Genetic Resources and Crop Evolution

Dear old peonies – for genebanks and gardeners. Microsatellite fingerprinting of herbaceous peonies in Fennoscandia

--Manuscript Draft--

Manuscript Number:	
Full Title:	Dear old peonies – for genebanks and gardeners. Microsatellite fingerprinting of herbaceous peonies in Fennoscandia
Article Type:	Regular research paper
Keywords:	genebank - genetic diversity - genetic resources - microsatellite - peony - simple sequence repeat (SSR)
Corresponding Author:	Pirjo Tanhuanpää Natural Resources Institute Finland (Luke) Jokioinen, FINLAND
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Natural Resources Institute Finland (Luke)
Corresponding Author's Secondary Institution:	
First Author:	Pirjo Tanhuanpää
First Author Secondary Information:	
Order of Authors:	Pirjo Tanhuanpää
	Sirkka Juhanoja
	Linnea Oskarsson
	Mari Marstein
	Merja Hartikainen
Order of Authors Secondary Information:	
Funding Information:	Maija ja Yrjö Rikalan Puutarhasäätiö — M.Sci. Merja Hartikainen
	Nikolai ja Ljudmila Borisoffin Puutarhasäätiö — M.Sci. Merja Hartikainen
Abstract:	Genetic diversity of 334 herbaceous peonies from Fennoscandia was analysed using 18 microsatellites (simple sequence repeats, SSR). The samples included peonies mostly from Finnish home gardens and nurseries (283) but also from Norwegian and Swedish peony collections. We wanted to concentrate on the following species: Paeonia anomala, P. × hybrida, P. humilis flore plena (nowadays called P. officinalis 'Nordic Paradox'), P. tenuifolia, and P. × festiva . The 18 microsatellites amplified a total of 249 alleles, and were used to calculate genetic distances between samples and to build a dendrogram. In the dendrogram, samples formed clear groups according to their species. Preliminary morphological observations were made from most of the Finnish home garden samples, and they mainly confirmed the outcome from genetical analysis. The results of the study will be used to create a Finnish gene resources collection of the most diverse and vigorous peonies, and to update the Norwegian and Swedish collections.

Dear old peonies – for genebanks and gardeners. Microsatellite fingerprinting of herbaceous peonies in Fennoscandia

Pirjo Tanhuanpää[1], Sirkka Juhanoja[2], Linnea Oskarsson[3], Mari Marstein[4], & Merja Hartikainen[1]

[1]Production Systems, Natural Resources Institute Finland (Luke), FI-31600 Jokioinen, Finland. E-mail: pirjo.tanhuanpaa@luke.fi; merja.hartikainen@luke.fi

[2]Formerly at: Production Systems, Natural Resources Institute Finland (Luke), FI-31600 Jokioinen, Finland. E-mail: ext.sirkka.juhanoja@luke.fi

[3] The Swedish National Gene Bank for Vegetatively Propagated Horticultural Crops, The Programme for Diversity of Cultivated Plants, The Swedish University of Agricultural Sciences, P.O. 57, S-230 53 Alnarp, Sweden. E-mail: linnea.oskarsson@slu.se

[4]MiA - Museene i Akershus, Strømsvegen 74, 2010 Strømmen. E-mail: mari.marstein@mia.no

With 5 tables, 3 figures, of which one also as electronic supplemental figure

Correspondence to:

Pirjo Tanhuanpää (ORCHID: 0000-0001-7431-975X)
Production Systems
Natural Resources Institute Finland (Luke)
FI-31600 Jokioinen
Finland
tel: +358 295326574
e-mail: pirjo.tanhuanpaa@luke.fi

Acknowledgements: The authors wish to thank Marja-Riitta Arajärvi, Minna Kavander, Pirkko Nykänen, Hannu Ojanen, and Anneli Virta for excellent technical assistance. Finnish citizens and nurseries are thanked for providing us the plant material. Vesa Koivu and Ahti Valli have offered valuable information of traditional peony species. The nursery Pionien koti has kindly placed photos at the project's disposal. The association of Finnish Nursery Growers is thanked for providing the network of peony growers. Finnish National Plant Genetic Resources Programme is acknowledged for supporting the plant call and data management**.** Thanks to the Norwegian UiA naturmuseum and botanical garden, NTNU Ringve botanical garden, UiT Tromsø arctic-alpine botanical garden and MiA – Museene I Akershus for providing plant material. Botanists Brynhild Mørkved and Martin Hajman in Tromsö have kindly helped with information concerning *P. officinalis 'Mollis'*. Thanks to the owners of Swedish private gardens who donated plants of their older peonies and contributed so generously to the gene bank collection.

Authors' contribution

Pirjo Tanhuanpää: Conceptualization, Methodology, Investigation, Resources, Writing – Original Draft, Writing – Review & Editing, Visualization.

Sirkka Juhanoja: Conceptualization, (Methodology), Investigation, Writing – Original Draft, Writing – Review & Editing.

Linnea Oskarsson: Investigation, Resources, Writing – Original Draft, Writing – Review & Editing.

Mari Marstein: Investigation, Resources, Writing – Original Draft, Writing – Review & Editing, Funding acquisition.

Merja Hartikainen: Conceptualization, (Methodology), Investigation, Resources, Writing – Original Draft, Writing – Review & Editing, Project administration, Funding acquisition.

All authors read and approved the final manuscript.

Abstract

Genetic diversity of 334 herbaceous peonies from Fennoscandia was analysed using 18 microsatellites (simple sequence repeats, SSR). The samples included peonies mostly from Finnish home gardens and nurseries (284) but also from Norwegian and Swedish peony collections. We wanted to concentrate on the following species: *Paeonia anomala, P. × hybrida, P. humilis flore plena* (nowadays called *P. officinalis* 'Nordic Paradox'), *P. tenuifolia,* and *P. × festiva*. The 18 microsatellites amplified a total of 249 alleles, and were used to calculate genetic distances between samples and to build a dendrogram. In the dendrogram, samples formed clear groups according to their species. Preliminary morphological observations were made from most of the Finnish home garden samples, and they mainly confirmed the outcome from genetical analysis. The results of the study will be used to create a Finnish gene resources collection of the most diverse and vigorous peonies, and to update the Norwegian and Swedish collections.

Key words: genebank - genetic diversity – genetic resources - microsatellite - peony – simple sequence repeat (SSR)

1. Introduction

Peonies (only one genus, *Paeonia*, in the family *Paeoniacea*) are native to Asia, South Europe and the western parts of North America (Hong 2010). First they were used as medicinal plants in Asia several thousand years ago because the Chinese believed that roots have medicinal properties (Hsu et al. 1986). In the late 1700s, they were started to be used as ornamental plants (Harding 1917), and nowadays they are among the most popular garden plants in temperate regions. Peonies are long-living perennial plants, and there are two types of them, tree peonies, which are shrubs with decidious leaves, and herbaceous peonies. When peonies are multiplied vegetative propagation is mainly used but some species can be propagated by seeds.

The current consensus of the number of known species in the genus *Paeonia* is 33 (Christenhusz and Byng 2016), and they can be divided into three sections: sect. *Moutan*, sect. *Paeonia*, and sect. *Onaepia* (Stern 1946). Sect. *Moutan* contains 9 woody species (e.g. *P. suffruticosa*) endemic to China; sect. *Paeonia* includes 25 herbaceous species with the widest distribution, mainly in the Mediterranean and Eastern Asiatic regions; and sect. *Onaepia* two herbaceous species, in the western North America and Mexico (Ji et al. 2012).

