153	0.005	0.068	0.07	0	0.011	0.106	0.021	0.083	0.027	0.026	0.129	0.154	0.106	0.036	0.06	0.2	0.162	0.193	0.169	0.204	0.191
OS014	0.135	0.163	0.131	0.124	0.006	0.041	0	0.062	0.072	0.074	0.044	0.029	0.019	0.065	0.077	0.132	0.089	0.042	0.044	0.113	0.111	0.142	0.125	0.128	0.115
OS013	0.139	0.172	0.135	0.13	0	0	0.045	0.066	0.07	0.073	0.054	0.058	0.024	0.039	0.07	0.14	0.143	0.046	0.032	0.129	0.128	0.161	0.136	0.154	0.131
OS012	0.055	0.074	0.054	0.06	0	0	0.006	0.004	0.004	0.004	0.004	0.003	0	0.001	0.001	0.064	0	0.002	0.001	0.071	0.059	0.068	0.065	0.058	0.067
OS011	0.037	0.038	0.049	0	0.083	0.142	0.149	0.162	0.158	0.17	0.172	0.186	0.136	0.161	0.184	0	0.25	0.159	0.161	0.043	0.034	0.052	0.039	0.064	0.046
OS009	0.001	0.015	0	0.051	0.078	0.153	0.164	0.188	0.177	0.191	0.191	0.187	0.151	0.186	0.205	0.067	0.244	0.168	0.149	0.035	0.043	0.075	0.057	0.083	0.068
OS008	0	0	0.015	0.04	0.107	0.196	0.206	0.206	0.195	0.2	0.223	0.221	0.183	0.202	0.251	0.062	0.256	0.2	0.185	0.054	0.044	0.058	0.056	0.069	0.081
OS007	0	0	0.001	0.036	0.075	0.15	0.16	0.174	0.162	0.17	0.184	0.177	0.144	0.173	0.201	0.053	0.217	0.161	0.145	0.031	0.037	0.045	0.046	0.05	0.061

Fig. 9. Pairwise parentage analysis for all *Orania sylvicola* samples from Singapore. "Source 1" is the putative parent, and "Source 2" is the putative offspring. A lower score (dark blue) indicates that for each homozygous loci in the parent, the offspring has at least one of the same alleles at the same loci, and hence a lower number of mismatched alleles. The higher the score, the higher the number of mismatches and less likely the two individuals share a parent-offspring relationship.

Table 3. Proposed parents of each assigned offspring from CERVUS results with a confidence of at least 95%, the heterozygosity (H) of each individual, comparison with the direct exclusion method (Fig. 9) and the offspring status. Biologically irrelevant assignments from CERVUS are marked with an (*) asterisk. H_o = Offspring heterozygosity; H_{P1} = Parent 1 heterozygosity; H_{P2} = Parent 2 heterozygosity.

Offspring	H_o	Parent 1	H_{P1}	Parent 2	H_{P2}	Direct exclusion result	H ratio	Offspring status
OS008	9.81	OS007	15.2	NA	NA	Same	0.65	Selfed
OS009	10.34	OS007	15.2	NA	NA	Same	0.67	Selfed
OS013	21.15	OS012	37.5	NA	NA	Same	0.56	Selfed
OS020	22.55	OS012	37.5	OS026	11.94	OS012 listed as the sole parent	0.6–1.89	Either outcrossed or selfed
OS021	11.94	OS012	37.5	OS026	11.94	OS012 listed as the sole parent	0.32–1	Either outcrossed or selfed
OS022	48.63	OS012	37.5	OS025*	44.43	OS012 listed as the sole parent	1.30	Outcrossed, but OS025 is not likely to be one of the parents as it is shorter than the offspring OS022
OS023	7.81	OS011	13.9	NA	NA	Same	0.56	Selfed
OS025	44.43	OS012	37.5	OS022	48.63	OS012 listed as the sole parent	0.91–1.18	Outcrossed
OS026	11.94	OS012	37.5	OS021*	11.94	OS012 listed as the sole parent	0.32	Likely selfed (OS021 is not one of the parents as it is still a seedling)

Table 3 (continued).

