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RESEARCH ARTICLE

Arising and development of the population of *Matteuccia struthiopteris* (L.) Tod. in the University Grove of Tomsk University

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Abstract

Matteuccia struthiopteris (L.) Tod. – Ostrich fern (Onocleaceae) is a circumboreal species widely distributed in the temperate zone of the northern hemisphere. We present the results of observations on the formation of *M. struthiopteris* population on a man-made lawn situated in the University Grove (Tomsk, Russia). The formation of the plant community on the new lawn occurred naturally, and sporophytes of the fern appeared naturally. During the observation period (2009–2025), we observed two wave of colonization of the lawn by *M. struthiopteris*, both waves coincided with covering of the lawn surface with a fresh soil. The first filling of the lawn occurred during its construction in 2007. The soil used for the lawn construction was taken from a native area located 6.65 km the southwest of the University Grove. The first *M. struthiopteris* sporophytes were found in 2009 in significant numbers: 263 individuals of early stages of ontogenesis. A new wave of colonization by spores occurred after a severe damage to the lawn and disruption of the established plant community in 2020 and subsequent refilling of the lawn with the new soil in 2023. By 2025, 315 new sporophytes of the initial stages of ontogenesis were added to sporophytes that survived the disruption. Thus, both waves of colonization of the lawn by *M. struthiopteris* were associated with its filling with the new soil, which means that the spores were presented in this soil. Both time, two years passed between the moments when the lawn was covered with the new soil and sporophytes became visible. This time was required for the germination of spores, development of gametophytes, fertilization and formation of sporophytes. No fertile (spore-bearing) sporophytes appeared during the whole observation period (17 years). For detection of the source of spores, from which the first wave of colonization occurred and *M. struthi-*

opteris population on the lawn formed, we conducted a molecular-genetic analysis of total DNA using ISSR method. The proven genetic identity of sporophytes from the lawn population to sporophytes growing at the site, where the soil was taken for the lawn construction, indicates that the spores were introduced to the lawn with this soil and that green spores of *M. struthiopteris* are capable of the formation of the spore bank, albeit a short-lived one.

Keywords

Matteuccia struthiopteris, fern, gametophyte, sporophyte, ontogenesis, spore dispersal, population, Western Siberia

Introduction

Colonization of free sites by plants and subsequent formation of vegetation cover are important biological issues that, at least for ferns, have received little previous detailed analyses. Ferns quickly colonize free sites in both native coenoses and anthropogenically disturbed areas. They can particularly colonize primary free areas such as lava fields, dunes, places after deglaciation, as well as newly formed free sites after fires, floods, etc. (Page 2002; Walker, Sharpe 2010; Gureyeva, Timoshok 2016). Ferns occupy a new area via spore dispersal or through vegetative reproduction. Long-distance dispersal of spores is likely highly important in the distribution of fern species. Most of the produced spores disperse within only a few meters (Wolf et al. 1991), but dispersal over distances of at least a thousand kilometers is also possible (Schneller, Liebst 2007; Kessler 2010), and dispersal of spores to high-altitude levels is also known (Page 1979a, 1979b). Most fern species have potentially hermaphroditic gametophytes; therefore, colonization of new habitats and establishment of new fern populations are possible by means of single spore arrival (Schneller, Holderegger 1996a). In the Siberian region, in particular, colonization of new sites by ferns has been suggested to primarily take place through spores and formation of gametophytes, whereas the expansion of the existing populations is more often determined by the ability of sporophytes for vegetative reproduction (Gureyeva 2001, 2002). Some fern species having spores with long-lasting viability can persist in soil and form a soil spore bank (Sheffield 1996; Schneller, Holderegger 1996b). Spores preserved in the soil have the potential to germinate when conditions become favorable, for example, in disturbed sites, where competition from other plants is reduced or absent (Sheffield 1996; Gureyeva 2002).

Some fern groups, such as Onocleaceae, Hymenophyllaceae, Osmundaceae, produce green chlorophyll-containing spores. These spores do not undergo a dormancy period, typically germinate in less than three days (mean = 1.46 days), and exhibit viability lengths of one year or less (mean = 48 days); however, exposure to darkness at temperatures of +3 … +5 °C promote viability preservation in chlorophyllous spores for up to two years (Lloyd, Klekowski 1970; Pence 2000; Kessler 2002, 2010).

The fern *Matteuccia struthiopteris* (L.) Tod. (Onocleaceae) produces chlorophyllous spores with the maximum of germination (95 %) within the first days after collecting. Its germination ability decreases at room temperature approximately to 80 % after one month. Less than 10 % of spores germinated after two months of storage, and less than 1 % of spores germinated after seven months of storage. Storage at low temperature and even in liquid nitrogen prolongs the viability of the spores, which makes the cryopreservation a potential method for long-term storage of green spores of ferns (Page 1979b; Kreshchenok et al. 2014).

Klekowski (1984) investigated *M. struthiopteris* as a plant with apical meristem based upon a single permanent apical cell. He found that such plants are more likely to accumulate unfavorable somatic mutations compared to those with stochastic apical meristem. *Matteuccia struthiopteris*, which forms extensive clones and seldom reproduces sexually, is supposed to have a high mutation load. These clones are genetic chimeras and are common in *M. struthiopteris*. In these clones, meiosis was normal but post-meiotic maturation of the spores was defective. According to Klekowski (1988), the clones of *M. struthiopteris* appear to have fixed dominant mutations that disturb normal sporogenesis and 94.5 % of spores have sporophytic lethals. On average, *M. struthiopteris* has 2.9 lethal equivalents per spore or 5.8 lethal equivalents per zygote. This is the highest genetic load documented in ferns to date. In Europe, von Aderkas (1983), who studied the development and sex expression of gametophytes in *M. struthiopteris* both in culture and in nature, noted that in multispore cultures, gametophytes demonstrate the greatest variety of sexual-morphological types (male, female, neuter and hermaphroditic). Field-found gametophytes also showed the least diversity, their populations contained mainly male or neuter gametophytes, while only two female gametophytes were found among 1299 samples, with no sporelings appeared during the growing season. D.R. Farrar (1976) also did not observe the establishment of *M. struthiopteris* gametophytes despite the huge number of spores produced (about 1 million per frond). He also reported about late maturation and shedding of spores in *M. struthiopteris* growing in Michigan (USA): its late-maturing fronds bear > 90 % unopened sporangia in December and > 80 % in March.