The biggest section of peonies, *Paeonia*, contains long-living perennial herbaceous species whose leaves and stems die during winter but roots and crowns stay underground resuming growth in spring. Herbaceous peonies are important traditional flowers in China but also highly valued as ornamentals in Europe and USA. They are very diverse what comes to morphology and also ploidy level (Hao et al. 2016). The basic chromosome number is 5 (Dark 1936). Hybridisation is important in nature as also in the development of new cultivars leading to triploid and tetraploid chromosome numbers.

In the Nordic countries, peonies have long been important as medicinal and ornamental plants. In Sweden, peonies (*P. x festiva* and *P. officinalis*) are mentioned in medical manuscripts from

the 16th century (Larsson 2009). Little is known about the introduction of peonies in Sweden, but in the 1680s *P. officinalis, P. x festiva, P. peregrina*, and *P. mascula* were included in the lists of plants grown in the botanical garden in Uppsala (Martinsson & Ryman 2007). Almost two hundred years later, the Gothenburg Garden Association's nursery had more than 60 cultivars of Chinese peonies (*P. lactiflora*) in its price list, many with French or English-sounding cultivar names (Pricelist from Gothenburg Garden Association 1864). In the late 19th century, *P. x festiva* 'Rubra Plena' was said to be one of the most common perennials in Swedish gardens (O.T. 1890). In Norway, peonies were first mentioned in a Norwegian Gardening book, Christian Gartner's *Horticultura* from 1694: «*Pæon* of all colours» (Balvoll and Weisaeth 1994). All the peonies covered by this study grew in the botanic garden in Oslo in 1823. There is even one called *hybrida*, but we do not know if it is the *P.* x *hybrida* we find in Nordic gardens today (Rathke 1823). In Finland, according to an old written document, peonies have been grown from the end of the 17th century, as a medicine for epilepsy (Ruoff 2002). In the 19th century peonies were grown in Finland as ornamentals and there were seeds from a few different peony species on the market. Peonies were also ordered from a nursery in St. Petersburg. Even though peonies have long been cultivated in Finland, there is no collection of peony genetic resources like in Norway and Sweden.

For genetic resources collections, it is very important to be able to recognise different species, as also cultivars. In addition to tens of peony species, there is a vast number of different cultivars, 7995 in 2007 (Jakubowski et al. 2007). Identification of different peony cultivars requires experience in recognising morphological traits of the flower and the plant. For this, sometimes two to 10 years have to be waited for bloom appearance. In addition, flower colour might vary depending on the growing site (Zhao et al. 2012). To simplify cultivar identification and to carry it on at an early stage of the plant, DNA markers can be used. Simple sequence repeat markers (SSRs, microsatellites) have been developed for tree peonies (Gai et al. 2012; Gao et al 2013; Guo et al. 2017; Homolka et al. 2010; Hou et al. 2011a, b; Wang et al. 2009; Wu et al. 2014; Yu et al. 2013; Zhang et al. 2011, 2012) and to a lesser extent for *P. lactiflora* (Cheng et al. 2011; Gilmore et al.

2013; Ji et al. 2014; Li et al. 2011; Sun et al. 2011; Wan et al. 2020). Except for identifying cultivars and species, DNA markers can be used to identify hybrid origin, to study genetic diversity and relationships, and for linkage mapping.

The aim of the present study was to collect leaf samples and roots from old peonies from Finnish home gardens and nurseries and to study their genetic diversity with SSR markers, and with the same set of markers, to evaluate genetic diversity of herbaceous peonies from Norwegian and Swedish peony collections. Finland, Sweden and Norway have always been strongly connected, both climatically and culturally. There has always been an active contact across borders. It is a tradition in all three countries to pass plants along to friends and relatives and to bring plants with you when you move. Therefore, it was justified to carry on a joint study combining plants from these three countries. We concentrated on the following species: *P. anomala, P. × hybrida, P. humilis flore plena* (nowadays called *P. officinalis* 'Nordic Paradox'), *P. tenuifolia,* and *P. × festiva*. The final goal is to create a Finnish collection of the most diverse and vigorous peonies with a good ornamental value. In addition, results of the study will be used to update Norwegian and Swedish collections, to exclude duplicates and to confirm some identities. Further, one aim is to create a joined Nordic plant genetic resources collection containing only unique genotypes.

2. Materials and methods

2.1. Plant material

Plant material contained peony samples from Finland, Norway and Sweden (Figs. 1, 2). In Finland, we first collected knowledge of the most rare peony species grown in private Finnish gardens and nurseries (Ruoff 2002; Peltola and Koivu 2007), and selected the following species for our study: *P. anomala, P. × hybrida, P. humilis flore plena, P. tenuifolia,* and *P. × festiva*, based on their danger of extinction, because they are not on production, and because they have been cultivated in Finland for a long time. To get peony samples from Finnish home gardens, a call was made in 2018-2019. We

wanted to collect oral tradition, photos and locations of selected peony species cultivated in Finland in 1950s or earlier. Owners of old peony varieties and landraces were asked to tell about their own plant by an online registration form (www.luke.fi/ilmoitakasvi). Registration continues still. Altogether 690 peony announcements were obtained, and the samples were given a number with a prefix 'LUKE' (referring to Natural Resources Center Finland, Luonnonvarakeskus in Finnish, abbreviation LUKE). A total of 335 plants from the announcements were chosen for the study. Peonies apparently (based on description and/or photos) not presenting the targeted species were not chosen. Otherwise, selection criteria included interesting cultivation history and especially the age of the plant. Leaf samples of the peonies were requested for DNA analysis and roots for planting samples to carry out later morphological and phenological observations. Finally, we got leaves from 284 samples (Fig. 1) for DNA extraction but not roots from all of them. Roots were planted in pots and kept outside during autumn, for winter they were put in a storage with a temperature below +5°C. In early spring they were transferred to a greenhouse and finally planted to a field in Luke's experimental station in Piikkiö (60°25´30''N, 022°31'00''E) in June 2019. Preliminary morphological observations were made in the greenhouse from 243 plants, which were classified as different species according to leaf shape, leaf hairiness, leaf colour, flower shape, and flower colour. In addition to peonies from home gardens, five reference samples were included: one *P. x festiva* 'Rosea Plena' (sample number: FIN-2019-75) and one 'Alba Plena' (FIN-2019-74) from a Finnish nursery, and three *P. lactiflora* samples (LUKE-5324, -5325, -5326) from Luke's exhibition garden Wendla. *P. lactiflora* samples were included in order to act as references for this peony group and also to test the functionality of SSRs, which were mainly derived from this species.

The name *P. humilis flore plena* was used in the call ('Juhannuspioni' in Finnish) but it would be better to use the name *P. officinalis* 'Nordic Paradox', which was registered by The American Peony Society (Jakubowski 2015). This peony is not wild-growing and therefore, the name should contain a cultivar name, 'Nordic Paradox'. *P. humilis* was a doubtful name already in 1810, when the

British started using the name *P. paradoxa*, and later *P. villosa* and *P. huthii* for the wild, single-flowered variety (Stern 1946).