Offspring	H_o	Parent 1	H_{P1}	Parent 2	H_{P2}	Direct exclusion result	H ratio	Offspring status
OS027	18.28	OS012	37.5	OS020*	22.55	OS012 listed as the sole parent	0.49	Likely selfed (OS020 is not one of the parents as it is still a seedling)
OS035	8.21	OS030	16.8	NA	NA	Same	0.49	Selfed
OS036	10.94	OS030	16.8	NA	NA	Same	0.65	Selfed
OS037	7.74	OS030	16.8	NA	NA	Same	0.46	Selfed
OS038	9.74	OS030	16.8	NA	NA	Same	0.58	Selfed

The exclusion-based shared alleles method checks for parent-offspring relationships based on patterns of Mendelian inheritance but does not consider the probability of mutation at the tested loci. We have included an arbitrary error rate of 1–2 errors per 1000 SNPs for one generation (i.e. a parent-offspring pair). While maximum likelihood approaches such as CERVUS are known to be less arbitrary as it allows for a particular confidence level (in our case 95%) to be defined, here we had to estimate that we sampled 50% of all candidate parents in Singapore. This was to account for the unsampled parents from the Tyersall population and this percentage was estimated based on observations of the population, although a full census of the number of reproductively mature offspring in the population was not carried out. As such, both techniques are influenced by their own errors and we accept the parentage assignments for offspring which are supported by both methods, either with one or both parents in agreement (Table 3).

For each parent-offspring pair, the ratio of heterozygosity between the two individuals was calculated (Table 3). If the heterozygosity in the offspring was close to 0.5 to that of the parent, it is likely that the offspring is a result of selfing. OS022 and OS025 had the highest heterozygosity amongst the samples overall, suggesting outcrossing, and they also stand out in our STRUCTURE plots (Figs. 2, 6), where they show a low level of potential introgression with populations of *Orania* in Peninsular Malaysia. It is possible that their other parent is an undetected *Orania* of cultivated origin; alternatively, they might be a result of introgression between the North and South populations. OS020 and OS021 had a range of heterozygosity values depending on which putative parent they were compared to, but as the results of CERVUS and direct exclusion did not agree on whether there was one or two parents, it was not possible to conclude if they were selfed or outcrossed. The rest of the offspring have lower heterozygosity values closer to 0.5, which means that they were likely selfed.

Historical records and collections

Mentions of *Orania sylvicola* in Singapore in historical records and the literature are detailed in Table 4, while data relating to *Orania sylvicola* specimens collected from Singapore are listed in Table 5.

Mapping the occurrence of **Orania sylvicola** *to topography and hydrology*

The populations of *Orania sylvicola* at Tyersall and BTNR (Table 1, Fig. 1), were mapped. In both populations, *Orania sylvicola* occurs in areas of high water flow (Figs. 10, 11A, 11B). It is possible that the extant individuals have been dispersed to their current locations from upstream parents by hydrochory, and/or that the species establishes better along streams.

Table 4. Historical records and literature mentioning the presence of *Orania sylvicola* in Singapore.

Source	**Description of *Orania sylvicola* in Singapore**
Murton (1881)	"Frequent in Singapore, notably at Captain Ross's residence near the Botanic Gardens" (present-day Tyersall Learning Forest, Fig. 12)
Cantley (1887)	"List of the Forest Department Palmetum, Singapore… Genus *Orania*… *O. macrocladus*" (part of present-day National Orchid Gardens (NOG) and near to Lawn Z in SBG)
Fox (1889)	"List of Plants in Lawn J… *Orania macroclada*" (Lawn J in this context refers to the Palmetum i.e. part of present-day National Orchid Gardens (NOG) and near to Lawn Z in SBG)
Ridley (1900)	"Rare in Singapore. Some trees at Chan Chu Kang. More abundant in Pulau Ubin." (Chan Chu Kang refers to present-day Nee Soon Freshwater Swamp)
Ridley (1925)	In Singapore present in "Chan Chu Kang; Woodlands; Pulau Ubin"
Turner (1990)	Recorded from Lawn F and Lawn Z at the Singapore Botanic Gardens
Turner et al. (1996)	Present in Nee Soon Swamp Forest
LaFrankie et al. (2005)	Listed as present in the 2-ha Centre for Tropical Forest Science (CTFS) plot in Bukit Timah Nature Reserve, but this could not be located by M.A. Niissalo (pers. obs.), suggesting a misidentification or local extirpation
Turner & Chua (2011)	Present in Bukit Timah Nature Reserve
Keim & Dransfield (2012)	"In Singapore *O. sylvicola* used to be found on mainland Singapore and a small offshore island (Ubin). However, this is based on reports and specimens collected by Ridley in 1891 (see Ridley 1900) at a time when a large part of Singapore was still covered with lowland tropical rainforest. The presence of *O. sylvicola* in Singapore and the surrounding islands has never been reported again."

