diversity

Article

Genetic Evaluation of a Wildtype Population of *Cornus mas* Accessions in Austria

Eduviges G. Borroto Fernandez [1,*], Negar Khayatzadeh [2,†], Gábor Mészáros [2], Simon Fink [1], Veronika Hanzer [1], Johann Sölkner [2] and Margit Laimer [1,*]

[1] Plant Biotechnology Unit, Department of Biotechnology, University of Natural Resources and Life Sciences, 1180 Vienna, Austria
[2] Division of Livestock Sciences, Department of Sustainable Agricultural Systems, University of Natural Resources and Life Sciences, 1180 Vienna, Austria
* Correspondence: eduviges.borroto-fernandez@boku.ac.at (E.G.B.F.); margit.laimer@boku.ac.at (M.L.)
† Current address: SUISAG Allmend 8, CH-6204 Sempach, Switzerland.

Abstract: *Cornus mas* grows in the "Cornelian Cherry Valley" in Lower Austria. The characterization of the biodiversity of this underutilized crop was undertaken to increase its visibility and to develop breeding parameters. To determine the genetic relationships among Austrian Cornelian cherries, a collection of 447 mainly wild accessions originating from four regions in Lower Austria were genotyped. Analyses of seven microsatellite loci detected a total of 74 alleles, with 10.57 being the average number per locus. Shannon's index was calculated to be 0.95 in locus CM008 and 1.80 in locus CM020. The average values of observed and expected heterozygosity were 0.54 ± 0.11 and 0.70 ± 0.12. The analyses revealed a high level of diversity. All accessions could be distinguished from each other, and wild and cultivated accessions could be separated. The pattern of grouping of the accessions based on the STRUCTURE (version 2.3.4) analyses suggested a greater number of subpopulations (K = 9). Karyotyping consistently yielded diploid chromosome numbers 2n = 2x = 18 for a millennial accession. The evaluations yielded a high degree of diversity, which provides excellent starting conditions for considerations of breeding strategies for the genetic enhancement of *C. mas* in Austria.

Keywords: genetic diversity; Cornelian cherry; population structure; admixture; SSRs

Citation: Borroto Fernandez, E.G.; Khayatzadeh, N.; Mészáros, G.; Fink, S.; Hanzer, V.; Sölkner, J.; Laimer, M. Genetic Evaluation of a Wildtype Population of *Cornus mas* Accessions in Austria. *Diversity* **2023**, *15*, 1031. https://doi.org/10.3390/d15101031

Academic Editor: Genlou Sun

Received: 29 August 2023
Revised: 14 September 2023
Accepted: 20 September 2023
Published: 24 September 2023

1. Introduction

Cornus is a genus of about 70 species of woody plants in the *Cornaceae* family, commonly known as dogwoods, most of which are used as ornamental plants, distinguished by their blossoms, berries, and distinctive bark [1].

Cornelian cherry (*Cornus mas* L.), together with *C. officinalis*, is one of the few species grown for the organoleptic characteristics of its fruits and its medicinal properties [2–6] The fruits are commonly olive-shaped, but they can also be found as being cylindrical, spherical, and pear-shaped [7], carrying a stony kernel. They are usually shiny red, varying in intensity from dark red and light red to yellow [8].

Cornelian cherry is a shrub or small tree distributed over Southern and Central Europe, the Black Sea basin, and the Caucasus [9,10] which occupies forest margins, woodland clearings, and light-exposed sites. Today, the Cornelian cherry is a minor wild fruit tree in Western Europe, but it represents a massively exploited natural resource in the Black Sea region. It is a very ancient crop plant in Ukraine and was mainly distributed by monasteries [10].

In the Late Iron Age, wild fruits like Cornelian cherry, together with *Crataegus* sp. and *Prunus spinosa*, contributed to the daily diet of settlements in Southern and Central Europe and can now be considered part of the so-called synanthropic vegetation [11]. The presence and importance of *C. mas* in the Danube region as a follower of human settlements (and thus as a highly valued forest species) has been described, even in the archeological literature.

There are indications that the red color of the fruits played an important role in mortuary practice [12]. In the Mesolithic cremation graves of Vlasac in the Danube Gorges, Cornelian cherry stones were recovered in high numbers, suggesting that Cornelian cherries could have had a role in complex funerary rituals performed by Mesolithic communities (9500 to 6500 BC). The dual function of Cornelian cherries in daily life at Vlasac, as a fresh fruit and a source of oil, was extended to the ritual sphere [12]. In Tuscany, the use of Cornelian cherries was also reported as being widespread in the Early Bronze Age and in the Etruscan period (3500–1000 BC) [13]. *C. mas* was described as a species that thrives well in floodplain habitats [14], which is in contradiction to the assumption that the plant is drought-tolerant and can grow and produce a valid yield under drought conditions. In fact, in the very dry years of 2017 and 2018, it was not possible to collect fruits from about half of the accessions. According to Werneck [14], *C. mas* has existed as a wild fruit but also as a cultivated fruit from the Neolithic period until 1850, i.e., for more than 4000 years.

In 1960, Werneck [14] stated that *C. mas* is conspicuously neglected in botany as well as in horticulture, which has led to its inclusion in the list of neglected and underutilized species (NUS) [15–17].

Given its natural area of distribution, its native status in many areas of Central Europe and the Eastern Alps has been doubted. Hegi [18] described *C. mas* as an element of the Southern European Pontic flora, which seems to have spread into Central Europe after the last Ice Age from Southeast to Central Europe through the Danube Gorges, a view that has been corroborated by archeological findings [12,19,20]. The Cornelian cherry has native status in Lower Austria [14].

Presently, the largest area of diversity of *C. mas* is in Lower Austria at the northern edge of the Flysch zone from Klosterneuburg to Amstetten and at the eastern edge of the Vienna basin; here, accessions with black-purple, red, yellow, and white fruits can be found [8,14]. In the Lower Austrian valleys of Pielach, Gölsen, and Triesting, Cornelian cherries, popularly named “Dirndl”, are spread in hills, open pastures, and meadows [8,16]. In the region, known as the “Cornelian cherry valley” (Dirndltal in German), active intervention to preserve and characterize the biodiversity of *C. mas* is being implemented [16,21] as changing climatic conditions have a considerable impact on wild-growing plants.

The selection and acceptance of the Cornelian cherry as an important healthy functional food [15,21–23] have been approached by establishing an in vitro collection of valuable germplasms of centenary Cornelian cherries [16], in parallel to a thematic garden as ex situ collection (https://info.bml.gv.at, accessed on 28 August 2023). In addition, the phenotypic characterization of a wildtype population of *C. mas* was carried out on fruits to evaluate their nutritional values [21,24].

Population genetics analyses can provide data on a variety of important evolutionary parameters, including standing levels of genetic variation, the partitioning of this variability within/between populations, overall levels of inbreeding, selfing versus outcrossing rates, effective population sizes, and the dynamics of recent population bottlenecks [25]. Beyond providing basic evolutionary insights, such analyses are also an important tool for developing effective management strategies for endangered and/or invasive species [26,27]. Owing to their codominant and highly polymorphic nature, simple-sequence repeats (SSRs) have been widely utilized in the study of genetic variation [28].

