



Проблемы сохранения биологического разнообразия  
и использования биологических ресурсов  
7-9 октября 2015, Минск, Беларусь

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Материалы III Международной научно-практической конференции,  
посвященной 110-летию со дня рождения академика Н.В. Смольского



Часть  
1

Часть 1

НАЦИОНАЛЬНАЯ АКАДЕМИЯ НАУК БЕЛАРУСИ  
Центральный ботанический сад  
Научно-практический центр по биоресурсам  
Институт экспериментальной ботаники им. В.Ф. Купревича  
Институт леса



## **Проблемы сохранения биологического разнообразия и использования биологических ресурсов**

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(7–9 октября 2015 г., Минск, Беларусь)

**В двух частях  
Часть 1**

**Секция 1. Ресурсы и биоразнообразие растительного мира:  
современное состояние, воспроизводство, охрана  
и устойчивое использование**

**Секция 2. Современные направления изучения  
ботанических коллекций для сохранения  
и рационального использования  
биоразнообразия растительного мира**

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## Developing an international model for *Paeonia lactiflora* Pall. (*Paeniaceae*) genetic resources conservation: integrating assessment of relative significance of historic cultivars for field genebanks with their genetic diversity

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**Summary.** The North American Plant Collection Consortium (NAPCC) working group to conserve cultivated *Paeonia* diversity is the source of data to set conservation goals and targets. An important NAPCC objective is to integrate cultivar assessment of variability in phenotypic traits with genetic diversity. Genetic diversity data for 53 accessions of *P. lactiflora* cultivars, species, and hybrids held by the University of Michigan Nichols Arboretum and the Central Botanical Gardens NAS Belarus is reported. Data indicate both institutions have strong genetic diversity in the total collections, yet both have 47–49 % unique accessions that may account unevenly for this diversity. More markers need to be employed, and more accessions from reference collections investigated to develop the scientific basis for a robust, prioritized, and systematized international *Paeonia* genetic resources conservation program.

**Резюме.** Миченер Д.С., Власова А.Б. Развитие международной модели сохранения генетических ресурсов *Paeonia lactiflora* Pall. (*Paeniaceae*): интегральная оценка значимости исторических сортов полевых генетических банков и их генетического разнообразия. Важной целью рабочей группы Североамериканского консорциума растительных коллекций (NAPCC) по сохранению разнообразия рода *Paeonia*, помимо создания ресурса для установки целей и задач, является интегрированная оценка изменчивости фенотипических признаков сортов и их генетического разнообразия. В статье представлены данные генетического разнообразия для 53 образцов *P. lactiflora*, включая сорта, виды и гибриды из коллекций Николс Дендрария Мичиганского университета и Центрального ботанического сада НАН Беларуси. Согласно данным, оба учреждения имеют сопоставимые генетически репрезентативные коллекции, хотя это разнообразие может быть распределено неравномерно у 47–49 % уникальных образцов учреждений. Предполагается увеличение маркеров и вовлеченных в исследование образцов из реферируемых коллекций для развития научно обоснованной базы и обеспечения надежной и систематизированной международной программы сохранения генетических ресурсов *Paeonia*.

**Introduction.** Living field genebanks for ornamental peonies are spatially limited for the number of accessions they can ultimately hold. An international consortium involving 13 peony collections is in development, and already holds over 1,400 named *Paeonia* L. cultivars of several species important in European and North American horticultural history and contemporary breeding. This communal holding represents significantly less than 40 % of the named *Paeonia* cultivars in the ICRA registry. It is clear that any expanded consortium can not keep apace with adding new cultivars as well as conserving additional historic cultivars as they are located. There is no consensus on how to determine which cultivars merit long-term inclusion duplication and sharing among collections for security. For conserving the domesticated genetic diversity of the species (specific alleles), all cultivars are suspected to be unequal contributors to the allelic diversity, but each may be independently significant as an artifact for interpreting cultural history. Issues in reconciling the two conservation-focused agendas (allelic diversity; culturally significant phenotypes) for a consensus prioritization scheme for herbaceous peonies are presented.