These observations suggest that spore reproduction requires a combination of specific factors needed for germination of spores, formation of gametophytes, fertilization, and formation and establishment of sporelings. Although the gametophyte is a sporophyte-independent small single-layered thallus growing on the surface of a substrate, it can be more resistant to unfavorable environmental conditions than the sporophyte (Page 1979a, 1979b; Gureyeva 2002). Gametophytes of the most temperate fern species are also known to survive freezing to –20 °C … – 40 °C, and can continue to grow after transfer to normal conditions and initiate sporophyte development (Sato 1982).

The observations suggest that *Matteuccia struthiopteris* is less capable of long-distance dispersal and formation of the viable population via spores compared to many other species. The complete absence of sporophytes generated sexually in

M. struthiopteris populations is evidenced by our many-year field investigations of the demographic structure of native populations of this species growing in the Siberian mountain forests, where this fern is very abundant (Gureyeva 2001, 2003, 2014). Despite comprehensive searches, we have not found gametophytes of *M. struthiopteris* in any of the studied populations. This finding gave us grounds to consider that self-maintenance of native populations of this species occurs only by vegetative reproduction of sporophytes. However, the existence of *M. struthiopteris* sporophyte populations in widely separated habitats cannot be explained other than by their development from spores via initial gametophyte formation.

The evidence to support this statement in the present study is the emergence of the *M. struthiopteris* population originated via spores. The first juvenile sporophytes of this fern were observed in 2009 on a new man-made lawn in the University Grove of Tomsk State University (Tomsk, Russia).

This observation provides clear evidence of the development of sporophytes after recent spore germination and successful gametophyte colonization. Therefore, the following questions were posed: (1) where is the source of spores from which the population originated, and (2) does this population have a chance for a long-term existence? Hence, the aims of this study were to detect the source of spores that established the *M. struthiopteris* population and identify the structure and dynamics of this population. The working hypotheses were as follows: (1) the population has arisen from spores dispersed to the lawn by wind from neighboring *M. struthiopteris* populations, and in this case, sporophytes should be genetically polymorphic or genetically identical to those in one of these populations; (2) spores were introduced with the soil used for the lawn construction, and in this case, sporophytes should be genetically identical to those growing in the place where the soil was taken.

Materials and methods

Ecological and biological characteristics of a studied fern, *Matteuccia struthiopteris*

Matteuccia struthiopteris (L.) Tod. – Ostrich fern (Onocleaceae) is a circumboreal species widely distributed in the temperate zone of the cold temperate and boreal regions of North America and Eurasia, especially in Asian part. The overall distribution of this species coincides with the boreal forest zone and mountain forest belt. The species prefers well-aerated moist and nutrient-rich soils, serving as an indicator of high permanent moisture associated with soil groundwater (Porfiriyev 1975). *M. struthiopteris* is widely distributed in the humid lowland and mountainous regions of Siberia, especially in the Altai-Sayan Mountain Country, and often dominates in the herbal cover of coniferous (*Abies sibirica* Ledeb. + *Pinus sibirica* Du Tour + *P. sylvestris* L.), small-leaved (*Populus tremula* L. + *Betula pendula* Roth) and mixed small-leaved coniferous forests. It also grows along the valleys of rivers and streams, forming thickets, pure or often mixed with *Athyrium filix-femina* (L.) Roth (Gureyeva 2001).

Sporophytes of *M. struthiopteris* are perennial plants with an average lifespan in native phytocoenoses of 40–58 years (Gureyeva 2001). The adult sporophyte of *M. struthiopteris* includes two types of rhizomes (shoots): short orthotropic, aerial, radially symmetrical rhizome, like a short stem, crowned with a funnel-shaped crown of large (up to 115–125 cm long) fronds, and long plagiotropic underground rhizomes (stolons), formed mainly at the base of orthotropic rhizome (Fig. 1A). The adult sporophyte of *M. struthiopteris* has three type of fronds: trophophylls (green photosynthetic fronds), sporophylls (fertile fronds bearing sporangia), and cataphylls (abortive fronds without stipe and blade). Fronds surround a large, dense, upward-directed terminal bud, which consists of the croziers of varying degrees of maturity (Fig. 1B). The terminal bud includes a significant number of croziers (30–57), and the number of fronds forming funnel-shaped rosette may attain 7–13(18) (Gureyeva 2001). The apical bud includes a multicellular structured apex located centrally with a single apical initial. Such apex described in detail by M.A. Romanova & V.Yu. Shalisko (2004). The frond primordia, which develop into young frond croziers, are located in the center of the terminal bud, with mature croziers disposed at its periphery. All types of fronds are long-lived, as their development from the primordia to mature croziers can last up to 5 years. The most long-lived part of the frond is the phyllopodium, which becomes part of the rhizome and persists for many years, as long as the rhizome remains alive. Green parts of the trophophyll (stipe and blade) are summer-green, i.e. they live one growing season (Gureyeva 2001, 2003, 2014).