Phenotypic characterization by microsatellites has been used for inferring genetic diversity and population structure of species [25,29]. They have been used to determine DNA diversity, gene flow, and genetic relationships in *Cornus* species [30–33]. Perceiving the potential of Cornelian cherries in Austria, available genomic markers (SSRs) [32] were used to assess the genetic diversity of 447 accessions from Lower Austria.

Since the different fruit sizes might be related to increasing levels of ploidy, karyotyping of a millennial *C. mas* accession [16] was performed. This will provide useful information to develop novel commercial cultivars from a locally adapted population. The characteristics considered to be desirable attributes are mainly based on different fruit shapes, smaller stones, and valuable bioactive chemical composition [21,32].

2. Material and Methods

2.1. Plant Material

The population of Cornelian cherry (*C. mas* L.) compared in this study included 447 individuals. Plants were selected from 41 sites at different altitudes (156 to 600 m above sea level), in 4 regions in Austria: 25 sites in the Pielach Valley (P) 5 sites in the Gölsen Valley (G), 9 sites in the Traisen Valley (T) and included two control populations in the Vienna region (V). The age of the plants was estimated from the information provided by the respective owners (Table 1 and Figure 1) [16]. The oldest plant is 1000 years old and therefore was chosen for karyotyping. Also, the growth habit of the individual accessions (upright or pending branches, shrub, or tree shape) was registered.

Table 1. A total of 447 samples were collected from 41 different sites at different altitudes in 4 regions: Pielach Valley (P) Gölsen Valley (G), Traisen Valley (T), and Vienna region (V). The orientation of the slope was mostly S (South), with only 2 sites oriented towards N (North).

Site	Region	Altitude	Exposition	Nr of Plants	Age of Trees (Years)
1	T	383	Forest border/S	9	100–150
2	P	320	Open pasture/S	10	50–200
3	P	344	Open pasture/S	9	100–200
			Forest border/S	5	
4	P	371	Plantation, not irrigated/S	12	25–100
5	T	521	Plantation, irrigated/S	5	>50
	T	521	Open pasture/S	5	25–100
6	P	344	Border/S	7	>100
7	P	372	Border/S	6	50–100
			Open pasture/S	7	
8	G	369	Border/S	15	100–200
9	G	369	Border/S	11	>200
10	G	600	Open pasture/S	20	>200
11	P	371	Border/S	15	100–400
		471	Border/S	15	
12	P	572	Hilltop	10	100–200
13	P	600	Border/S	6	100–400
			Hilltop	6	
14	P	600	Border/S	9	50–200
			Hilltop	10	
15	P	580	Border/S	6	200–400
			Hilltop	6	
16	P	600	Border/S	12	50–100
17	P	320	Open pasture/S	2	>100
18	T	350	Open pasture/S	5	100–200
19	T	350	Open pasture/S	11	100–200
20	T	350	Open pasture/S	4	100–200
21	T	420	Border/S	6	100–200
		350		6	

Table 1. *Cont.*

Site	Region	Altitude	Exposition	Nr of Plants	Age of Trees (Years)
22	T	570	Border/S	11	>100
23	T	356	Open pasture/S	4	>200
24	P	600	Border/S	10	200–400
			Hilltop	10	
25	P	600	Border and valley/N	12	>100
26	P	372	H steep hill/S	5	100–200
			Open pasture/S	6	
27	V	156	Plantation, not irrigated/S	1	>100
				1	25
28	P	400	Border/S	6	100–200
			Hilltop	5	
29	P	280	Plantation irrigated/S	11	>10
30	G	600	Border/S	6	200–500
			Hilltop	6	
31	P	500	Border/S	8	100–200
			Hilltop	9	
32	P	600	Border/S	3	>200
33	P	550	Open pasture/S	16	50 –200
34	P	600	Open pasture/S	2	>200
35	P	400	Border/S	10	100–200
36	P	400	Open pasture/S	10	100–200
37	P	460	Open pasture/S	5	>400
38	G	450	Open pasture/S	10	100–200
39	T	600	Open pasture/S	2	400–1000
40	V	280	Plantation, irrigated/S	20	>50
41	P	550	Border/N	8	100–200

Plants in these regions represent a landscape-defining element in a predominantly cattle farming and forestry-shaped environment. Centennial individuals are typically found in open pastures, on borders between properties or toward forest areas, on very dry, stony hilltops, and even in closed forest stands. In most cases, these stands are rather steep hillsides with an exposition towards the south, with rather difficult accessibility. In only a few cases, vigorous plants were found in sites with an exposition towards the north or with high humidity (Table 1). A few sites could be considered as plantations, with or without irrigation. They yielded important information during the sampling years exhibiting particular conditions of drought.

The control population consisted of grafted cultivars of *C. mas*, which are very popular in Austria, e.g., 'Bulgarico', 'Flava', 'Kanzanlak', 'Schönbrunner Gourmet' and 'Schumener', but also novel breeding lines and plants from the Botanical Garden Vienna and other sites in the Vienna region, e.g., Lobau, an alluvial forest habitat. One large-fruited sample from Iran was included in the genetic analyses as a potential outgroup.

For any breeding program, it is a prerequisite to select the most appropriate crossing partners. For this purpose, it is necessary to determine the genetic distance/vicinity of the wild-growing population. Since the main aim was to identify the genetic variability of the huge population of *C. mas* present in the region, the selection of the accessions attempted to encompass both positive as well as negative traits that could be observed. Plants were included in the sampling process based on distinguished properties, e.g., attractivity and farmers' preferences or particular traits described as unfavorable, like not fruit-bearing, the formation of black fruits, or exceptional anthocyanin levels. Individuals sampled per site were initially planned to be 10, but finally varied from 2 to 30, reflecting different locations, expositions, and the presence of interesting specimens.