**Methods.** Ornamental display collections and field genebanks of historic to contemporary peony cultivars are important tools in cultivar conservation. The need for multi-site institutional consortia to collection and manage such diversity has been formalized in North America with the North American Plant Conservation Consortium (NAPCC) of the American Public Garden Association (<https://www.publicgardens.org/napcc>) The advantages of a consortium include collaborative inventories to assess cultivar rarity, the ability to conserve duplicate specimens, and the sharing of knowledge. However, managing historic and contemporary ornamental cultivar collection networks presents fundamental issues to resolve in conservation biology. The central long-term problems are: a) the physical number of spaces in each institution as well as

any consortium is limited such that accessions will have to be evaluated for acceptance/retention for holding a space, b) the authenticity of historic materials is presumed by cultivar name but this method has been demonstrated to be erroneous – a serious issue when the presumed identical cultivar is present in multiple sites, and: c) the same cultivar displays a range of phenotypes when grown in the diverse climatic conditions of the different host institutions (leading to questions of authenticity). Assessing and managing cultivar diversity by the cultivar name is the current practice but ultimately a limited approach since each cultivar is a “black box” for the genetic diversity it carries – some are likely trivial changes at the genomic level yet may have large phenotypic differences. Consistently applied molecular methods are required, as are the concepts to integrate the two approaches into an integrated conservation scheme.

Genetic fingerprinting of plant material – *Paeonia lactiflora* L. cultivars, species and interspecific hybrids from the field collections of CBG and MBGNA with 4 pairs of SRAP primers described previously [1]. Calculation of genetic diversity indices and the number of rare alleles were performed using GenAlex [2].

Results. For ornamental peonies, 14 institutions have begun to collaborate in the NAPCC peony consortium (Table 1). The Central Botanical Garden, Minsk, though not a formal partner is included as the first European collaborator. The group currently holds 1,467 *Paeonia* cultivars, of which 939 (64 %) are unique (only at one institution). When re-calculated only for the herbaceous cultivars, 11 of the institutions hold 1,108 herbaceous cultivars, of which 665 (60 %) are unique. Since unique cultivars are likely only one step from extinction, a means to prioritize their evaluation is needed. The obvious step of duplicating and dispersing all unique cultivars is an incorrect approach. This counter-intuitive situation is due to the spatial limits of the collections – duplicating the unique accessions (for security through redundancy) means the overall capacity to carry total cultivar diversity is equivalently reduced (since space is limited). This problem is severe even at each institution. For the institutions that specially collect herbaceous ornamental peonies ( $n = 11$ ) the extent of herbaceous uniqueness ranges from a high of 49,2 % ( $n = 274$ ) at the Botanical Garden of the Academy of Sciences in Minsk to a low of 25,7 % at Cornell University in New York. (Tabl. 1). A similar pattern is present for ornamental peonies when the collections scope is expanded to include tree, intersectional, and herbaceous cultivars (data not presented, available on request).

In a consortium, each institution has its own focus. When the focus is depth of a breeder's production, or a set of breeders (as from a country or region), the issue of cultivar-specific merit will benefit from molecular passportization. For example, the American breeder Pleas, was a woman active in the late 1800s to early 1900s at a time when peony breeding in North America was dominated by men. She introduced 55 cultivars, of which only one, 'Jubilee' is still common in the trade, and 11 (20 %) are have escaped extinction in the consortium. Much of her work was considered unimportant by subsequent peony specialists, but the criteria are not clearly articulated. If any of the remaining 44 likely extinct cultivars are located, as through continued consortium expansion or discovery in private peony collections, do they bring additional genomic diversity to merit inclusion? How many cultivars are enough to represent any breeder's output, given the subtle variations in phenotypes among closely related cultivars? Genetic passportization will be an essential tool in establishing genomic criteria for inclusion. These can then be weighed with historical and phenotypical criteria to establish an overall priority for inclusion.

Earlier we showed the possibility to differentiate the cultivars of herbaceous *Paeonia* on the basis of elaborated system of SRAP molecular markers [1]. It was revealed that delimitation and identification of *P. lactiflora* cultivars including interspecific hybrids. In this study allelic profiles for locus generated with 4 pairs of SRAP primers of 53 accessions with the European, American and former Soviet Union origin, as well as 4 species allowed to calculate descriptive statistics of the genetic diversity of the *P. lactiflora* cultivars according to their geographic origin (Tabl. 2). The number of alleles ( $N_a$ ) identified with this marker system ranged from 1.513 (American cultivars) to 1.867 (historic European pedigree accessions). The number of effective alleles was highest in European landraces and wild species (1.316 and 1.304, respectively). Slightly similar  $N_e$  indices were revealed for American and Soviet origin cultivars (1.285 and 1.289, accordingly).