Plagiotropic underground rhizomes (stolons) are formed on the orthotropic rhizome. Stolons are perennial, they develop from "detached meristem" (a term by Wardlaw 1946) located in internodes of the orthotropic rhizome. Commonly, well-developed stolons are formed at the base of the orthotropic rhizome and can reach 1.5–3 m in length. The stolons bear cataphylls consisted only of a phyllopodium as neither a stipe nor a blade is initiated. This distinguishes the cataphyll of the stolon from the cataphyll of the orthotropic rhizome. Stolons grow underground for a long time, and then change the direction to form an above-ground orthotropic rhizome bearing fronds (Fig. 1C) (Nekhlyudova, Filin 1993; Gureyeva 2001, 2003). New plagiotropic stolons are formed at the basal part of the newly formed orthotropic rhizomes. Plagiotropic stolons transition to orthotropic growth in areas with a lower density of other orthotropic rhizomes bearing the fronds, resulting in their location at relatively equal distances from one another. Therefore, native *M. struthiopteris* populations represent a peculiar "net" of interwoven plagiotropic parts of shoots, with the orthotropic parts (rosette rhizomes) located at knots (Gureyeva 2014). Thus, *M. struthiopteris* is the clonal plant, its native populations represent the clones containing genetically identical individuals (ramets) formed mainly by vegetative reproduction (Fig. 1C) (Gureyeva 2001, 2003). The clonal structure of populations and the predominance of vegetative reproduction over sexual one in this species were also emphasized in the works by A. Odland (2007) and M. Grzybowski & M. Kruk (2015). Some studies have shown that new plants of sexual origin are absent within dense *M. struthiopteris* populations presumably due to a strong allelopathic effect inhibited the growth of new individuals (Prange, von Aderkas 1985).

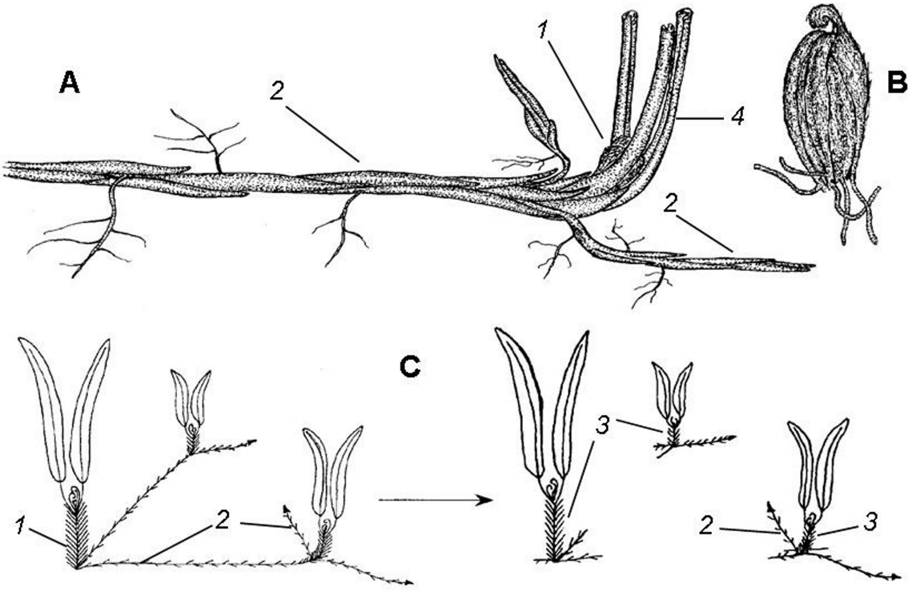

Figure 1. Structure of the sporophyte and scheme of the vegetative reproduction of *Matteuccia struthiopteris*. **A** – transition of the stolon to the formation of an orthotropic rhizome and development of new stolons at its base; stolons are covered by cataphylls; terminal bud is surrounded by stipes of living fronds; **B** – terminal bud of the orthotropic rhizome; **C** – scheme of the vegetative reproduction of *M. struthiopteris*: sporophyte with orthotropic rhizomes and stolons; formation of sporophytes of vegetative origin after their separation. **1** – orthotropic rhizome; **2** – stolons; **3** – separated sporophytes of vegetative origin; **4** – stipes of fronds. By Gureyeva (2001, 2003).

Methods for demographic and genetic studies

Observations on the developing population of *Matteuccia struthiopteris* were conducted on a new man-made lawn situated along the west wall of the Scientific Library building in the University Grove of Tomsk State University (Tomsk, Russia). For the lawn construction, the soil was removed from this site and new nutrient-rich soil was brought in early summer of 2007. The soil for the lawn filling was collected from a natural site near the village of Kislovka, situated 6.65 km (in a straight line) southwest of Tomsk. Seeds of cultivated plants (grasses) were sown on the new lawn surface; however, most seedlings failed to establish, which led to the natural formation of the plant community. We first discovered very young fern sporophytes on this lawn in 2009. Starting that year, we monitored the development of sporophytes on a 15×3 m lawn plot annually until 2025, with the exception of 2020, when the library building wall was undergoing renovation. The lawn was completely covered with boards, the vegetation cover was almost completely destroyed due to lack of light, and extensive bare soil patches emerged on the lawn surface. Observations were resumed in the lawn in 2021, after the completion of the repairs, and continued until 2025. In 2021–2022, the plant community recovered. At the end of May 2023, the lawn was covered with new soil without removing the previous layer. The new soil was seeded with lawn grass. However, high humidity and shade from the building's wall prevented seed germination and plant establishment, and most of the seedlings died. Thus, plants colonized the new soil spontaneously, and some plants developed from rhizomes or seeds remaining in the old layer of soil. It should be noted that the lawn herbal cover was mown annually 2–3 times during the growing season. Figure 2 demonstrates the changes in the vegetation cover of the lawn.