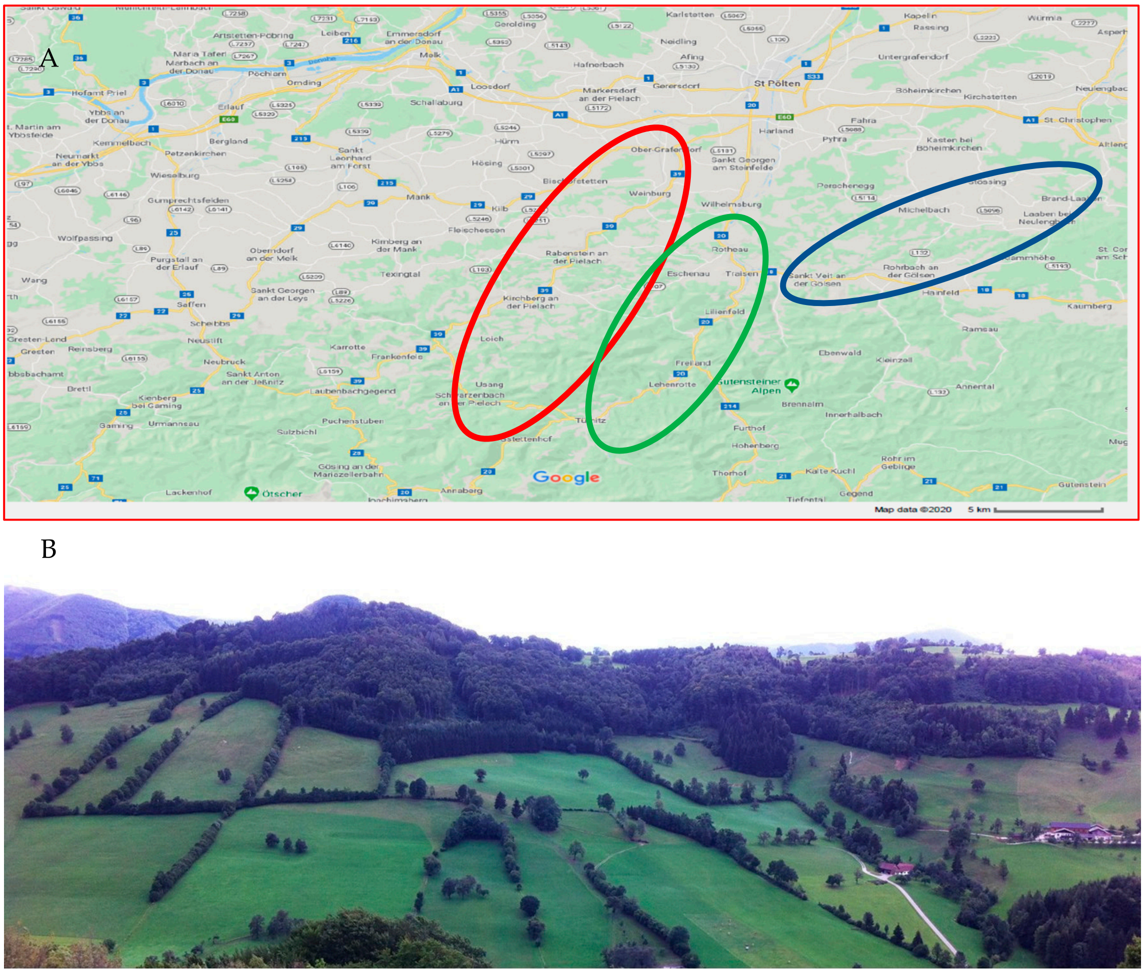


Figure 1. Geographic location of three regions sampled in Lower Austria (**A**) Pielach Valley (red), Traisen Valley (green), and Gölsen Valley (blue); (**B**) Typical exposition of Cornelian cherries in borders (between pastures), free-standing in open pastures and on the forest borders (Table 1).

2.2. DNA Extraction and PCR

Total DNA (gDNA) was extracted from the flower buds of 447 individual plants from 41 different sites (Table 1) using the DNeasy Plant Mini Kit (QIAGEN) according to the supplier's instructions.

Seven gSSRs developed for *C. florida* and *C. mas* [32] (Table 2) were used to screen the gDNA from 447 accessions. PCR was carried out in a total 10 µL reaction mixture using 5 µL of 2XQIAGEN Multiplex (Qiagen, Hilden, Germany), 1 µL containing 2 µM of each primer, and 150–200 ng DNA of total genomic DNA. Optimized conditions (Table 2) were used to amplify microsatellite fragments using an ABI PCR cycler and fragment analyses were performed on an ABI PRISM® 3100 DNA Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sizing of fragments was carried out with a GeneScan 3.7 and Genotyper® 2.0 software (Applied Biosystems Foster City, CA, USA), utilizing the internal GeneScan™ 500 LIZ™ dye Size Standard (Applied Biosystems Foster City, CA, USA).

Table 2. Characteristics of seven SSR loci and size description used in this study, developed for *Cornus florida* (CF) and *C. mas* (CM) [32].

Locus	Primer Sequence (5′-3′)	Repeat Motif	Size Range (bp) [32]	No. of Alleles [32]
CF55	L: tggagtagggcaaaagatcaagag R: tccagggaatgttcggtagattag	$(GT)_7T(TG)_{10}$	144–181	2
CM008	L: tcgttaatgtgaaattggaacg R: caccgtacacgcaaagtcc	$(GT)_{11}$	158–194	6
CM020	L: tggcagactaggttcttgttagc R: ctccactgtctggcttacttgg	$(TG)_{10}$	188–233	2
CM026	L: gaattcatgtaatgttgttgtctgc R: cctgcatataattcaggtaaagagc	$(CA)_{14}$	179–203	7
CM031	L: taccctctcttgctctttgtcc R: aaacaatcaaacccaaacaacc	$(AG)_{26}(TG)_{13}$	191–224	11
CM037	L: aacacagagaaacacgtgcaa R: tggagatctttgaagaacagga	$(TG)_{20}$	172-203	10
CM043	L: gtccacacctgttgttcagc R: ggttgcaatgctttcttggt	$(TG)_{16}(TA)_5$	213–235	8

2.3. *Chromosome Preparation*

The oldest accession in this study (1000 years), a national monument tree [16] served for chromosome preparation. In vitro cultures from the PBU collection, were used for root induction [16]. Feulgen staining [34] modified by Greilhuber and Temsch [35] was performed on root meristems used for metaphase spread preparation [36]. Brightfield microscopy imaging (Olympus BX61 Upright Brightfield Microscope, Center Valley, USA) was conducted using standard objectives and filter sets for ultraviolet blue (DAPI), green (FITC), and red (Cy3) excitation brightfield. The appropriate pseudo-colors were superimposed using the software Cell^F version 2.8 (Olympus, Center Valley, USA). Images were digitally captured at 1000X magnification.

2.4. *Data Analyses*

To reveal the genetic structure of the selected accessions of *C. mas*, a model-based cluster analysis using STRUCTURE version 2.3.4 [29] was used. A burn-in period and Markov chain Monte Carlo (MCMC) repetitions set to 5000 and 10,000, respectively, were implemented. The admixture model with correlated allele frequencies was selected. The range of 2 to 15 discrete ancestral populations was screened (K = 1–15) with three iterations for each. The inference of the best K was performed using the delta K method [37] to determine the most appropriate K value. The results were visualized using Microsoft Excel (2019).

For each of the defined loci, Simple-Sequence Repeats (SSR) allelic composition was determined in 447 accessions. Putative alleles were indicated in alphabetical order (A for the smallest fragment). The program POPGENE version 1.32 [38] was used to calculate allele frequencies at each locus, number of observed allele (Na), effective number of alleles (Ne), Shannon's information index (I), observed (Ho) and expected (He) heterozygosity, measured as described by Levene [39] and Nei [40], inbreeding coefficients (F_{IS}) and Wright's statistics or fixation index (F_{ST}) and gene flow (Nm), estimated from the formula $Nm = 0.25\ (1/F_{ST} - 1)$ by Nei [41]. Nei's genetic identity (I) and genetic distances between populations (D) [42] were also calculated using POPGENE (version 1.32).