Table 1. Uniqueness values of herbaceous peony cultivars in the major collections engaging in the North American Plant Conservation Consortium – Peony multi-site project in the next several years\*

Institution	Location	No Total accessions	No Unique genotypes	Unique, %
Central Botanical Garden	Belarus, Minsk	274	135	0.49
Nichols Arboretum	USA, Michigan	253	115	0.45
Winterthur	USA, Delaware	69	29	0.42
Boerner Botanical Garden	USA, Wisconsin	117	42	0.36
Royal Botanical Garden, Hamilton	Canada, Ontario	321	104	0.32
Lauritzen Botanical Garden	USA, Nebraska	62	19	0.31
Barnes Foundation	USA, Pennsylvania	116	35	0.30
New York Botanical Garden	USA, New York	172	50	0.29
Montreal Botanical Garden	Canada, Quebec	236	63	0.27
Minnesota Landscape Arboretum	USA, Minnesota	194	51	0.26
Cornell Plantations	USA, New York	70	18	0.26
<i>Alaska Botanic Garden</i>	USA, Alaska	52	3	0.06
<i>Brooklyn Botanical Garden</i>	USA, New York	2	1	0.50
<i>Scott Arboretum</i>	USA, Pennsylvania	10	0	0.00

\* Alaska Botanical Garden, Brooklyn Botanical Garden, and Scott Arboretum (in italics) are excluded from the discussion since their collections are focused on tree peonies (Brooklyn, Scott) and their unique N totals 4. The total herbaceous cultivar diversity is 1108 cultivars; the sum of N (1948) includes duplicate cultivars (not unique).

The mean value of expected heterozygosity for all analyzed groups total 0.186, ranging from 0.201 for European cultivars to 0.173 for USA. The  $H_e$  values were very similar to unbiased expected heterozygosity values, showing a mean value of 0.201 (with minimum value 0.187 for USSR cultivars and maximum 0.215 for wild species). Despite the limited number of analyzed accessions of American origin they show relatively genetic diversity parameters and were distributed spatially (evenly) on PCoA, as shown earlier [1].

Table 2. Genetic diversity of *P. lactiflora* cultivars of European, American and Soviet origin

Group	N	No/ Pp*, %	Na	Ne	I	He	uHe
Wild species	4	56.64	1.566 (0.498)	1.304 (0.031)	0.288 (0.025)	0.188 (0.017)	0.215 (0.020)
Europe	30	86.73	1.867 (0.341)	1.316 (0.030)	0.323 (0.021)	0.201 (0.016)	0.205 (0.016)
USSR	15	69.03	1.690 (0.464)	1.289 (0.031)	0.286 (0.023)	0.181 (0.016)	0.187 (0.017)
USA	4	51.33	1.513 (0.502)	1.285 (0.032)	0.264 (0.026)	0.173 (0.018)	0.198 (0.020)
Grand Mean over all loci and Groups	13.250 (0.502)	65.93 (7.86)	1.9912	1.298 (0.015)	0.290 (0.012)	0.186 (0.008)	0.201 (0.009)
Total	53						

\* Abbreviations: Mean numbers measured over Loci for each Pop: No/Pp – Number/ Percentage of Polymorphic Loci (%); Na – number of different alleles; Ne – number of effective alleles; I – Shannon's Information Index; He – expected heterozygosity; uHe – unbiased expected heterozygosity.

Private loci were detected for Wild, Europe and USSR groups (2.13; 7.21 and 2.61 %, correspondingly). Of the loci with frequency greater than 5 %, the biggest number (24) of such bands were shared by a European group with up to 50 % of the remaining groups, the smallest (9) – by the involved in analysis wild species (Fig. 1, data not presented).