Norwegian samples for the peony collection were collected through a project financed by the Norwegian Gene Resources Center between 2003 and 2008. Botanists and other professionals visited garden owners in different parts of Norway, interviewing them and collecting plants. Gardens with a selection of traditional plants were preferred. The collected plants were planted in separate departments in the botanical gardens in Kristiansand, Oslo, Trondheim, and Tromsø, and at some local museums. Information about the plant's local growing history was documented. From the Norwegian collection, 20 samples were selected to the study and leaf samples sent to Luke. (Table 1, Fig. 1).

Leaf samples from Sweden were collected from peonies preserved in the Swedish National Gene Bank for Vegetatively Propagated Horticultural Crops. The genebank is located at the Swedish University of Agricultural Sciences and contains 2200 older cultivars of fruits, berries, ornamentals, and vegetables. The genebank was inaugurated in 2016 and the cultivars preserved were collected through nationwide inventories of garden plants grown in Sweden before 1940 or 1950, depending on plant species. The majority of the cultivars preserved in the genebank were collected from private gardens all around Sweden and in addition to the plants, the histories and traditions associated with them were also documented. The inventories were initiated and implemented by the Programme for Diversity of Cultivated Plants, Sweden's national programme for plant genetic resources. All in all, 75 accessions of peonies are preserved in the Swedish National Gene Bank. Of these, 25 belong to the species selected by Luke for genetic testing, and leaf samples of them were sent to Luke in spring 2018 (Table 2, Fig. 1).

E.Z.N.A® SP Plant DNA kit (Omega Bio-tek, Norcross, GA, USA) was used for DNA extractions from frozen peony leaves. In some samples DNA quality was low (indicated by low A260/A280 and A260/A230 ratios) and created problems in SSR amplification. Therefore, DNA from

these samples was further purified using a general protocol of ethanol precipitation. DNA concentrations were measured using a NanoDrop™ One/One^{C} Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific Ltd, Vantaa, Finland).

2.2. SSR analyses

For the diversity study, 44 SSRs developed for *P. lactiflora* and 12 for *P. suffruticosa* from different studies (Cheng et al. 2011; Gilmore et al. 2013; Ji et al. 2014; Li et al. 2011; Sun et al. 2011; Wu et al. 2014) were selected. Functioning of SSRs was first tested in three *Paeonia* species: *P. anomala, P. lactiflora* (two different genotypes), and *P. x hybrida*. Those amplifying well in this first trial were further analysed for their polymorphism in five species (16 individuals): *P. anomala, P. lactiflora, P. x hybrida* (four genotypes), *P. officinalis* (two genotypes), and *P. x festiva* (three genotypes), and in five samples with undefined species from Finnish home gardens. Eighteen best SSRs (Table 3) were selected and multiplexed for final analyses. The SSRs were amplified in three PCR reactions according to results from Multiplex Manager v1.2 program (http://multiplexmanager.com). To separate and visualise amplified products, an ABI PRISM® 310 Genetic Analyzer (Thermo Fisher Scientific Ltd, Vantaa, Finland) was used. The forward primer of each primer pair was labelled with a fluorescent dye, FAM™ (5-carboxyfluorescein), NED™, VIC® or PET®. The PCR amplification conditions were as follows: 32 cycles of 30 s at 95°C, 90 s at 57°C , and 30 s at 72°C in a BioRad C1000 thermal Cycler (Bio-Rad, Hercules, California, USA). The program started with an initial denaturation step of 5 min at 95°C and was followed by a final extension step of 30 min at 60°C. The PCR amplification was performed in a total volume of 10 µl, containing 5 µl Master mix from Qiagen Type-it® Microsatellite PCR Kit (Qiagen, Helsinki, Finland), 5 ng of DNA, and 67-400 nM each primer. PCR products were diluted 1/50 for the ABI runs. GeneMapper® software 5 was used for allele size estimation.

2.3. Data analyses

The study contained plants with different and often unknown ploidy levels, and it was impossible to know the dosages of the SSR alleles. Therefore, allele phenotypes were scored using a binary code (1/0) for the presence or absence of allele peaks. Hence, each SSR allele was treated as a separate marker locus when calculating genetic distances between individuals. Some SSRs might also represent multiple loci (P05 and Pae100, Gilmore et al. 2013).

Based on the Dice coefficient, a dissimilarity index between samples was counted with DARwin software version 6.0.014 (Dissimilarity Analysis and Representation for Windows, Perrier and Jacquemoud-Collet 2006) using a bootstrap value of 1000 replications. The dissimilarity matrix was used for building an unweighted neighbor-joining (NJ, Saitou and Nei 1987) tree. Polymorphism information content (PIC) of the SSRs was calculated with a free online computer program (Abuzayed et al. 2017) using the formula by Roldan-Ruiz et al. (2000).

3. Results

Fifty-six SSRs were selected from published articles to study genetic diversity in herbaceous peonies. Based on their functioning and polymorphism, the best 18 SSRs were used for final analyses of 334 peony samples. Six of the selected SSRs (33 %) were from *P. suffruticosa* and 12 (67 %) from *P. lactiflora* (Table 3). Two of the selected SSRs contained a trinucleotide repeat, and the rest dinucleotide repeats. The PIC values of the SSRs varied from 0.08 (Pmg180) to 0.26 (Sy4) with a mean of 0.16. The 18 SSRs amplified a total of 249 alleles, the number of alleles per SSR varying from 4 (Pae115) to 33 (PS004). SSRs from *P. suffruticosa* produced more alleles (mean 18/SSR) than those from *P. lactiflora* (mean 12/SSR) but PIC value was greater in SSRs from *P. lactiflora* (0.18 vs. 0.13).

Genetic distances between samples were visualised with an NJ tree (Fig. 3 and Fig. S1). The samples formed clear groups, which were named according to already identified species samples ('references') from Norway, Sweden, and Finland (Table 4): 1) *P. x festiva* group, 71

samples, 2) *P. x hybrida* group, 58 samples, 3) *P. anomala* group, 33 samples, 4) alleged *P. tenuifolia* group (based only on morphological observations, no references), 7 samples, 5) *P. lactiflora* group, 79 samples, 6) *P. humilis fl. pl./P. officinalis* group, 77 samples, and 7) *P. officinalis* 'Mollis' group, 8 samples. In addition, one yellow-flowered peony (LUKE-4338) did not clearly cluster into any group. There were duplicates in all groups, the amount varying from 0 to 75% among the samples from Finnish home gardens (Table 4). All the reference samples went to their corresponding groups. The two uncertain *P. officinalis* samples (SWE-2018-21 and -22) from Sweden proved to be *P. officinalis*. Within each group, some subgroupings could also be observed, e.g. in *P. humilis fl. pl./P. officinalis* group, there were clearly separate groups for *P. humilis fl. pl.* and for *P. officinalis,* and in addition, three separate samples (LUKE-5021, -4607, and -4793) which did not cluster into either group.

Even though the total amount of polymorphic markers (= SSR alleles) was 249, the number of polymorphic markers in each group varied greatly because some SSRs did not amplify or were monomorphic in certain groups (Table 5). Therefore, discrimination between samples within a group was based on 38 (*P. x festiva)* – 116 (*P. anomala*) markers. The SSRs worked the best in *P. anomala* and *P. lactiflora:* the number of polymorphic SSRs and the number of alleles were the highest among all groups (Table 5). Some groups contained private alleles, in the *P. x festiva* group the most, 16 (results not shown).