3. Results

Plants were visited, samples collected, and data recorded several times a year over the past six years. DNA was successfully extracted from 447 accessions of high quality to allow further analyses.

Microsatellite analyses allowed the observation of 74 alleles across 7 SSR loci for 447 individuals in total. The number of alleles per each SSR locus ranged from 6 (locus CM008) to 18 (locus CM043) (Table 3). On average, the number of alleles per locus was 10.57. Effective numbers of alleles ranged from 2.12 (locus CF55) to 6.14 (locus CM043) and the average value was 3.86. Shannon's index ranged between 0.95 in locus CM008 and 1.80 in locus CM020 (Table 3).

Table 3. Microsatellite characterization for seven loci in 447 *C. mas* accessions.

Locus	No. of Alleles (A)	No. of Effective Alleles (N_e)	Index	Observed heterozygosity (H_o) *	Expected Heterozygosity (H_e) **	F_{ST}	Nm
CF55	8	2.1299	1.0723	0.3964	0.5311	0.0590	3.9890
CM008	6	2.2210	0.9498	0.5203	0.5504	0.1001	2.2473
CM020	12	4.9265	1.7978	0.5103	0.7979	0.1128	1.9655
CM026	8	3.7798	1.5510	0.5000	0.7363	0.0890	2.5596
CM031	10	4.7836	1.7488	0.6937	0.7918	0.0680	3.4267
CM037	12	3.0062	1.4218	0.5135	0.6681	0.1497	1.4197
CM043	18	6.1419	2.1014	0.6689	0.8381	0.0498	4.7714
Mean	10.5714 ± 3.9521	3.8556 ± 1.5062	1.5204 ± 0.4090	0.5433 ± 0.1036	0.7020 ± 0.1227	0.0891	2.5574

Index = Shannon's information index (Lewontin, 1972). * Expected homozygosity and heterozygosity were computed using Levene [39]. ** Nei's [40] expected heterozygosity.

The overall allele frequency across 7 SSR loci and all populations ranged from 0.001 in loci CF55, CM020, CM037, and CM043 to 0.65 in locus CF55.

The expected heterozygosity (H_e) is a fundamental measure of genetic diversity in a population, describing the expected proportion of heterozygous genotypes under Hardy-Weinberg equilibrium [40]. The H_o varied between 0.39 (locus CF55) and 0.69 (locus CM031) and the H_e between 0.53 (locus CF55) and 0.84 (CM043), respectively. The average of observed and expected heterozygosity were 0.54 ± 0.11 and 0.70 ± 0.12 (Table 3).

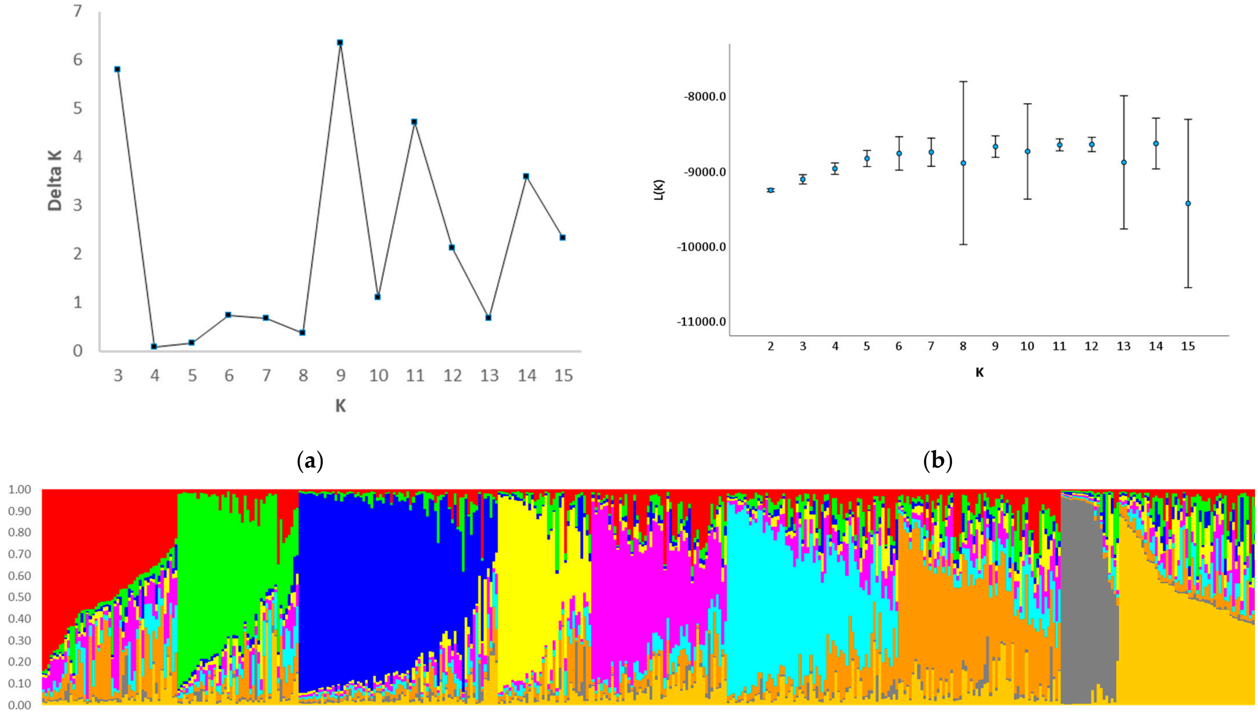
The F_{IS} measures the deficiency or excess of heterozygosity [43,44]. Locus CM031 showed the lowest $F_{IS} = -0.02$ and the highest $F_{IS} = 0.38$ was observed in locus CM026. The fixation index (F_{ST}) was between 0.05 for locus CM043 and 0.15 for locus CM037. Higher levels of gene flow were observed for locus CM043 to be 4.77 and the lowest levels 1.43 were observed for locus CM037.

Based on the genetic diversity measurements (number of effective alleles, Shannon index, expected heterozygosity) CM002 and CM031 show the highest polymorphism in these subpopulations. The expected heterozygosity is higher than the observed heterozygosity except for allele CM008, indicating a smaller effective population size. The F_{ST} is above 0.1 for CM008, CM002, and CM037, indicating more fixation for these alleles rather than the others in these subpopulations.

The results of population structure analyses, assuming the admixture model in STRUCTURE (version 2.3.4), showed an optimum of 9 ancestral populations (K = 9). The individuals in population 4 showed a clearer background, implying less migration and admixture (cross-pollination) compared to the other clusters. Based on genetic distance results from POPGENE (version 1.32) population 4 is identified always as a separate group (outgroup).