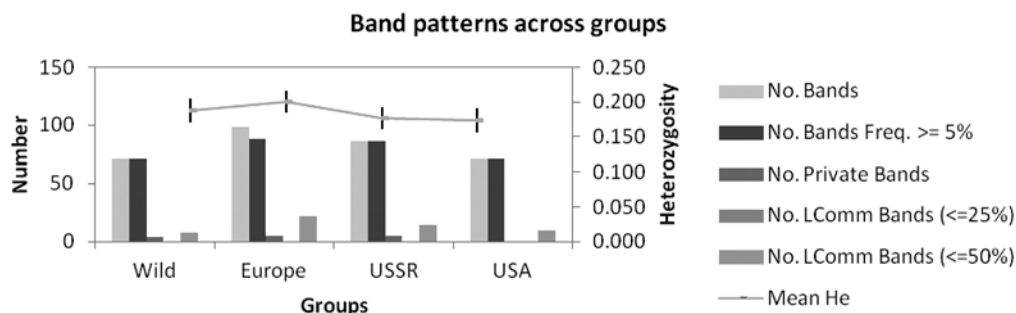


Fig. 1. Band patterns across groups of cultivars by geographic origin and wild species of *Paeonia*.

Genetic diversity of *Paeonia* germplasm resources were previously reported for different groups, species and cultivars of diverse geographic origin assessed by using molecular markers. The estimated percentage of polymorphic loci for the tree peonies of different origin ranged from 98.1 % for Japanese material, 92.3 % for French material; 88.9 % – for wild species, and 69.1 % for American cultivars, although sample sizes were small ( $n = 12, 3, 2$  and  $3$ , respectively) [3]. The number of alleles ( $N_a$ ) and observed heterozygosity ( $H_o$ ) for Chinese herbaceous peony germplasm estimated by SSRs were 6.1 and 0.3574, respectively [4]. Gilmore and colleagues presented that Shannon-Weiner index ( $H$ ) assessed using SSR polymorphism for herbaceous peonies was higher than for other estimated groups – Itoh and tree [5].

Another perspective of using genetic markers is assessing overall collection diversity. On the basis of the frequency of alleles and grouping the accessions by site (CBG/ MBGNA) it is possible to estimate the genetic diversity of entire collection and their value as germplasm conservation repositories. As an example genetic diversity parameters for 23 accessions from CBG and 30 from MBGNA were measured and similar aggregated values were found across each collection (Tabl. 3).

Table 3. Genetic diversity of *Paeonia lactiflora* resources of CBG an MBGNA collectionsn

Group	N	Pp*, %	Na	Ne	I	He	uHe
CBG	23.000	83.19	1.690 (0.067)	1.320 (0.029)	0.330 (0.021)	0.206 (0.015)	0.211 (0.016)
MBGNA	30.000	81.42	1.673 (0.067)	1.319 (0.030)	0.322 (0.022)	0.202 (0.016)	0.206 (0.016)
Grand Mean over loci and groups	26.500 (0.233)	82.30 (0.88)	1.681 (0.047)	1.320 (0.021)	0.326 (0.015)	0.204 (0.011)	0.208 (0.011)
Total	53						

\* Abbreviations as in Tabl. 2.

For a more precise and comprehensive assessment of each collections genetic diversity it is necessary to include all 538 accessions (CBG+MBGNA) in the analysis. Especially interesting is to investigate the genetic diversity numbers in sets by breeder, which may allow evaluating the contribution of particular cultivars and breeders to modern diversity of cultivated *P. lactiflora*. In addition is to infer their original breeding stock and exchange or use of new cultivar among the breeder as they developed subsequent cultivars according to their aesthetic objective and the genetic restriction of their available material. This integrated approach of using cultivar genetic diversity analysis and traditional phenotypic character specific to each cultivar will allow

development of conceptual tools to document and rank cultivars for their individual contribution to the aggregate germplasm conservation. This is a critical need for international consortia as NAPCC so that true diversity is captured, managed, and conserved, rather than using cultivar phenotype as indicators of genetic-diversity conservation. Additional perspectives needed includes marker systems as ISSR, RAPD and SSR, as well as whole genome high throughput screening to address pedigree and breeder contribution, and exchange of the now-certified reference material. For this robust model more cultivars, more markers, and more collaborators will be needed.

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