Observations of *M. struthiopteris* sporophytes were carried out annually at the end of June or in early July, before the first grass cutting, when the plants had reached full development. The number of sporophytes and the ontogenetic stage of each of them were recorded. The division into ontogenetic stages was carried out on the basis of morphological and biological characteristics using the method of discrete description of ontogenesis proposed by T.A. Rabotnov (1950) and A.A. Uranov (1975) for angiosperms and modified for ferns by N.I. Shorina (1991 a, b) and I.I. Gureyeva (1990, 1996, 2001, 2003). Demographic structure of the population was assessed as a proportion of each ontogenetic group (in percent). Sixteen years of observations (2009–2025, except 2020) made it possible to reveal the dynamics of the number of sporophytes and describe the demographic structure of the developing population.

For analysis of the genetic structure of populations, we used the ISSR (Inter Simple Sequence Repeat) method, which is multilocus and covers the entire genome. To identify the initial population producing spores that gave rise to formation of the population on the lawn (hereinafter referred to as the lawn population), the living material was collected from several sites. These are the lawn population, the population growing at the site where the soil for the lawn was taken, and three more populations within the potential range of wind dispersal of spores (Table 1). Taking into account the clonal organization of *M. struthiopteris* populations revealed earlier (Gureyeva 2003, 2014), we selected few individuals from each population for analysis (6).

Total genomic DNA was extracted using "DiamondDNA Genomic DNA Extraction Kit" (Barnaul, Russia) in accordance with the manufacturer's protocol. The quality and quantity of DNA were assessed using a spectrophotometer "Implen P330". Short di- and tri-nucleotide microsatellite repeats were used as primers in the polymerase chain reaction (PCR) for ISSR-analysis of DNA polymorphism. A total of 26 primers were tested, and six primers were then selected. The optimal annealing temperature for the primers was evaluated, and the reaction was optimized for $MgCl_2$ and Taq-polymerase concentration. In general, the conditions of reaction and primers, which reproduced the greatest number of bands, were selected (Table 2). In total, 102 discernible bands were generated. The number of fragments yielded per primer varied from 13 to 25, the proportion of polymorphic loci was high (85.7–100 %). This indicates that the ISSR markers detect sufficient polymorphism for DNA typing in the genetic study of *M. struthiopteris* populations. To determine the length of the amplified DNA fragments, a standard was entered into the first and last lanes of the gel. In this study, 100bp + marker (Thermo Scientific, Latvia) was used as the standard.

Figure 2. The changes in the vegetation cover of the lawn in different years: **A** – well-developed plant community 13 years after the lawn construction (July 4, 2019); **B** – the lawn surface of after the building's wall repair (June 17, 2020); **C** – new layer of soil with sparse plants (June 16, 2023); **D** – plant community developing on the lawn (June 18, 2025). Photos by Irina I. Gureyeva.

Table 1. Sampled populations of *Matteuccia struthiopteris*

Population code	Locality and characteristics of populations
LW (Lawn population)	Russia, Tomsk, Tomsk State University, University Grove, a new man-made lawn along the west wall of the Scientific Library building, 56º28'05.4" N 84º56'57.1" E. The lawn population appeared presumably in 2007, includes young sporophytes only.
GR (Grove population)	Russia, Tomsk, Tomsk State University, University Grove, an old man-made lawn near the north wall of the Scientific Library building. 56º28'06.4" N 84º56'57.9" E. The population grows 20 m from LW, includes young sporophytes only.
RV (Ravine population)	Russia, Tomsk, Tomsk State University, Siberian Botanical Garden, in the ravine, 56º28'01.3" N 84º56'48.3" E. The population grows 160 m from LW in the ravine with a small-leaved forest, includes many well-developed spore-bearing sporophytes.
ST (Stream population)	Russia, Tomsk, Tomsk State University, Siberian Botanical Garden, the stream near the west boundary of the Garden territory, 56º28'00.9" N 84º56'34.1" E. The population grows 365 m from LW at the wet site of the stream valley overgrown with shrubs, includes separate spore-bearing sporophytes.
KS (Kislovka population)	Russia, Tomsk Region, environs of the village of Kislovka, south-west from Tomsk, 56º25'37.3" N 84º53'16.5" E. The population grows 6,65 km from LW at the open wet site in the valley of the Kislovka River, where soil was taken in 2007 for the lawn construction. The population includes mainly well-developed spore-bearing sporophytes.

Note: Distances between the populations were measured in a straight line.

Table 2. Primers and optimal temperature of annealing selected for ISSR analysis

Primer	Annealing temperature, °C	Number of bands	Polymorphic bands, %
17898B (CACACACACACAGT)	50	25	100
17899B (CACACACACACAGC)	56	14	96.4
HB11 (GTGTGTGTGTGTCC)	49	17	85.7
17898A (CACACACACACAAC)	52	13	96.3
844B (CTCTCTCTCTCTCTCTGC)	56	15	100
HB10 (GAGAGAGAGAGACC)	48	18	100

The ISSR bands at a given locus were scored as 1 (present) or 0 (absent) to create a binary matrix set (bands of the same size were considered to belong to the same locus). The dataset was analyzed using statistical methods. Genetic identity (I) and standard genetic distance (D) between the populations were calculated using the method by M. Nei (1972). The genetic structure of *M. struthiopteris* populations was estimated based on the ISSR dataset using STRUCTURE Version 2.3.4 (Pritchard et al. 2000). This software analyzes the distribution of genetic patterns within and among the populations and assigns samples to the groups that exhibit similar variation patterns. STRUCTURE uses a Bayesian clustering approach with Markov Chain Monte Carlo (MCMC) estimation. The MCMC process starts by randomly assigning samples to a user-defined number of groups (which is represented as K value and usually taken as the probable number of populations plus 2–3). The K value ranged from 2 to 8. The program was run for 100,000 of burn-in repetitions and 500,000 of MCMC simulations for each K. The optimal K value, which indicates the number of genetically different clusters in the dataset, was determined from 10 replicates for each K value (Evanno et al. 2005). To detect the number of genetically homogeneous groups (K) that best fits the data, we used Structure Harvester version 6.0 (Earl, Holdt 2012), which implements the method by G. Evanno et al. (2005). DNA extraction and ISSR analysis were conducted in the Laboratory of Structural and Molecular Analysis of Plants (Tomsk State University, Tomsk, Russia) in 2018.