Table 4 (continued).

Source	**Description of *Orania sylvicola* in Singapore**
Loo et al. (2015)	"*Orania sylvicola* is critically endangered with a few individuals left in the wild in Singapore. It is a distinctive single-stemmed feather palm in the Catchment Nature Reserve[*], as it is the only native canopy arecoid palm that is not spiny." [*]Likely refers to the Central Catchment Nature Reserve that includes the Nee Soon Swamp Forest, from which *Orania sylvicola* was historically known to occur based on herbarium specimens but is no longer extant
Merklinger (2016)	"The [SBG] rain forest is home to several rattan species as well as two *Orania sylvicola*, which could have always been there"
Taylor (2016)	"A third palm represented by large, old specimens is the native, but now very rare, Ibul (*Orania sylvicola*), found in the Gardens' Rain Forest as well as near Corner House Gate, where its progeny are also thriving"

Table 5. Herbarium specimens of *Orania sylvicola* collected from Singapore.

Collector and number	**Date**	**Locality**	**Notes**	**Herbarium and barcode number**
Ridley 3144	1891	Chan Chu Kang		SING [SING0017047; SING0017048]; K [K000667894]
Ridley 3146	1891	Pulau Ubin		SING [SING0017045; SING0017046]; K [K000667895]
Kiah SFN 26132	8 Jan 1933	SBG Lawn Z	40–50 ft tall	SING [SING0039293]; K [K000667916]

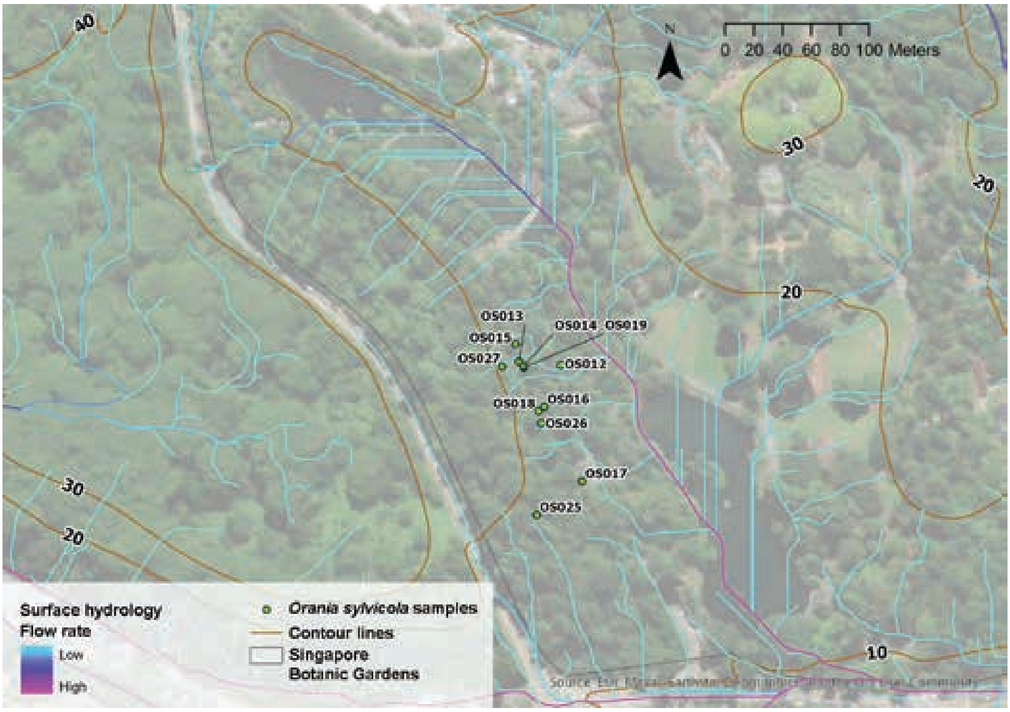


Fig. 10. The occurrence of sampled *Orania sylvicola* specimens at the south-western end of the Singapore Botanic Gardens (grey outline), mapped against hydrological flows based on surface topography.