Preliminary morphological evaluation from the Finnish home garden samples was done in greenhouse in Piikkiö from 243 samples. From 17 plants the species could not be defined due to poor growth or if the plant did not bloom at all. From the remaining 226 samples, only two (LUKE-4940 and -4387) gave controversial results compared to genetical analysis (Table 4). LUKE-4940 clustered in the dendrogram to *P. anomala* group but was (clearly) separate from the other samples. The SSRs worked in this sample partly like in *P. anomala* and partly like in *P. x hybrida*: P05 amplified normally like in *P. anomala* (does not work in *P. x hybrida*) but on the other hand, Sy2 did not function and Sy4 was monomorphic like in *P. hybrida* (Table 5). Morphologically this sample

seemed to be *P. x hybrida,* however, containing also characters from *P. anomala.* Actually, LUKE-4940 can be *P. intermedia*, which has long been thought to be a subspecies of *P. anomala*, even though Hong (2010) thinks that it is a species of its own. LUKE-4387 clustered genetically into the *P. officinalis* 'Mollis' group but morphologically to *P. humilis fl. pl.*, however, this plant did not bloom in greenhouse. According to the photo sent by the owner, LUKE-4387 seems to be 'Mollis', so the genetical result is correct. The morphological identification of samples in the 'Mollis' group was not straightforward but the five samples from home gardens were classified as undefined. Only one of these plants flowered in the greenhouse, and it seemed to be 'Mollis'. The final identification of most of the samples according to morphological and phenological observations in two years' field trial will be reported later in another article. However, some of the samples did not survive the first winter which diminishes the number of morphological results.

4. Discussion

Genetic diversity in peony samples from Swedish and Norwegian peony collections, and from Finnish home gardens and nurseries was assessed with 18 SSRs. The call for old peonies from Finnish home gardens was pointed at obtaining the following species: *P. anomala, P. × hybrida, P. humilis flore plena* (nowadays called *P. officinalis* 'Nordic Paradox'), *P. tenuifolia,* and *P. × festiva*. In addition to these, samples representing *P. lactiflora* and *P. officinalis* were also received. In the dendrogram, different species were clearly separated into their own groups and the identity of a group could be ascertained with Finnish reference samples and already identified samples from Norwegian and Swedish collections. The separation into different species groups was facilitated due to the fact that some SSRs were group-specific, e.g. did not amplify at all or were monomorphic in certain groups. But on the other hand, due to a lower number of polymorphic markers in these groups, it perhaps was not possible to differentiate samples leading to a high number of duplicates. Another reason for about half of the samples from Finnish home gardens being duplicates might be that well-growing peonies have been spreading out around Finland for decades because people have given peony

roots to each other. On the other hand, in *P. anomala* group, which had the highest number of polymorphic markers, nearly all samples from home gardens and nurseries were of different genotype, only two samples being genetically identical. The fact that this species is mainly propagated by seeds also explains the high number of different genotypes.

The informativeness level of markers can be assessed with PIC values, which reflect diversity and distribution of alleles. In the present study, the PIC values were mostly in the category of low (< 0.25, Botstein et al. 1980), the mean being 0.16. One reason for this is that the SSRs were developed in a different species than in which they were used, and therefore, did not amplify of were monomorphic in some species groups. In addition, SSRs had to be scored as dominant markers due to unknown ploidy levels, and this also diminishes PIC values. In a comparable study of rhubarb, PIC values were similar, varying from 0.05 to 0.16 with a mean of 0.12 (Tanhuanpää et al. 2019).

There has been controversy of the species identity within the *P. anomala* complex, which contains herbaceous peonies in Central Asia, Siberia, and adjacent northeastern European regions (Hong and Pan 2004). *P. x hybrida* of Pallas in this complex was according to A. P. de Candolle (1818) a garden hybrid between *P.anomala* and *P.tenuifolia*, occurring also in the wild (Stern 1946). On the other hand, Anderson (1818) regarded *P. x hybrida* as synonymous with *P. tenuifolia* for the first time, and after taxonomic revision, Hong and Pan (2004) were of the same opinion. In our study, *P. x hybrida*, *P. anomala*, and *P. tenuifolia* belonged to a bigger cluster, within which they each formed their own subgroups suggesting that *P. x hybrida* and *P. tenuifolia* are different species. However, because there were only 7 *P.tenuifolia* samples, and they represented only three different genotypes, more samples are needed to verify this observation.

The cultivar name of some reference samples was known (Tables 1 and 2). Samples under the same cultivar name should be genotypically identical because one could suppose them being vegetatively propagated. However, this was not always the case. *P. x festiva* cultivars ‘Rosea

Plena' and 'Rubra Plena' seemed not to be uniform and they did not even cluster into their own groups. However, differences between samples were small because the amount of polymorphic SSRs in the 'Rosea Plenas' and 'Rubra Plenas' was not big, 3 and 8, respectively. In addition, there was uncertainty in the interpretation of some SSRs. Therefore, more markers would be needed to verify this result. The three Norwegian *P. officinalis* 'Mollis' samples were not identical but according to the importer's diaries both seeds and living plants have been imported and the plants have been propagated from seeds for sale in Norway, which might be a reason for variation. Norwegian samples are twice as high (about 80 cm) than described in other places in Europe. Of the four *P. officinalis* 'Nordic Paradox' samples, one from Norway located in another duplicate group than the other from Norway and the two from Sweden. However, the difference dealt only one somewhat uncertain allele and therefore, these four samples can be regarded as the same genotype.

There are several studies on genetic diversity in tree peonies (Gao et al. 2013; Guo et al. 2018; He et al. 2020; Ji et al. 2012; Wang et al. 2014; Wu et al. 2014) but very few in herbaceous peony species and cultivars, and especially in European cultivars. Gilmore et al. (2013) used 21 SSRs to distinguish 93 cultivars in tree, intersectional and herbaceous peonies, and the herbaceous group was separated into three major groups: *P. officinalis*, *P. lactiflora* and *P. lobata*.

5. Conclusions

The results of this genetical diversity study of peonies from Finnish home gardens will be later combined with morphological and phenological observations from these plants, and used in selecting the most diverse peony individuals for the Finnish national peony collection. Some of these peonies will also be introduced into nursery production. In addition, the results will be used for updating the Swedish and Norwegian collections.

Declarations

Funding

Maiju ja Yrjö Rikalan Puutarhasäätiö and Nikolai ja Ljudmila Borisoffin Puutarhasäätiö have financed the study. The Norwegian Agriculture Agency provided financial support for collecting the Norwegian material.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and material

The datasets generated during the study are available from the corresponding author on reasonable request.