Results and discussion

Features of spore maturation and dispersal in *Matteuccia struthiopteris*

Spores are produced by sporophylls (fertile spore-bearing fronds) that are located vertically in the center of a well-formed rosette and surrounded by trophophylls (sterile green fronds) (Fig. 3A). Sporophylls appear much later than trophophylls, in the middle of summer, turn brown by autumn, and retain their vertical position until autumn of the next year. Sporangia containing spores are arranged in sori located on the lower surface of pinnae of the sporophylls, and are covered by the revolute pinna margins (Fig. 3B, D). In Siberia, spores mature in autumn, but the pinnae of fertile fronds remain with hardened revolute margins, which prevent sporangia from opening until winter. Sporophylls bearing unopened sporangia persist through winter and protrude from under the snow cover (Fig. 3C). Spore dispersal can start in winter, primarily within unopened sporangia. At this time, the edges of sporophylls unfold under the influence of frost (Fig. 3E), and sporangia containing spores fall into the snow, and then, after snow melting, they are deposited upon the soil. If sporophylls are completely covered with snow, this process occurs in spring. Overwintered sporophylls remain upright, and many retained spores dissipate in spring and even in summer of the year following the spore maturation.

Low winter and spring temperatures in Siberia preserve the viability of green spores in *M. struthiopteris*, allowing the spores to germinate in the late spring or early summer of the year following their maturation. Thus, the long period of spore dispersal in *M. struthiopteris* can be considered as an adaptation to prevent spore germination in autumn, which could lead to the death of gametophytes during overwintering. As a result, spores germinate in more favorable environmental conditions during the next growing season, and the developed gametophytes are able to produce antheridia and archegonia, undergo fertilization, and form sporelings.

D.R. Farrar (1976) also reported about late maturation and spore dispersal in *M. struthiopteris* growing in Michigan (USA): its late-maturing fronds bear > 90% unopened sporangia in December and > 80% in March.

Figure 3. Spore-bearing sporophytes and sporophylls of *Matteuccia struthiopteris*: **A** – funnel-shaped rosette with trophophylls and sporophylls (in the centre) (Kislovka, July 7, 2019); **B** – sporophylls with mature and immature spores (Kislovka, August 17, 2025); **C** – sporophylls protruded from under the snow cover (Kislovka, November 22, 2020); **D** – lower and upper sides of sporophyll matured in autumn 2018 (Kislovka, September, 29, 2018); **E** – overwintered sporophyll (Kislovka, May 12, 2019). Photos by Irina I. Gureyeva.

Ontogenesis of *Matteuccia struthiopteris* sporophyte

The method of discrete description of ontogenesis of ferns (Shorina 1991 a, b; Gureyeva 1990, 1996, 2001, 2003) employed in the study revealed the following periods and stages of sporophyte ontogenesis: (1) pre-reproductive period with the stages of embryo, sporeling (analog of a seedling in flowering plants), juvenile, immature and virginal (adult sterile) sporophyte, (2) reproductive period with the stages of young fertile (spore bearing), middle-age fertile and old fertile sporophyte, and (3) post-reproductive period with the stage of senile sporophyte. In the pre-reproductive period, only vegetative organs (fronds and rhizomes) develop, the transition to the reproductive period is marked by the ability of sporophyte to produce spores, and in the post-reproductive period, the sporophytes lose the ability for spore production. In the population developing on the lawn over 17 years of observations, we discovered the juvenile, immature and virginal (adult sterile) sporophytes generated sexually. These ontogenetic stages for *M. struthiopteris* have been observed and described for the first time. Adult fertile and senile sporophytes were studied earlier in native populations, embryos and sporelings were described in gametophyte culture (Gureyeva 2001), but in 2024 and 2025, sporelings were also discovered in the lawn population.

Development of the sporophyte starts with the stage of embryo (em). The embryo looks like a meristematic prominence situated on the ventral surface of the gametophyte. This stage starts after the fertilization of the ovule and ends with the formation of the first root and frond of sporeling. Sporeling (sl) has the first twice- to four- or eight-lobed frond (fronds) and the first root (roots). The gametophyte remains attached at the base of the sporeling. The first fronds are 0.8–1.5 cm long. The stage of juvenile sporophyte (j) starts after the death of the gametophyte, with fronds of the juvenile type arranged in the rosette. Fronds expand sequentially, each subsequent frond is more complicated (the number and dissection of pinna pair increases) and larger than the previous one. The rhizome is short, orthotropic. During this stage, fronds of the juvenile sporophytes increase from 1–2 to 10–12 cm in length, and from 1 to 4 in number. Based on frond shape and size, we divided this stage into three sub-stages: earlier juvenile (j1), middle juvenile (j2), and late juvenile (j3) (Fig. 4 B–D). Immature sporophyte (im) (Fig. 4 E) is the transition stage from a juvenile sporophyte to an adult one. During this stage, the sporophytes increase in size, frond dissection becomes more complicated but still differs from that of the adult sporophyte. The rhizome still does not have dead parts. The fronds of the immature sporophyte increase from 10–15 to 20–30 cm in length. Virginal (adult sterile) sporophyte (v) (Fig. 4 F) exhibits adult features: fronds acquire the dissection, shape, and size typical of the species. The stolons start to form at the base of the orthotropic rhizome. The fronds increase to 35–50 cm in length.