Based on the admixture model generated in the software STRUCTURE (version 2.3.4) with an elevated delta (K = 9) (Figure 2), K = 3, K = 4, and K = 11 were also plotted. The

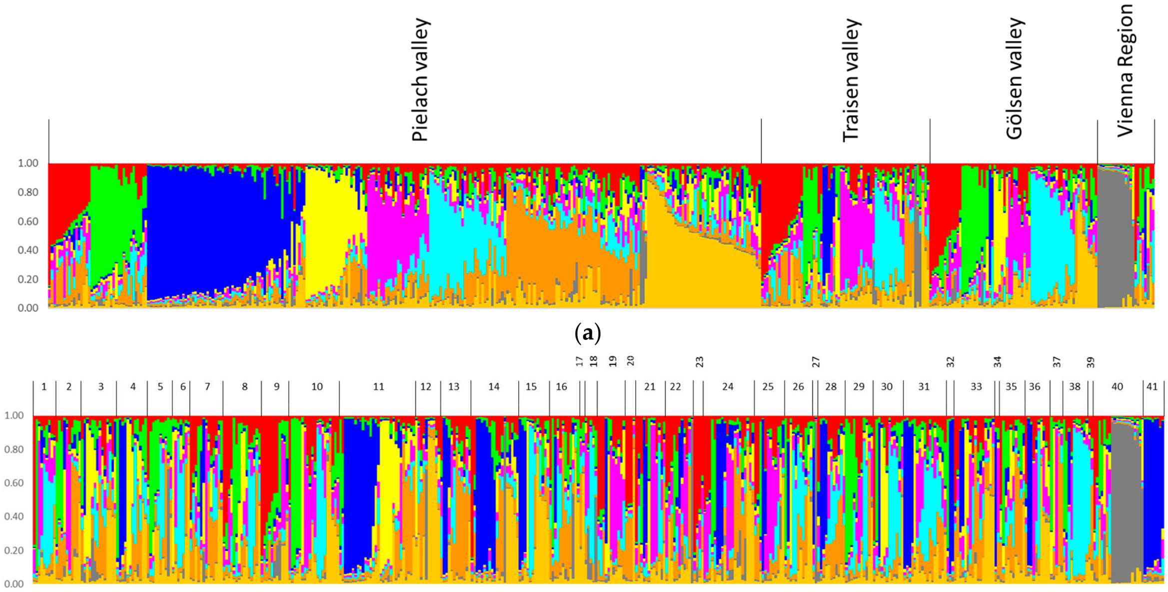
K = 9 was used to further describe the individuals, grouped by valley and site (Figure 3) revealing the exact distribution of genotypes.



(**a**) (**b**)

(**c**)

Figure 2. Genetic structure of 447 *C. mas* individuals evaluated by Structure (version 2.3.4) (**a**) K model with delta K values calculated from 2 to 15 (**b**) The number of subpopulations determined by the L(K) (**c**) Population structure analysis of the 447 individuals at K = 9.



(**a**)

(**b**)

Figure 3. Genetic analyses (based on K = 9) of 447 *C. mas* individuals represented (**a**) by Q and by valley/region and (**b**) by site.

In total three yellow-fruited accessions were found in the study region. They can be clearly distinguished from each other and the yellow-fruited cultivar 'Flava' (Figure 4). Fruits appear either yellow or with a light orange flush on the skin (Figure 4). This indicates the potential for the occurrence of three spontaneous mutations related to the color of the fruit skin and flesh.

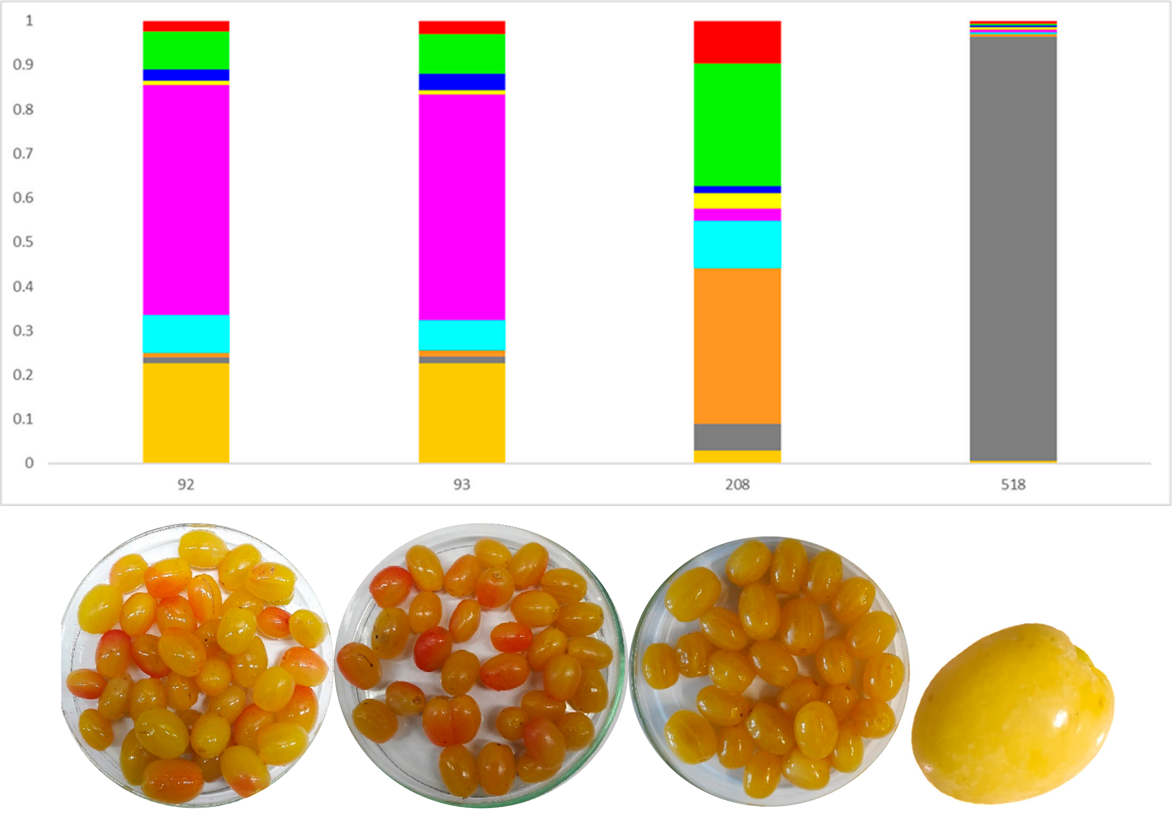


Figure 4. Genetic analyses (K = 9) and fruits of yellow-fruited accessions (92, 93, 208) allow a clear distinction between each other and the yellow-fruited cultivar 'Flava' (518).

A representative metaphase karyotype of the millennial accession (Laimer et al., 2021) is shown in Figure 5. The basic chromosome number observed corresponded to the diploid numbers 2n = 2x = 18, as previously reported [36,45,46].