References

Abuzayed M, El-Dabba N, Frary A, Doganlar S (2017) GDdom: an online tool for calculation of dominant marker gene diversity. Biochem Genet 55: 155–157

Anderson G (1818) A monograph of the genus *Paeonia*. Trans Linn Soc London. 12(1):248-283

Balvoll G, Weisæth G (1994) Horticultura. Norsk hagebok frå 1694 av Christian Gartner. Page 70. Landbruksforlaget. Oslo

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32: 314-331

De Candolle AP (1818) Regni Vegetabilis Systema Naturale 1: 386-394. Paris

376 Cheng Y, Kim C-H, Shin D-I, Kim S-M, Koo H-M, Park Y-J (2011) Development of simple sequence
377 repeat (SSR) markers to study diversity in the herbaceous peony (*Paeonia lactiflora*). J Med Plants
378 Res 5: 6744-6751

379 Christenhusz MJM, Byng JW (2016) The number of known plants species in the world and its annual
380 increase. Phytotaxa 261: 201-217

381 Dark S (1936) Meiosis in diploid and tetraploid *Paeonia* species. Genetics 32: 353-372

382 Gai S, Zhang Y, Mu P, Liu C, Liu S, Dong L, Zheng G (2012) Transcriptome analysis of tree peony
383 during chilling requirement fulfillment: assembling, annotation and markers discovering. Gene 497:
384 256-262

385 Gao Z, Wu J, Liu Z, Wang L, Ren H, Shu Q (2013) Rapid microsatellite development for tree peony and
386 its implications. BMC Genomics 14: 886

387 Gilmore B, Bassil N, Nyberg A, Knaus B, Smith D, Barney DL, Hummer K (2013) Microsatellite marker
388 development in peony using next generation sequencing. J Amer Soc Hort Sci 138: 64-74

389 Guo Q, Guo L-L, Zhang L, Zhang L-X, Ma H-L, Guo D-L, Hou X-G (2017) Construction of a genetic
390 linkage map in tree peony (*Paeonia* Sect. *Moutan*) using simple sequence repeat (SSR) markers. Sci
391 Hortic 219: 294-301

392 Guo L, Guo D, Zhao W, Hou X (2018) Newly developed SSR markers reveal genetic diversity and
393 geographical clustering in *Paeonia suffruticosa* based on flower colour. J Hortic Sci Biotech 93: 416-
394 424

395 Hao L, Ma H, Texeira da Silva JA, Yu X (2016) Pollen morphology of herbaceous peonies with
396 different ploidy levels. J Amer Soc Hort Sci 141: 275-284

397 Harding A (1917) The book of the peony. Lippincott, Philadelphia, PA / London, UK

398 He D, Zhang J, Zhang X, He S, Xie D, Liu Y, Li C, Wang Z, Liu Y (2020) Development of SSR markers in
399 *Paeonia* based on *De Novo* transcriptomic assemblies. PloS ONE 15 (1): e0227794

400 Homolka A, Berenyi M, Burg K, Kopecky D, Fluch S (2010) Microsatellite markers in the tree peony,
401 *Paeonia* x *suffruticosa* (Paeoniacea). Amer J Bot 97:e42-e44

402 Hong D-Y (2010) Peonies of the world: Taxonomy and phytogeography. Royal Botanic Gardens,
403 Richmond, Surray, UK. 302 pages

404 Hong D-Y, Pan K-Y (2004) A taxonomic revision of the *Paeonia anomala* complex (Paeoniaceae). Ann
405 Missouri Bot Gard 91: 87-98

406 Hou XG, Guo DL, Cheng SP, Zhang JY (2011a) Development of thirty new polymorphic microsatellite
407 primers for *Paeonia suffruticosa*. Biol Plant 55: 708-710

408 Hou XG, Guo DL, Wang J (2011b) Development and characterization of EST-SSR markers in *Paeonia*
409 *suffruticosa* (Paeoniacea). Am J Bot 11: e303-e305

410 Hsu H, Chen Y, Shen S, Hsu S, Chen C, Chang H (1986) Oriental materia medica: A concise guide.
411 Oriental Heeling Arts Inst, Keelung, Taiwan. 932 pages

412 Jakubowski R (2015): Nomenclature. The American Peony Society Bulletin No.374: 7-8

413 Jakubowski R, Hollingsworth D, Nordick J, Buchite H, Schroer C (2007). Peonies 1997-2007. Amer
414 Peony Soc, Gladston, MO

415 Ji L, Wang Q, Teixeira da Silva JA, Yu XN (2012). The genetic diversity of *Paeonia* L. Sci Hortic 143: 62-
416 74

417 Ji L, Teixeira da Silva JA, Zhang J, Tang Z, Yu X (2014) Development and application of 15 novel
418 polymorphic microsatellite markets for sect. *Paeonia* (*Paeonia* L.). Biochem Syst Ecol 54: 257-266

419 Larsson I (2009). Millefolium, rölika och näsegräs: medeltidens svenska växtvärld i lärd tradition. Kungliga
420 Skogs- och lantbruksakademien, Stockholm

421 Li L, Cheng F-Y, Zhang Q-X (2011) Microsatellite markers for the Chinese herbaceous peony *Paeonia*
422 *lactiflora* (Paeoniacea). Am J Bot 98: e16-e18

423 Martinsson K, Ryman S (2007). Hortus Rudbeckianus: an enumeration of plants cultivated in the Botanical
424 Garden of Uppsala University during the Rudbeckian period 1655-1702. Uppsala: Acta Universitatis Upsaliensis

425 O.T. (1890). Pioner. Svenska Trädgårdsföreningens tidskrift, no 9. Stockholm

426 Peltola R, Koivu V (2007) Pionit. Kustannusosakeyhtiö Tammi, Helsinki, Finland. ISBN 978-951-31-
427 3370-2
428
429 Perrier X, Jacquemoud-Collet JP (2006). DARwin software. http://darwin.cirad.fr/darwin
430
431 Pricelist from Göteborgs Trädgårdsförening (1864) Region Västra Götaland and City of Gothenburg
432 Archives, Trädgårdsföreningen i Göteborg, F VII a: 1, 2

433 Rathke J (1823) Enumeratio plantarum horti botanici Universitatis Regiae Fredericianae
434 Christianiensis. Gröndahl, Christiania.

435 Roldan-Ruiz I, Dendauw J, Bockstaele EV, Depicker A, Loose MD (2000) AFLP markers reveal high
436 polymorphic rates in ryegrass (*Lolium* spp.). Mol Breed 6: 125–134
437
438 Ruoff E (2002) Vanhoja suomalaisia puutarhoja. Otavan kirjapaino Oy, Helsinki, Finland
439
440 Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic
441 trees. Mol Biol Evol 4: 406–425
442

443 Sun J, Yuan JX, Wang BS, Pan J, Zhang DM (2011) Development and characterization of 10
444 microsatellite loci in *Paeonia lactiflora* (Paeoniacea). Am J Bot 98: e242-e243

445 Stern FC (1946) A study of the genus *Paeonia*. Royal Horticulture Society, London

446 Tanhuanpää P, Suojala-Ahlfors T, Hartikainen M (2019) Genetic diversity of Finnish home garden
447 rhubarbs (*Rheum* spp.) assessed by simple sequence repeat markers. Genet Resour Crop Evol 66: 17-
448 25

449 Wan Y, Zhang M, Hong A, Zhang Y, Liu Y (2020) Characteristics of microsa tellites mined from
450 transcriptome data and the development of novel markers in *Paeonia lactiflora.* Genes 11(2): 214
451

452 Wang J, Xis T, Zhang J, Zhou S (2009) Isolation and characterization of fourteen microsatellites from a
453 tree peony (*Paeonia* x *suffruticosa).* Conserv Genet 10: 1029-1031

454 Wang X, Fan H, Li Y, Sun X, Sun X, Wang W, Zheng C (2014) Analysis of genetic relationships in tree
455 peony of different colors using conserved DNA-derived polymorphism markers. Sci Hortic 175: 68-73