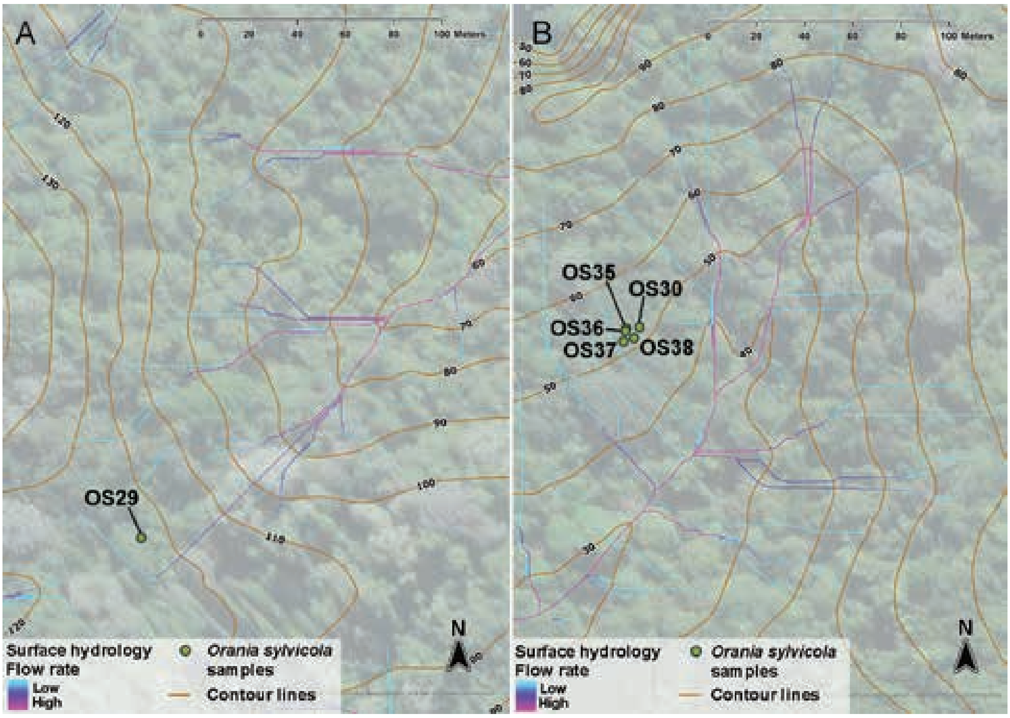


Fig. 11. The occurrence of sampled *Orania sylvicola* specimens at the Bukit Timah Nature Reserve, mapped against hydrological flows based on surface topography.

Discussion

The origin of **Orania sylvicola** *in Singapore*

There is some genetic differentiation of *Orania sylvicola* into two populations in Singapore. However, this differentiation is much weaker than between the samples in Singapore and those from Peninsular Malaysia, which suggests that the Singapore plants form their own genetic unit. This separation is supported by the pairwise F_{ST} values across all populations (Table 2). While the F_{ST} values between the two countries could have been overestimated because of the small sample sizes, their separation is also obvious in the Neighbour-Net plot (Fig. 2) as well as in the STRUCTURE analysis (Figs. 3, 4).

As the species was under-represented in the herbarium specimen record, the literature sources became critical to understanding its historical distribution. In particular, Murton (1881) mentions that *Orania sylvicola* was "frequent in Singapore, notably at Captain Ross's residence near the Botanic Gardens". Captain John Dill Ross was a trader who lived at a house called the "Woodneuk" in Tyersall and he recounted selling his house to the then Sultan of Johor (Ross, 1897). The house became known as Istana Woodneuk, and old maps from the National Archives of Singapore showed that it was located in Tyersall Learning Forest (Fig. 12). This early record of the Tyersall population, coupled with the lack of records of the species being cultivated in Singapore, indicates that the existing *Orania sylvicola* at Tyersall Learning Forest is very likely part of the original vegetation. Since all other Singapore specimens are more closely related to the Tyersall specimens than the Peninsular Malaysia specimens, we can conclude that both North and South populations are of in-situ origin. Based on this evidence, we conclude that all the plants of *Orania sylvicola* in Singapore are of native origin.