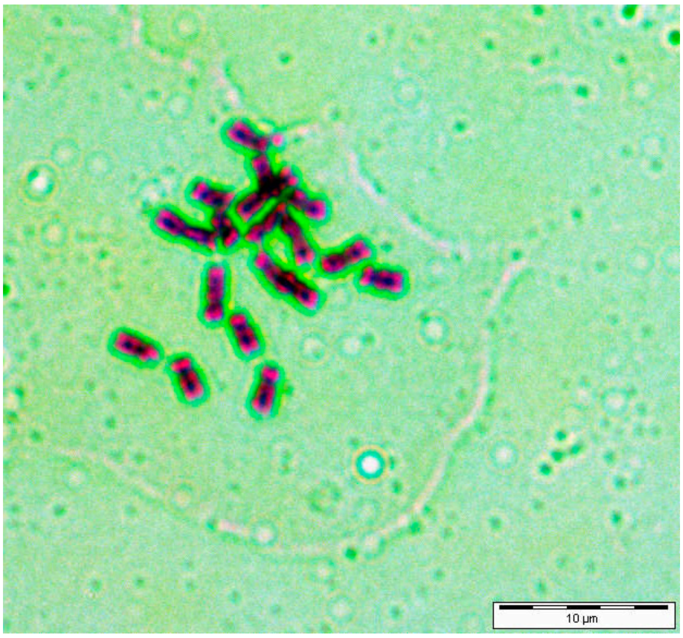


Figure 5. Mitotic metaphase chromosomes of millennial *Cornus mas* L. (2n = 2x = 18) (bar 10 µm).

4. Discussion

The conservation and use of plant genetic resources are two closely linked, important aspects for future generations [47]. As a measure for the conservation of important gene pools, in vivo and in vitro gene banks are commonly used [16,48–50]. A thorough evaluation of the genetic resources of the native genotypes is essential for selecting the most useful genotypes for future breeding programs, including traits such as tolerance to variable temperature ranges, e.g., increasing temperatures, fluctuating availability of humidity or even drought, resistance to diseases from wild genotypes to be crossed into cultivars [51,52]. Gepts [47] therefore advocated the use of molecular markers to make informed decisions about the composition of a gene bank, allowing the identification of plant accessions even in the vegetative stage, i.e., without fruit.

Overall, the genetic analyses have shown that the chosen methods allow the distinguishing of all 447 plants studied from each other, i.e., that the genetic variation is very high (Figure 2C). This indicates that cross-pollination might predominate, regardless of the presence of insects or wind. This also means that there is a good basis for selecting breeding material. The distribution of genotypes among the valleys studied (Figure 3) could also reflect a different altitudinal distribution of the occurrence, but this would need to be investigated in more detail. Unfortunately, the efforts to define the growth habit of the individual accessions were rendered difficult in several cases due to pruning interventions of the owners.

The wild forms of Cornelian cherries occurring in Lower Austria differ from the known selections (in grey) of *C. mas* to be clearly distinguished (Figure 3). Interestingly, the analyses also allowed the identification of three accessions as cultivars, which the owners planted on a special occasion (as part of hedge day plantings), where the plants were provided by local institutions and nurseries (plant no. 177 identified as variety ‘Jolico’, plant 259 and plant 298).

The higher number of alleles per each SSR locus (6–18) in relation to the ones reported by Wadl et al., (2014) could be explained by the more than 10-fold higher number of samples (Table 3). The mean He in the analyses was 0.70, while Wadl [32] reported a He mean of 0.58, and Hassanpour [33] described a mean He of 0.42. The higher values obtained with the Austrian *C. mas* accessions indicate that the seven regions addressed by the SSR analyses were useful in the study of genetic structure in Austrian accessions.

Generally, the variability among the accessions of the different sites was high. Although the numbers of analyzed accessions varied between the valleys (Figure 3A). Certain patterns are present in a comparable amount in the three valleys, while others show a prevalence mainly in the Pielach Valley and are absent or scant in the other two valleys.

When analyzing the distribution pattern by site (Figure 3B) an additional feature can be observed: while most of the sites contain highly variable patterns, individual sites show a high number of similar genotypes, e.g., site 11, site 38, and site 41. The formation of subpopulations observed in these sites therefore could be indicating human selective intervention. This anthropogenic influence might correspond to the preference of the individual previous owners, e.g., for dark or light red fruit. The selection made by the owners over the past generations, in combination with the phenotypic characterization [21], allows the recommendation of the selection of breeding partners.

Another interesting feature is the appearance of yellow-fruited accessions. The cultivar ‘Flava’ is one of the oldest cultivars, first mentioned in the 17th century, with medium-sized, translucent yellow, very tasty fruit with a light pineapple aroma [53,54].

Wadl [32] refers to the yellow-fruited selections (Alosza, Florianka, and Jantarnyi) descending from a single wild, yellow-fruited mutant discovered in the Crimean Forest by the horticulturalist Kefeli [55]. Klymenko [53] also mentions that even as early as 1843 “varieties with yellow fruit were very rare” in the wild and that in Ukraine it is thought that wild genotypes no longer exist in their natural habitat. Therefore, it was with much interest that the three yellow-fruited accessions—with distinctly lighter green leaves than red-fruited accessions found in the Pielach and Traisen valleys—were studied

(Figure 4). The observed lower allergen content of these accessions might be of particular interest [56].

Seed propagation and long-term human selection have given rise to a great diversity of trees [32]. Only recently have breeding programs been aimed at the development of large-fruited and high-yielding trees in several countries, mainly in Ukraine [52], Austria [8], Bulgaria, Poland, Slovakia, Czech Republic, Serbia, but also in Turkey, Russia, Georgia, and Azerbaijan [32].

5. Conclusions

This is the first investigation using SSR markers to determine the genetic relationships among Cornelian cherry in Austria. The evaluations revealed a high degree of diversity, allowing the distinguishing of every single accession, which provides excellent starting conditions for considerations of breeding improvement. This might be highly useful for population genetic studies and for assessing the degree of clonal reproduction of *C. mas* in both wild and cultivated populations in Austria. In combination with the de novo assembly of a reference genome of *C. mas* [57], this will contribute to accelerating and promoting the genetic improvement of *C. mas*.

Author Contributions: M.L. and E.G.B.F. designed the experiment, carried out the analyses and wrote the paper. N.K., G.M. and J.S. assisted with the genetic analyses and reviewed the text. S.F. performed the karyotyping analyses. V.H. maintained the tissue cultures. All authors have read and agreed to the published version of the manuscript.