456 Wu J, Cai C, Cheng F, Cui H, Zhou H (2014) Characterisation and development of EST-SSR markers in
457 tree peony using transcriptome sequences. Mol Breeding 34: 1853-1866

458 Yu HP, Cheng FY, Zhong Y, Cai CF, Wu J, Cui HL (2013) Development of simple sequence repeat (SSR)
459 markers from *Paeonia ostii* to study the genetic relationships among tree peonies (Paeoniaceae). Sci
460 Hortic 164: 58-64

461 Zhang JM, Liu J, Sun HL, Yu J, Wang JX, Zhou SL (2011) Nuclear and chloroplast SSR markers in
462 *Paeonia delavayi* (Paeoniaceae) and cross-species amplification in *P. ludlowii*. Am J Bot 98: e346-348

463 Zhang J, Shu Q, Lui Z, Ren H, Wang L, De Keyser E (2012) Two EST-derived marker systems for cultivar
464 identification in tree peony. Plant Cell Rep 31: 299-310

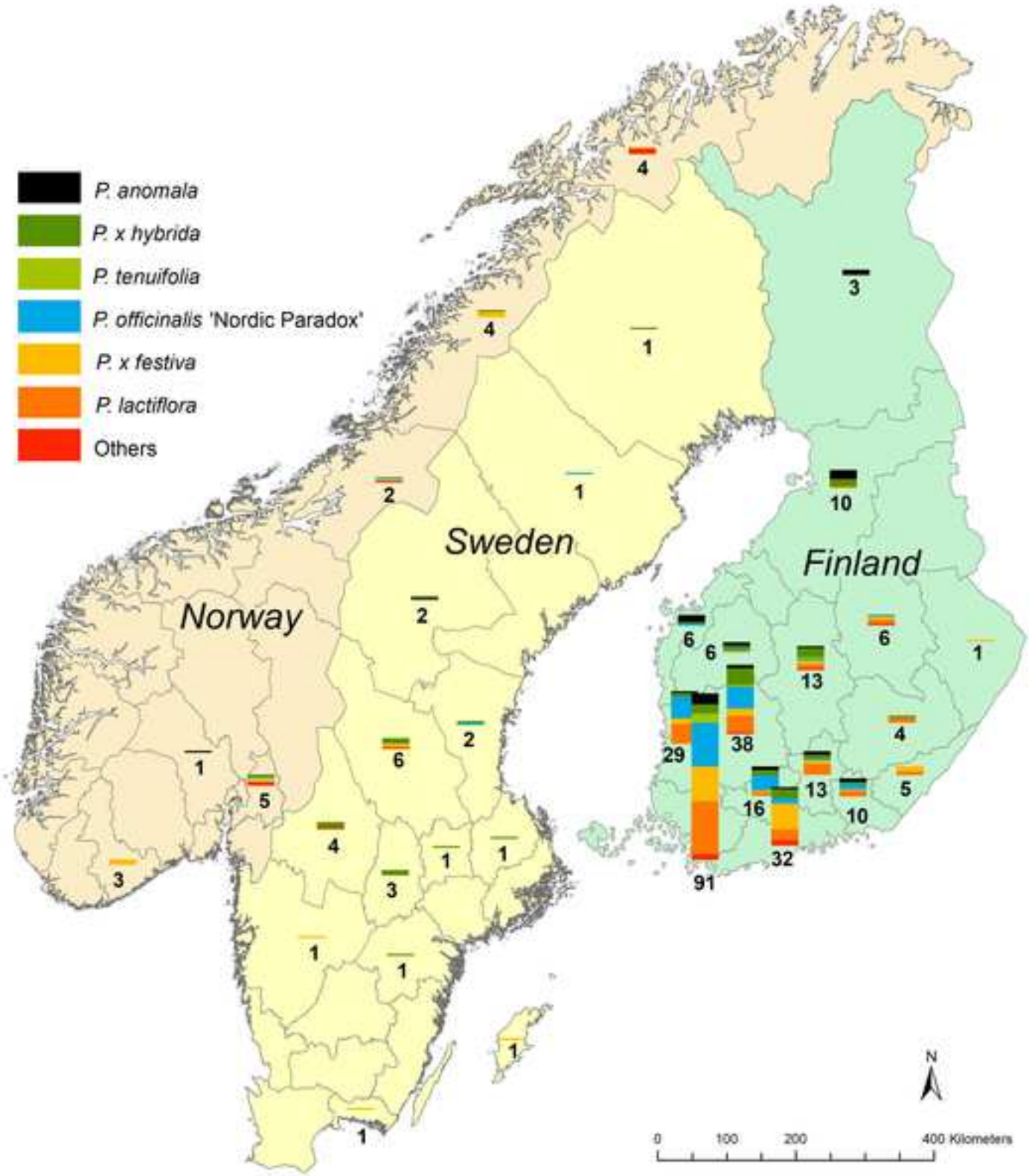
Zhao DQ, Hao ZJ, Tao J (2012) Effects of shade on plant growth and flower quality in herbaceous peony (*Paeonia lactiflora* Pall.). Plant Physiol Biochem 61: 187–196

Figure captions

Fig. 1. Geographical map of the peony samples in the study according to their location to provinces. The class 'others' contains peonies from the following groups: *P. officinalis* and *P. officinalis* 'Mollis.

Fig. 2. Photos of different peony species taken by Mari Marstein, except *P. tenuifolia* by Mikko Uusi-Honko.

Fig. 3 and Electronic supplementary Fig. S1. The dendrogram of 334 peony samples, of which 25 are from Sweden (prefix SWE), 20 from Norway (prefix NOR), and the rest from Finland: 284 from home gardens (prefix LUKE) and 5 references (LUKE-5324, -5325, -5326 = *P. lactiflora*, FIN-2019-74 = 'Alba plena', FIN-2019-75 = 'Rosea plena'). Confidence levels greater or equal to 50% from bootstrap analysis of 1000 replicates are indicated.



P. anomala

P. x festiva 'Rubra plena'

P. x festiva 'Rosea plena'

P. x hybrida

P. lactiflora

P. officinalis

P. officinalis 'Mollis'

P. officinalis 'Nordic Paradox'

P. tenuifolia

Table 1. Twenty peony samples from the Norwegian collection.