The reproductive period starts with the stage of young fertile sporophyte (sp1) and is marked by the appearance of the first sporophylls bearing sporangia. The number of trophophylls is 3–4 with a length of 50–85 cm; 1–2 sporophylls start to form. The rhizome is large, orthotropic, 5–10 cm tall, with well-developed plagiotropic stolons, some of which are long and have an orthotropic rhizome bearing fronds (a daughter sporophyte). Middle-age fertile sporophyte (sp2) has the maximal number of sporophylls (3–5) and trophophylls (5–14), 70–125 cm long. All organs of the middle-age fertile sporophyte reach their maximal size. The orthotropic rhizome (10–25 cm tall) produces a maximal number of stolons, some of them reach 1.5–3 m in length; most of the stolons bear the orthotropic rhizome with the rosette of fronds (daughter sporophytes). The separation of the daughter sporophytes through the dying off and destruction of stolons starts. The reproductive period finishes with the stage of old fertile sporophyte (sp3). During this stage, the number of trophophylls and sporophylls decreases to 2–4 and 1, respectively; trophophylls decrease in size to 43–79 cm long. The rhizome, which was orthotropic at previous stages, becomes ascending due to the die-off of its lower part. The ability to produce new stolons is decreased or absent.

The life of the sporophyte finishes by the stage of senile sporophyte (s). At this stage, the sporophyte loses the ability to produce sporophylls and spores. Trophophylls are 2–3 in number, 28–45 cm long. The form and dissection of the frond are similar to those in immature or even in juvenile sporophytes. The rhizome is large, but the size of the died-off part exceeds that of the living one. The rhizome lies on the soil horizontally due to the destruction of the basal part. All stolons formed during previous stages are separated or dead.

The demographic structure of the *Matteuccia struthiopteris* population on the lawn

The first sporophytes of *Matteuccia struthiopteris* were discovered in a developing population (LW) on the new lawn in 2009. Based on the analysis of publications concerning the *M. struthiopteris* spore reproduction (von Aderkas 1983; Klekowski 1985, 1988) and our own findings, we conclude that we have the opportunity to observe a unique spontaneous "natural experiment" in the emergence of a population of this species from spores. Considering the time required for the development of gametophytes from spores, their fertilization with subsequent formation of sporelings, and the fact that the soil for the lawn construction was brought in the early summer of 2007, we can state that the gametophyte population arose that same year. Thus, by 2025, the observed lawn population (LW) of sporophytes had already existed for 18 years. The growing season in 2007 was warm, the average temperature even in August and early September was 15 °C (17.7 °C daytime, 12.4 °C night), which created suitable conditions for the development of gametophytes, fertilization, and formation of sporelings. Spores may have been introduced to the lawn in two ways: (1) with the soil used for the lawn construction, and (2) by wind from the populations having fertile sporophytes. Fertile sporophytes were present in three of the populations studied – RV, ST, and KS. Populations RV and ST were situated closer to the lawn population (160 and 365 m) than KS population (6,65 km).

Consequently, dispersion of spores by wind was more likely from nearest places than from distant one. The absence of adult spore-bearing sporophytes in the GR population during the observation period ruled out the possibility of spore dispersal from there.

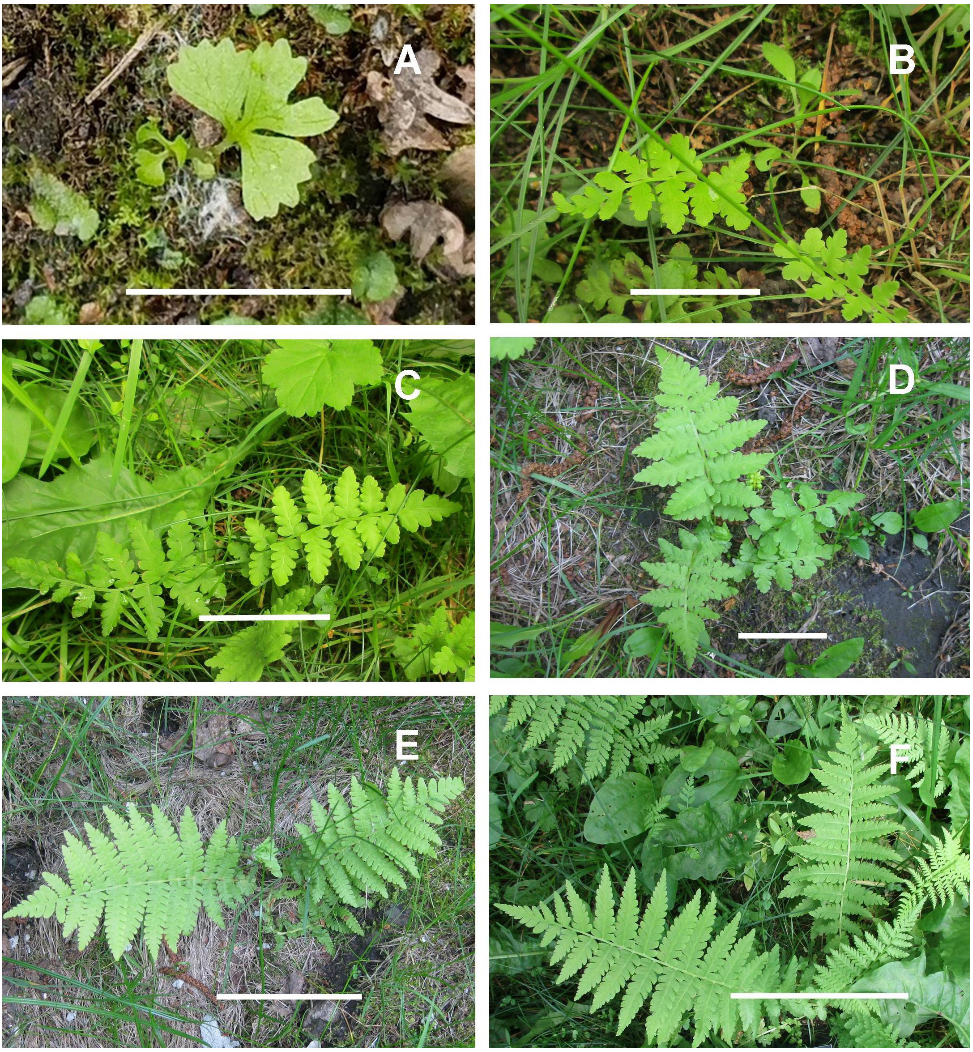

Figure 4. Ontogenetic stages of the pre-reproductive period of the sporophyte of *Matteuccia struthiopteris*, observed in the lawn population in the University Grove: **A** – sporeling (sl); **B** – early juvenile sporophyte (j1); **C** – middle juvenile sporophyte (j2); **D** – late juvenile sporophyte (j3); **E** – immature sporophyte (im); **F** – virginal sporophyte (v). Scale bars: **A** – 1 cm, **B** – 3 cm, **E** – 5 cm, **F** – 10 cm. Photos by Irina I. Gureyeva.