Funding: Open access funding provided by the University of Natural Resources and Life Sciences Vienna (BOKU). Research Grants 101176 and 101692 are supported by the Federal Ministry for Agriculture, Forestry, Regions and Water Management and the Land Niederösterreich, Austria.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The owners of the plants in the region are thanked for not only allowing us to collect the plant material, but also for providing valuable insight into the traditional knowledge still available in their families. The technical assistance of Mansky S. with the microsatellite analyses is acknowledged.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. World Flora Online. Version 1.1. Published on the Internet. 2020. Available online: https://wfoplantlist.org/plant-list (accessed on 28 August 2023).
2. Eyde, R.H. ComprehendingCornus: Puzzles and progress in the systematics of the dogwoods. *Bot. Rev.* **1988**, *54*, 233–351. [CrossRef]
3. Czerwińska, M.E.; Melzig, M.F. *Cornus mas* and *Cornus officinalis*—Analogies and Differences of Two Medicinal Plants Traditionally Used. *Front. Pharmacol.* **2018**, *9*, 894. [CrossRef] [PubMed]
4. Brindza, P.; Brindza, J.; Tóth, D.; Klimenko, S.; Grigorieva, O. Slovakian *Cornelian cherry* (*Cornus mas* L.): Potential for cultivation. *Acta Hortic.* **2007**, *760*, 433–437. [CrossRef]
5. Ercisli, S.; Yilmaz, S.O.; Gadze, J.; Dzubur, A.; Hadziabulic, S.; Aliman, Y. Some Fruit Characteristics of Cornelian Cherries (*Cornus mas* L.). *Not. Bot. Horti Agrobot. Cluj-Napoca* **2011**, *39*, 255–259. [CrossRef]
6. Szczepaniak, O.M.; Kobus-Cisowska, J.; Kusek, W.; Przeor, M. Functional properties of *Cornelian cherry* (*Cornus mas* L.): A comprehensive review. *Eur. Food Res. Technol.* **2019**, *245*, 2071–2087. [CrossRef]
7. Dokoupil, L.; Řezníček, V. Production and use of the *Cornelian cherry*—*Cornus mas* L. *Acta Univ. Agric. Silvic. Mendel. Brun.* **2012**, *55*, 49–57. [CrossRef]
8. Pirc, H. Enzyklopädie der Wildobst-und Seltenen Obstarten. Leopold Stocker Verlag: Graz, Austria, 2015; pp. 92–112. In *Enzyklopädie der Wildobst-und Seltenen Obstarten*; Leopold Stocker Verlag: Graz, Austria, 2015; pp. 92–112.
9. Da Ronch, F.; Caudullo, G.; Houston Durrant, T.; de Rigo, D. Cornus mas in Europe: Distribution, habitat, usage and threats. In *European Atlas of Forest Tree Species*; San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A., Eds.; Publications Office of the EU: Luxembourg, 2016; pp. 82–83.
10. D'Antuono, L.; Kolesnov, A.; Fedosova, K.; Jorjadze, M.; Boyko, N.; Mudryk, M.; Bignami, C. *Cornelian cherry*: An Important Local Resource and Promising Health Promoting Fruit Plant of The Black Sea Area. *Acta Hortic.* **2014**, *1017*, 299–307. [CrossRef]

11. Dálnoki, O.; Jacomet, S. Some aspects of Late Iron Age agriculture based on the first results of an archaeobotanical investigation at Corvin tér, Budapest, Hungary. *Veg. Hist. Archaeobot.* **2002**, *11*, 9–16. [CrossRef]
12. Filipović, D.; Fotirić Akšić, M.; Dabić Zagorac, D.; Natić, M. Gathered fruits as grave goods? *Cornelian cherry* remains from a Mesolithic grave at the site of Vlasac, Danube Gorges, south-east Europe. *Quat. Int.* **2020**, *541*, 130–140. [CrossRef]
13. Bellini, C.; Mariotti-Lippi, M.; Secci, M.M.; Aranguren, B.; Perazzi, P. Plant gathering and cultivation in prehistoric Tuscany (Italy). *Veg. Hist. Archaeobotany* **2008**, *17*, 103–112. [CrossRef]
14. Werneck-Willingrain, H.L. Bodenständige Obsthölzer der Gegenwart, Früh-und Urgeschichte in den Ostalpen. *Schr. Ver. Verbreit. Nat. Kennt. Wien* **1960**, *100*, 181–221.
15. Brindza, J.; Toth, D.; Brindza, P.; Grygorieva, O.; Sajbidor, J.; Kucelova, L. Forgotten and Less Utilised Plant Species as Functional Food Resources. In *Functional Properties of Traditional Foods*; Springer: Boston, MA, USA, 2016; pp. 209–232.
16. Laimer, M.; Zeiser, M.; Hanzer, V.; Fernandez, E.G.B. In vitro conservation of centennial Austrian *Cornelian cherry* accessions. *Plant Biotechnol. Rep.* **2021**, *15*, 289–298. [CrossRef]
17. Karapatzak, E.; Dichala, O.; Papanastasi, K.; Manthos, I.; Ganopoulos, I.; Karydas, A.; Badeka, A.V.; Kosma, I.S.; Kyrkas, D.; Yfanti, P.; et al. A Multifaceted Evaluation Approach for Greek Native Neglected and Underutilized Forest Fruit Trees and Shrubs as Natural Sources of Antioxidants: Consolidating the Framework for Their Sustainable Agronomic Exploitation. *Plants* **2023**, *12*, 1642. [CrossRef] [PubMed]
18. Hegi, G. *Illustrierte Flora von Mitteleuropa*; Pichlers Witwe & Sohn: München, Germany, 1926; Volume 5, pp. 1567–1722.
19. Filipović, D.; Allué, E.A.; Borić, D. Integrated carpological and anthracological analysis of plant record from the Mesolithic site of Vlasac, Serbia. *J. Serb. Archaeol. Soc.* **2010**, *26*, 145–161.
20. Filipović, D.; Jovanović, J.; Rančić, D. In search of plants in the diet of Mesolithic-Neolithic communities in the Iron Gates. In *From Hunter-Gatherers to Farmers: Human Adaptations at the End of the Pleistocene and the First Part of the Holocene*; Mărgărit, M., Boroneanţ, A., Eds.; Cetatea de Scaun: Targovişte, Romania, 2017; pp. 93–111.
21. Borroto Fernández, E.G.; Mokhber, A.; Zeiser, M.; Laimer, M. Phenotypic Characterization of a Wild-Type Population of Cornelian Cherries (*Cornus mas* L.) from Austria. *Erwerbs-Obstbau* **2022**, *64*, 673–683. [CrossRef]
22. Dinda, B.; Kyriakopoulos, A.M.; Dinda, S.; Zoumpourlis, V.; Thomaidis, N.S.; Velegraki, A.; Markopoulos, C.; Dinda, M. *Cornus mas* L. (Cornelian cherry), an important European and Asian traditional food and medicine: Ethnomedicine, phytochemistry and pharmacology for its commercial utilization in drug industry. *J. Ethnopharmacol.* **2016**, *193*, 670–690. [CrossRef]
23. Bayram, H.M.; Ozturkcan, S.A. Bioactive components and biological properties of *Cornelian cherry* (*Cornus mas* L.): A comprehensive review. *J. Funct. Foods* **2020**, *75*, 104252. [CrossRef]
24. Borroto Fernandez, E.G.; Laimer, M. Züchterische Aspekte der Kornelkirsche. *Besseres Obst.* **2021**, *1*, 18–21.
25. Ellis, J.R.; Burke, J.M. EST-SSRs as a resource for population genetic analyses. *Heredity* **2007**, *99*, 125–132. [CrossRef]
26. Hedrick, P.H. Conservation genetics: Where are we now? *Trends Ecol. Evol.* **2001**, *16*, 629–636. [CrossRef]
27. Sakai, A.K.; Allendorf, F.W.; Holt, J.S.; Lodge, D.M.; Molofsky, J.; With, K.A.; Baughman, S.; Cabin, R.J.; Cohen, J.E.; Ellstrand, N.C.; et al. The population biology of invasive species. *Annu. Rev. Ecol. Syst.* **2001**, *32*, 305–332. [CrossRef]
28. Eser, E.; Topçu, H.; Kefayati, S.; Sütyemez, M.; Islam, R.; Kafkas, S. Highly polymorphic novel simple sequence repeat markers from Class I repeats in walnut (*Juglans regia* L.). *Turk. J. Agric. For.* **2019**, *43*, 174–183. [CrossRef]
29. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of Population Structure Using Multilocus Genotype Data. *Genetics* **2000**, *155*, 945–959. [CrossRef]
30. Wang, X.; Trigiano, R.; Windham, M.; Scheffler, B.; Rinehart, T.; Spiers, J. Development and characterization of simple sequence repeats for flowering dogwood (*Cornus florida* L.). *Tree Genet. Genomes* **2007**, *4*, 461–468. [CrossRef]
31. Wadl, P.A.; Hatmaker, E.A.; Fussi, B.; Scheffler, B.E.; Trigiano, R.N. Isolation and characterization of microsatellite loci for *Cornus sanguinea* (Cornaceae). *Appl. Plant Sci.* **2013**, *1*, 1300012. [CrossRef] [PubMed]
32. Wadl, P.A.; Szyp-Borowska, I.; Piórecki, N.; Schlarbaum, S.E.; Scheffler, B.E.; Trigiano, R.N. Development of microsatellites from *Cornus mas* L. (Cornaceae) and characterization of genetic diversity of cornelian cherries from China, central Europe, and the United States. *Sci. Hortic.* **2014**, *179*, 314–320. [CrossRef]
33. Hassanpour, H.; Hamidoghli, Y.; Habibollah, S. Estimation of genetic diversity in some Iranian cornelian cherries (*Cornus mas* L.) accessions using ISSR markers. *Biochem. Syst. Ecol.* **2013**, *48*, 257–262. [CrossRef]
34. Feulgen, R.; Rossenbeck, H. Mikroskopisch-chemischer Nachweis einer Nukleinsäure vom Typus der Thymonukleinsäure und die darauf beruhende elektive Färbung von Zellkernen in mikroskopischen Präparaten. *Hoppe-Seylers Z. Phyiol. Chem.* **1924**, *135*, 203–248. [CrossRef]
35. Greilhuber, J.; Temsch, E.M. Feulgen densitometry: Some observations relevant to best practice in quantitative nuclear DNA content determination. *Acta Bot. Croat.* **2001**, *60*, 285–298.
36. Borroto Fernandez, E.G.; Fink, S.; Temsch, E.M.; Laimer, M. Flow cytometry and karyotyping of in vitro grown Cornus mas genotypes to evaluate absolute genome size and ploidy. *Caryologia*, 2023; *in preparation*.
37. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the number of clusters of individuals using the software structure: A simulation study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [CrossRef]
38. Yeh, F.C.; Boyle, T.J.B. Population genetic analysis of codominant and dominant markers and quantitative traits. *Belg. J. Bot.* **1997**, *129*, 156–159.
39. Levene, H. On a Matching Problem Arising in Genetics. *Ann. Math. Stat.* **1949**, *20*, 91–94. [CrossRef]