Accession number	Species/hybrid	Cultivar	Municipality	Province
NOR-UiA-2003-0248	*P. x festiva*	'Rubra Plena'	4213 Tvedestrand	Agder
NOR-UiA-2006-0135	*P. x festiva*	'Rubra Plena'	4206 Farsund	Agder
NOR-GH-2008-09	*P. x festiva*	'Rubra Plena'	3034 Nes	Viken (Akershus)
NOR-UiT-2002-56	*P. x festiva*	'Rubra Plena'	1813 Brönnöy	Nordland
NOR-UiT-2002-298	*P. x festiva*	'Rubra Plena'	1837 Melöy	Nordland
NOR-UiT-2015-399	*P. x festiva*	'Rubra Plena'	1806 Svolvær	Nordland
NOR-UiA-2001-1028	*P. x festiva*	'Rosea Plena'	4215 Lillesand	Agder
NOR-NTNU-2004-501	*P. x festiva*	'Rosea Plena'	5053 Inderöy	Tröndelag
NOR-GH-2007-17	*P. x festiva*	'Rosea Plena'	3033 Ullensaker	Viken (Akershus)
NOR-UiT-2010-70	*P. x festiva*	'Rosea Plena'	5402 Harstad	Troms
NOR-GH-1980-01	*P. officinalis*	'Nordic Paradox' [1]	3034 Nes	Viken (Akershus)
NOR-NTNU-2005-254	*P. officinalis*	'Nordic Paradox' [1]	5037 Levanger	Tröndelag
NOR-GH-2009-09	*P. x hybrida*		3030 Lilleström	Viken (Akershus)
NOR-UiT-2004-120	*P. x hybrida*		1849 Hamaröy	Nordland
NOR-UiT-1993-982	*P. x hybrida*		0729 Færder	Vestfold
NOR-GH-2006-23	*P. officinalis*		3026 Aurskog-Höland	Viken (Akershus)
NOR-UiT-2004-207	*P. officinalis*	'Mollis'	5401 Tromsö	Troms
NOR-UiT-2004-181	*P. officinalis*	'Mollis'	5401 Tromsö	Troms
NOR-UiT-2010-153	*P. officinalis*	'Mollis'	5401 Tromsö	Troms
NOR-GH-2009-10	*P. anomala*		3007 Ringerike	Viken (Buskerud)

[1]'Nordic Paradox' is nowadays called *P. officinalis* because *P. humilis* is not anymore an accepted term (Jakubowski 2015).

Table 2. Twenty-five peony samples from the Swedish collection.

Sample number	Species/hybrid	Cultivar	Municipality	Province
SWE-2018-1	*P. x festiva*	'Rubra Plena'	Tranemo	Västra Götaland
SWE-2018-2	*P. x festiva*	cf 'Rubra Plena'	Falun	Dalarna
SWE-2018-3	*P. x festiva*	'Rubra Plena'	Floda	Dalarna
SWE-2018-4	*P. x festiva*	cf 'Rosea Plena'	Hasslö	Blekinge
SWE-2018-5	*P. x festiva*	cf 'Mutabilis Plena'	Klintehamn	Gotland
SWE-2018-23	*P. officinalis*	'Nordic Paradox'='Flore Pleno'[1]	Trönödal	Gävleborg
SWE-2018-24	*P. officinalis*	'Nordic Paradox'='Flore Pleno'[1]	Sidensjö	Västernorrland
SWE-2018-6	*P. x hybrida*		Kristinehamn	Värmland
SWE-2018-7	*P. x hybrida*		Falun	Dalarna
SWE-2018-8	*P. x hybrida*		Hagfors	Värmland
SWE-2018-9	*P. x hybrida*		Smedjebacken	Dalarna
SWE-2018-10	*P. x hybrida*		Kälarne	Jämtland
SWE-2018-11	*P. x hybrida*		Gagnef	Dalarna
SWE-2018-12	*P. x hybrida*		Borensberg	Östergötland
SWE-2018-13	*P. x hybrida*		Täby	Stockholm
SWE-2018-14	*P. x hybrida*		Odensbacken	Örebro
SWE-2018-15	*P. x hybrida*		Gyttorp	Örebro
SWE-2018-16	*P. x hybrida*		Dyltabruk	Örebro
SWE-2018-17	*P. x hybrida*		Brevens Bruk	Örebro
SWE-2018-18	*P. x hybrida*		Öjebyn	Norrbotten
SWE-2018-19	*P. x hybrida*		Delsbo	Gävleborg
SWE-2018-20	*P. x hybrida*		Grunnebacka	Värmland
SWE-2018-21	*P. officinalis?*		Filipstad	Värmland
SWE-2018-22	*P. officinalis?*		Gustavs	Dalarna
SWE-2018-25	*P. anomala*		Östersund	Jämtland

[1]'Nordic Paradox' is nowadays called *P. officinalis* because *P. humilis* is not anymore an accepted term (Jakubowski 2015).

Table 3. SSRs used in the genetic diversity analysis of 334 peonies.

SSR	Developed from	Developed by	Repeat motif	Primers	Fluorescent label	Multiplex no.*	Allele size range (bp)	No. of alleles	PIC	PIC range
AG8073	*P. suffruticosa*	Homolka et al. 2010	(AG)10	TCAGCTAATATGGGTGTTTC ATCAAAGTGGAAGTTCTACAGT	VIC	2	192-250	21	0.17	0.006-0.498
AT8051F	*P. suffruticosa*	Homolka et al. 2010	(AT)5	GGTATCAATCCGTGTGC GCGAAAATTTAGATGAGTGT	FAM	3	175-193	8	0.14	0.006-0.317
P05	*P. suffruticosa*	Wang et al. 2009	(AG)9	TCGCCCAACCTGTCGTGGAGAT TTGAATAGAGCGGAATGGAAAA	NED	2	276-314	21	0.12	0.006-0.5
P06	*P. suffruticosa*	Wang et al. 2009	(TC)5CCC(TC)5(CA)8	GTTATAGAACCACTGACAT TGAGAGACAAATAATCGTG	FAM	2	304-333	8	0.1	0.006-0.246
P20	*P. lactiflora*	Li et al. 2011	(TC)9(CA)6	CTG AGA AGC ACT ATG TTC AT ACA CCA AAA CCA TTA CAC A	NED	2	90-115	12	0.23	0.006-0.455
Pae03	*P. lactiflora*	Gilmore et al. 2013	(CT)8	GCTGCGAGATATGTGGTTCA CAGCAACTTTAGAGAGAGGGAGA	FAM	1	76-115	14	0.25	0.006-0.498
Pae100	*P. lactiflora*	Gilmore et al. 2013	(AT)7	ACCATTCAAGGTGAGCTTCC TCCAGATATATTCCCTCACCCTA	PET	3	175-349	18	0.21	0.006-0.476
Pae115	*P. lactiflora*	Gilmore et al. 2013	(TA)9	CTTTCCGAATTCTGCACCAC CGAACTCGGGAAGTCAAAAA	FAM	2	112-117	4	0.13	0.006-0.31
Pmg165	*P. lactiflora*	Sun et al. 2011	(GA)18	AAGAAACCTACCTCAATCAGTC TTCTTTCATCTCCCTTCTACAC	FAM	1	184-249	23	0.12	0.006-0.474
Pmg180	*P. lactiflora*	Sun et al. 2011	(GA)19	TTCTCCAACCCTTGAATAGCTC TCTCCTCCTCCACCATTACCAC	NED	2	179-211	15	0.08	0.006-0.275
PS004	*P. suffruticosa*	Wu et al. 2014	(CCA)5	GTGCTTAGCCTCTAATCTG CTTTGCTCCAAGTCTGTC	PET	2	215-336	33	0.14	0.006-0.453
PS153	*P. suffruticosa*	Wu et al. 2014	(CT)10	ATGTCCAAACTGGCAATA CCCTCCCTCAACACTTAC	FAM	3	250-273	15	0.09	0.006-0.328
Sy1	*P. lactiflora*	Ji et al. 2014	(TCT)23	TGTTTTATACAGACCGACGACATCTC GATTTTGTGGTGCTCCATTAAATATG	FAM	1	329-353	6	0.22	0.006-0.5
Sy2	*P. lactiflora*	Ji et al. 2014	(AC)9	GCTATACCTTGATAATCAACATTCAACC ATTGTAAGTTTTGGAACTTTTCCTCTAA	VIC	1	268-276	4	0.15	0.006-0.355
Sy4	*P. lactiflora*	Ji et al. 2014	(TC)15	AACCGATTGGGAACTCTTGAAAT GGGATAAGAAATGAAAGGGAAGGT	VIC	3	289-315	10	0.26	0.018-0.499
Sy5	*P. lactiflora*	Ji et al. 2014	(GA)13GG(GA)2	GTCGTAAGACAACTTGGGGTAAATCG TGTGGGTCTACTCGTAATCCTATCAT	NED	3	229-288	6	0.1	0.03-0.22
Sy7	*P. lactiflora*	Ji et al. 2014	(TG)2C(GT)8	GAGCAATGAACAAGCTCAAGAAACT ACAATCAACGGTCCTGTCAACCT	VIC	1	162-186	9	0.21	0.024-0.455
Sy15	*P. lactiflora*	Ji et al. 2014	(TG)10	AAAAGCAATCCCAGCCAGTTAG TTTCCCCATTCCAAGGTAAAGAT	FAM	2	149-211	22	0.18	0.006-0.499

* SSRs were amplified in three separate PCR reactions.