The dynamics of the total number of sporophytes, the number of sporophytes of each stage, and the ontogenetic spectrum over the observation period are shown in Figure 5.

In the first year of observations (2009), the population of *M. struthiopteris* sporophytes in the lawn (LW) included a total of 263 sporophytes. By 2019, the total number of sporophytes decreased from 263 to 95, meaning the loss of 63.9% of the individuals. The death of sporophytes may have been tied to competition from flowering plants, which number increased from seven species in 2009 to 25 in 2019. In addition, annual grass mowing, which was carried out 2–3 times during the growing season, could have a significant negative impact for *M. struthiopteris* sporophytes. This negative effect is especially important for ferns, as sporophytes are forced to develop new fronds after each mowing. Under this regime, each new generation of fronds develops from immature croziers, and since the rhizome does not accumulate sufficient reserves, the plant becomes gradually depleted. This could lead to plant death. A decrease in the number of sporophytes between 2020 and 2023 was due to the reconstruction of the building and the filling of the lawn with new soil. We observed the minimum number of sporophytes (26) in 2023. A new increase in the sporophyte number occurred in 2024 (44), and especially in 2025, when the number of sporophytes increased to 372.

In the first year of observations, the population included only juvenile sporophytes of the early sub-stages (j1 and j2). The number of individuals in both of these groups was maximal in the year of their detection and then decreased gradually from 2009 to 2019.

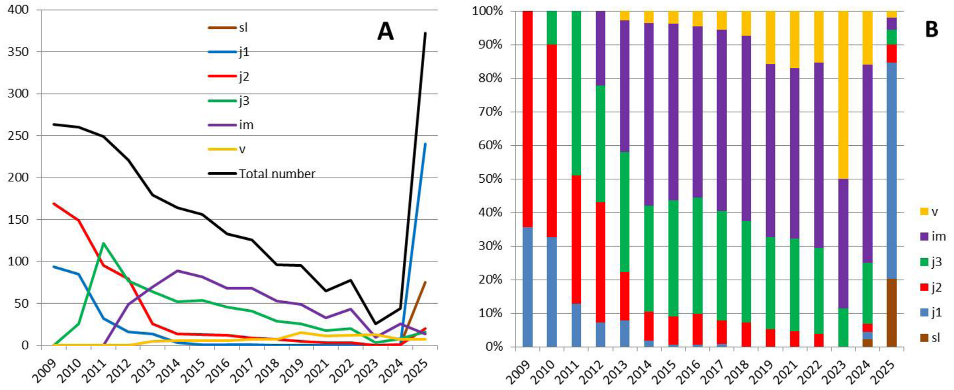

Figure 5. Dynamics of the number of sporophytes of *Matteuccia struthiopteris* of different ontogenetic stages (**A**) and dynamics of the demographical structure of population (**B**) developing on the lawn in the University Grove (LW) from 2009 to 2025. Vertical axes: **A** – the number of sporophytes of each ontogenetic stage, **B** – proportion of each ontogenetic group in population (in percent); horizontal axes: **A** and **B** – years of observations.

The number of j1-sporophytes was minimal from 2015 to 2017, and in 2018, this group disappeared. Group j3 appeared in 2010, its number reached a maximum in 2011, and then decreased by 2019. Immature (im) sporophytes appeared in 2012, reached a maximum in 2014, and then their quantity started to decrease. The first virginal (v) sporophytes appeared in 2013, their number remained at the same level in 2014 and 2015, and then their participation increased by 2019. A decrease in the sporophyte number in all ontogenetic groups is associated with both the death of individuals and the transition from the previous ontogenetic stage to the next one.

In 2020, when the lawn was severely damaged because of the repair of the library building's wall, observations were not conducted, but they were resumed in 2021. Only 65 sporophytes of *M. struthiopteris* were found after the repair, mainly the individuals of the j3- and im-stages. In 2022, the plant community on the lawn recovered, the number of plant species reached 21, and the number of sporophytes of *M. struthiopteris* reached 78, including sporophytes of j2-, j3-, im- and v-stages with predomination of j3- and im-sporophytes. An increase in the number of j3 and im sporophytes was not due to the emergence of new sporophytes. It is possible that some sporophytes survived the growing season of 2021 in a dormant state, without producing fronds, and therefore were not counted. It should be noted that despite a decrease in the number of sporophytes of each stage, their participation in the population structure from 2019 to 2022 remained approximately at the same level.

In the late May 2023, the lawn was covered with a layer of new soil before the plants began to grow. Only 26 sporophytes of *M. struthiopteris* mainly of the im- and v-stages and four species of flowering plants were able to break through this soil layer by the end of June. However, in June 2024, we discovered new sporophytes of M. struthiopteris – sporelings (sl) and early juvenile (j1) emerged through sexual reproduction. Finally, at the end of June 2025, we observed a great increase in the number of sporelings and early juvenile sporophytes. This can be due to the presence of free sites where spore germination, development, and fertilization of gametophytes occurred during the 2023 growing season. Gametophytes and sporelings are very small (up to 2–5 mm and 10–15 mm, respectively) and therefore were not detected with the naked eye during the counts in 2023 and 2024. By the end of observations in June 2025, we discovered 372 sporophytes, mainly those of the early stages of ontogenesis – sl (75) and j1 (240). In this case, we can assume the second wave of *M. struthiopteris* colonization of the lawn in 2023 and formation of new sporophytes through sexual reproduction.