Likely source populations and human-mediated dispersal

The South population is mainly located in the Tyersall Learning Forest in the southern part of SBG with a few individuals located elsewhere in SBG. As most of the individuals in this population are located at Tyersall Learning Forest, it can be inferred that the source of this population is at the Tyersall Learning Forest itself. All other individuals located elsewhere in SBG are a result of dispersal (human-mediated or otherwise) from this source population. The North population is found in both BTNR and the northern part of SBG, specifically in Lawn Y (Corner House Gate), Lawn Z (Marsh Garden) and plot V3 of the SBG Rain Forest. Between BTNR and SBG, it is more likely that BTNR is the source population, given that there is slightly more genetic diversity among the BTNR individuals (Fig. 5). In addition, Lawn Y and Lawn Z were known sites of plant introduction, as Lawn Y was the site of an old experimental nursery (Fox, 1889), while Lawn Z was close to or part of the Palmetum which contained cultivated palms, and *Orania sylvicola* was also one of the species recorded there (Cantley, 1887; Fox, 1889). However, it is unclear why *Orania sylvicola* has not been collected in BTNR prior to 2019, despite Ridley's collections from other now-extinct *Orania sylvicola* populations in Singapore (Table 5). On the other hand, there is an

Fig. 12. Extract from a map dated between the 1860s and 1870s showing the land where the residence of Captain Ross is located, the earliest mentioned locality of *Orania sylvicola* in Singapore. The road running across the middle-top to bottom called ‘Garden Road’ is today known as Tyersall Road, which had been realigned westward in 2016, and part of what historically used to be the land around Captain Ross’s residence is now within the boundary of the Singapore Botanic Gardens. This same patch of land is where *Orania sylvicola* is found today. (Map reproduced with permission from the Singapore Land Authority)

Orania sylvicola specimen collected by Kiah in 1933 from Lawn Z (Table 5), although it cannot be proven that this is from the same individual that still exists today. As such, while there are no herbarium specimens or documentation from BTNR, it was likely that the North population was introduced to the SBG, leading to both populations being represented in SBG.

Gene flow and potential conservation actions

There is limited genetic mixing between these two populations despite their close proximity in SBG, and only two confirmed incidences of outcrossing (Table 3). As most palms are insect-pollinated, especially within Arecoideae (Henderson, 1986), this suggests that the insect pollinator could be limited by its specific habitat requirement or movement to other *Orania* individuals (e.g., ants). In Singapore, water could play a role in the seed dispersal of *Orania*, as hinted from the prevalence of extant *Orania* populations along streams and areas of high hydrological flows in Tyersall and BTNR, although there are no present-day permanent streams in these areas. Further research is necessary to understand the pollination and dispersal of this species. Another observation is that shorter individuals (with the height as a proxy for life stage) have lower heterozygosity or genetic diversity (Fig. 8), which means that the seedling generation is more inbred than the parental generation.

Conservation actions are required to improve the genetic diversity in the shrinking and increasingly inbred *Orania sylvicola* populations in Singapore. The two identified genetic clusters should be recognised as distinct plant material transfer zones for *Orania sylvicola* in Singapore. Both clusters need to be represented in propagation efforts and local reforestation projects. Seeds of local provenance are preferable for restoration projects to avoid the genetic contamination of local populations, and to increase restoration success through better seedling establishment, survival and growth of locally adapted plant material. Studies of other critically endangered palm species that exist in isolated metapopulations, such as *Beccariophoenix madagascariensis* Jum. & H.Perrier and *Tahina spectabilis* J.Dransf. & Rakotoarin. in Madagascar (Shapcott et al., 2007; Shapcott et al., 2020) have shown that the re-introduction of genetically diverse individuals from other populations will reduce the risk of inbreeding. While it is important to maintain the unique genetic diversity of each population, human-mediated disturbances have disrupted the natural processes maintaining this diversity and the risk of outbreeding depression is unwarranted (Frankham et al., 2011). Conservation measures to facilitate gene flow and increase genetic diversity will be a lifeline for native plant species with limited cross pollination and dispersal capabilities in the midst of anthropogenic disturbances. The presence of native in-situ and ex-situ *Orania sylvicola* individuals within the grounds of the SBG further illustrates the value of botanic gardens' living collections and their potential for the recovery of threatened plant species in the wild (Abeli et al., 2019).

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