Figure

Click here to access/download;Figure;Fig. 3.pptx

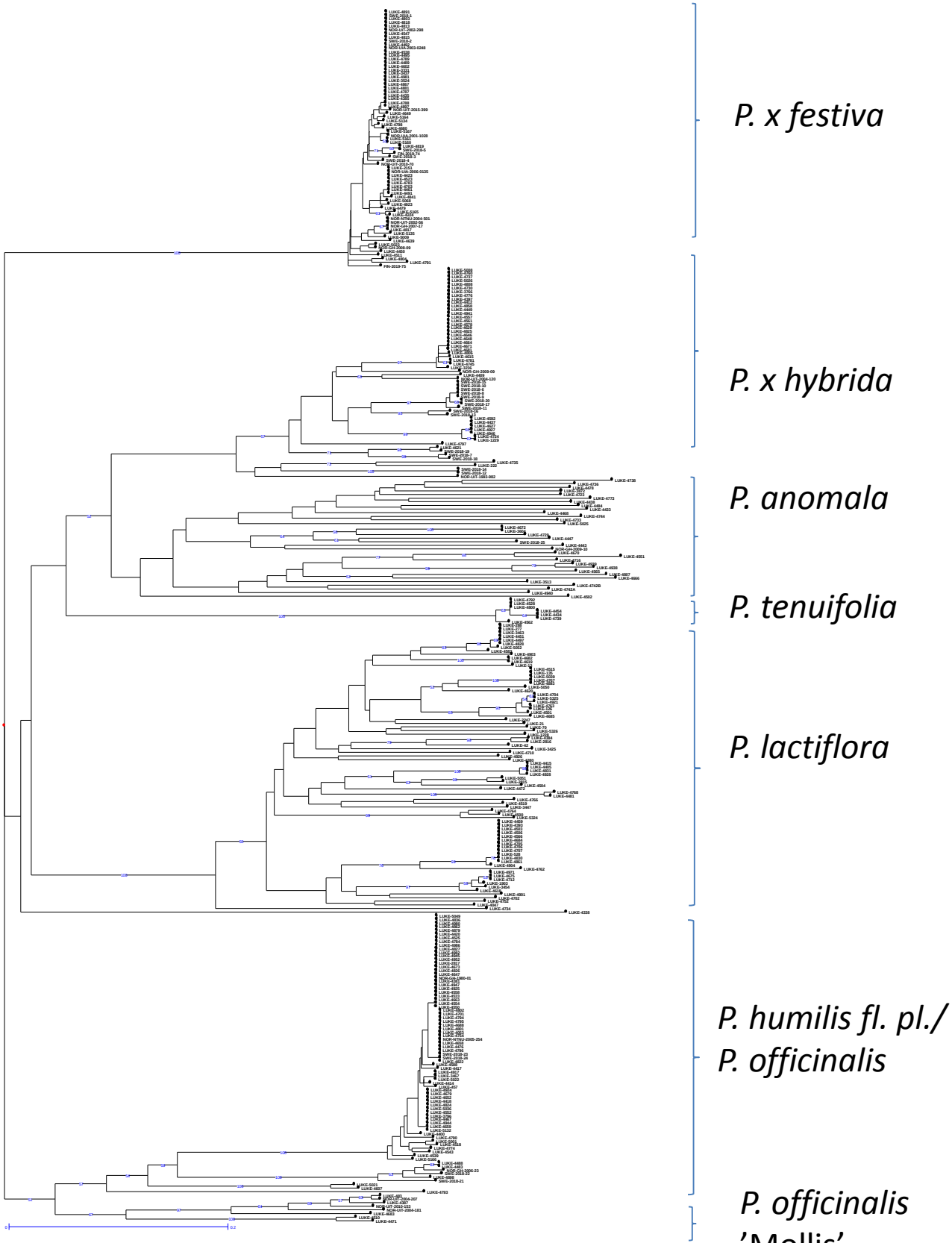


Table 4. The number of samples in each peony group in the NJ tree. One sample (LUKE-4338) did not clearly cluster into any group.

				Reference samples from			Samples from Finnish home gardens			
									Morphology described	
Group no.	**Group name**	**Total no. of samples**		**Finland**	**Norway**	**Sweden**	**Total**	**Different genotypes**	**Total**	**Inconsistency[2]**
1	*P. x festiva*	**71**		2	10	5	54	26 (48 %)	42	0
2	*P. x hybrida*	**58**			3	15	40	12 (30 %)	35	0
3	*P. anomala*	**33**			1	1	31	30 (97 %)	28	1
4	*P. tenuifolia*	**7**					7	3 (43 %)	4	0
5	*P. lactiflora*	**79**		3			76	49 (65 %)	57	0
6	*P. humilis fl. pl. / P. officinalis*									
	- *P. humilis fl. pl.*	**68**			2[1]	2[1]	64	16 (25 %)	54	0
	- *P. officinalis*	**6**	**77**		1	2	3	2 (67 %)	1	0
	- separate samples	**3**					3	3 (100 %)	0	0
7	*P. officinalis* 'Mollis'	**8**			3		5	5 (100 %)	5	1
	Total	**333**		**5**	**20**	**25**	**283**	**146**	**226**	**2**

[1]These samples are 'Nordic Paradox', which is nowadays called *P. officinalis* because *P. humilis* is not anymore an accepted term (Jakubowski 2015).

[2]inconsistency between genetical analysis and morphological evaluation.

Table 5. Amplification of 18 SSRs in different peony species groups. Groups with less than 10 samples have been omitted (*P. tenuifolia* , 7 samples and *P.officinalis* 'Mollis' group, 8 samples).

Group no.	Group name	No. of samples	No. of polymorphic SSRs	No. of polymorphic alleles	SSRs not amplified	Monomorphic SSRs
1	*P. x festiva*	71	14	38	Sy2, Sy5	Pae03, Sy1
2	*P. x hybrida*	58	14	45	P05, P06, Sy2	Sy4
3	*P. anomala*	33	17	116	P06	
5	*P. lactiflora*	79	17	90		Sy1
6	*P. humilis fl. pl. / P. officinalis*	77	15	77 (48)[1]	Pae115, Sy5	Sy1

[1]Fourty-eight if the three separate samples (see text) are not included. Nine alleles amplify only in *P. officinalis.*