40. Nei, M. Analysis of Gene Diversity in Subdivided Populations. *Proc. Natl. Acad. Sci. USA* **1973**, *70*, 3321–3323. [CrossRef] [PubMed]
41. Nei, M. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **1978**, *89*, 583–590. [CrossRef] [PubMed]
42. Nei, M. Genetic Distance between Populations. *Am. Nat.* **1972**, *106*, 283–292. [CrossRef]
43. Fyfe, J.L.; Bailey, N.T.J. Plant breeding studies in leguminous forage crops. I. Natural cross-breeding in winter beans. *J. Agric. Sci.* **1951**, *41*, 371–378. [CrossRef]
44. Ritland, K.; Jain, S.K. A model for the estimation of outcrossing rate using n independent loci. *Heredity* **1981**, *47*, 35–52. [CrossRef]
45. Zonneveld, B.J.M.; Leitch, I.J.; Bennett, M.D. First nuclear DNA amounts in more than 300 angiosperms. *Ann. Bot.* **2005**, *96*, 229–244. [CrossRef]
46. Truta, E.; Capraru, G.; Roșu, C.M.; Surdu, S.; Giorghita, G.; Rati, V. Preliminary studies concerning chromosome constitution of some *Cornus mas* L. genotypes (Bacau district). *J. Exp. Mol. Biol.* **2009**, *10*, 1–4.
47. Gepts, P. Plant genetic resources conservation and utilization: The accomplishments and future of a societal insurance policy. *Crop Sci.* **2006**, *46*, 2278–2292. [CrossRef]
48. Rop, O.; Mlcek, J.; Kramarova, D.; Jurikova, T. Selected cultivars of *Cornelian cherry* (*Cornus mas* L.) as a new food source for human nutrition. *Afr. J. Biotechnol.* **2010**, *9*, 1205–1210.
49. Yilmaz, K.U.; Zengin, Y.; Ercisli, S.; Orhan, E.; Yalcinkaya, E.; Taner, O.; Erdogan, A. Biodiversity, ex-situ conservation and characterization of *Cornelian cherry* (*Cornus mas* L.) genotypes in Turkey. *Biotechnol. Biotechnol. Equip.* **2009**, *23*, 1143–1149. [CrossRef]
50. Available online: https://info.bml.gv.at/im-fokus/forschung/projekte-und-berichte/landwirtschaft/vielfaeltigedirndl.html (accessed on 28 August 2023).
51. Ercisli, S.; Orhan, E.; Esitken, A.; Yildirim, N.; Agar, G. Relationships among some cornelian cherry genotypes (*Cornus mas* L.) based on RAPD analysis. *Genet. Resour. Crop Evol.* **2008**, *55*, 613–618. [CrossRef]
52. Hassanpour, H.; Hamidoghli, Y.; Samizadeh, H. Some fruit characteristics of Iranian cornelian cherries (*Cornus mas* L.). *Not. Bot. Horti Agrobot.* **2012**, *40*, 247–252. [CrossRef]
53. Klymenko, S. The *Cornelian cherry* (*Cornus mas* L.): Collection, preservation, and utilization. *J. Fruit Ornam. Plant Res.* **2004**, *12*, 93–98.
54. Swanson, M.A. *Cornus mas* L. Cultivar Selection Based on Hardiness and Propagation. Master's Thesis, North Dakota State University, Fargo, ND, USA, 2018.
55. Simirenko, L.P. *Crimean Commercial Horticulture*; Printing House of J. H. Kushnerev: Moscow, Russia, 1912; Volume 1, 748p.
56. Gierlinger, S. Proteomic Investigations of Different Tissues and Phenotypes of *Cornus mas*. Bachelor's Thesis, BOKU-Universität für Bodenkultur, Vienna, Austria, 2017.
57. Borroto Fernandez, E.G.; Zoccarato, L.; Laimer, M. Sequencing and De Novo Assembly of *Cornus mas* genome in Austria using the PacBio and Hi-C technology. *J. Syst. Evol.* 2023; *in preparation*.