There are available data by E.J. Klekowski (1988) on mutations disturbing normal sporogenesis and 94.5 % of spores with sporophytic lethals, and data by P. von Aderkas (1983) and D.R. Farrar (1983) who also did not observe the establishment of *M. struthiopteris* gametophytes despite a huge number of produced spores. Contrary to these data, the existence of the lawn population of sexually generated sporophytes proves that the spores of *M. struthiopteris* are able to germinate and form gametophyte, which in turn are capable of fertilization and forming long-term existing sporophytes (17 years old in our observations).

Genetic structure and differentiation of *Matteuccia struthiopteris* populations

The analysis of total genomic DNA of 30 samples of *M. struthiopteris* sporophytes from the five populations listed in Table 1 revealed a high level of genetic differentiation between the populations (AMOVA, FST = 0.672, $P < 0.0001$). Variation within the population is 33 %, and among the populations – 67 %, which confirms that all of the studied populations are separate (Table 3).

Table 3. Analysis of molecular variance (AMOVA) for five populations of *Matteuccia struthiopteris*

Source of variance	DF	SS	MS	Estimated variance	% variance
Among populations	4	136.200	34.050	5.247	67
Within populations	25	64.167	2.567	2.567	33
Total	29	200.367	–	7.814	100

Note: DF (Degrees of freedom), SS (Sum of Squares), MS (Mean Squares) – statistical values.

The smallest Nei's genetic distance between *M. struthiopteris* populations is characteristic of the pairs LW and KS (0.072), and GR and KS (0.074), while the greatest distance is characteristic of LW and RV (0.275). Respectively, genetic identity is greatest between LW and KS (0.931), and GR and KS (0.929), and the smallest genetic identity is observed between LW and RV (0.760) (Table 4). This suggests that the spores that started to develop in both LW and GR populations came to these locations from KS. Since sporophytes appeared simultaneously in large numbers, at least in LW, it can be assumed that they were not wind-dispersed but were present in the soil used for the lawn construction. It should be noted that the soil used to make the new lawn (LW) was also partly poured into the adjacent old lawn (GR). This explains the high genetic identity of GR and KS populations: some sporophytes of this population originated from spores contained in the soil from KS partly poured into GR.

Table 4. Nei's original measures of genetic distance (above diagonal) and genetic identity (below diagonal) among the populations of *Matteuccia struthiopteris*

Populations	LW	RV	ST	GR	KS
LW	1.000	0.275	0.192	0.123	**0.072**
RV	0.760	1.000	0.249	0.147	0.215
ST	0.825	0.780	1.000	0.100	0.124
GR	0.884	0.863	0.905	1.000	0.074
KS	**0.931**	0.807	0.883	0.929	1.000

Note: maximum genetic identity and minimum genetic distance are shown in bold; LW, RV, ST, GR, KS are the population codes (Table 1).

In the STRUCTURE processing, a clear peak in the value of ΔK (382.6) is at K = 4 (Fig. 6); therefore, the optimal number of groups that best fits our dataset is K = 4. The STRUCTURE accumulation diagram shows that the two populations – LW and KS – belong to the same group, i.e. they are very similar in allele frequencies, while RV, ST, and GR populations differ from each other and belong to three groups (Fig. 7). This diagram clearly shows that sporophytes from the lawn population (LW) and sporophytes from the population near the village of Kislovka (KS) are genetically identical. Therefore, this is only possible if the lawn population developed from the spores introduced to the lawn with the soil taken from the nearby KS population. Some individuals from RV, GR, ST, KS populations showed genetic admixture between three or four groups, indicated in gray, blue, yellow, and red (Fig. 7). This suggests an exchange of the genetic material between the populations in previous years, likely through colonization by the spores from different locations. However, in general, the placement of each population in a separate genetic group is the result of clonal organization of *M. struthiopteris* populations, with each resulting population maintained through vegetative reproduction and no new colonization by spores. Individuals in such populations are mostly genetically homogeneous.

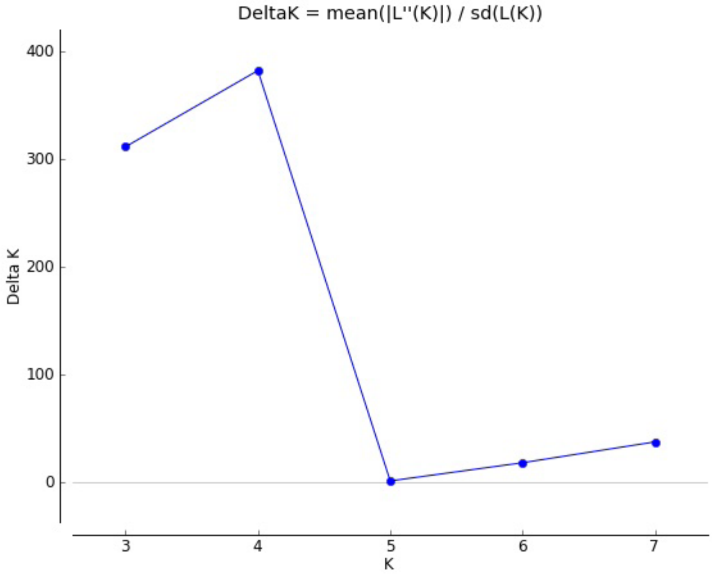

Figure 6. Results of the Bayesian assignment analysis obtained using the STRUCTURE Harvester.

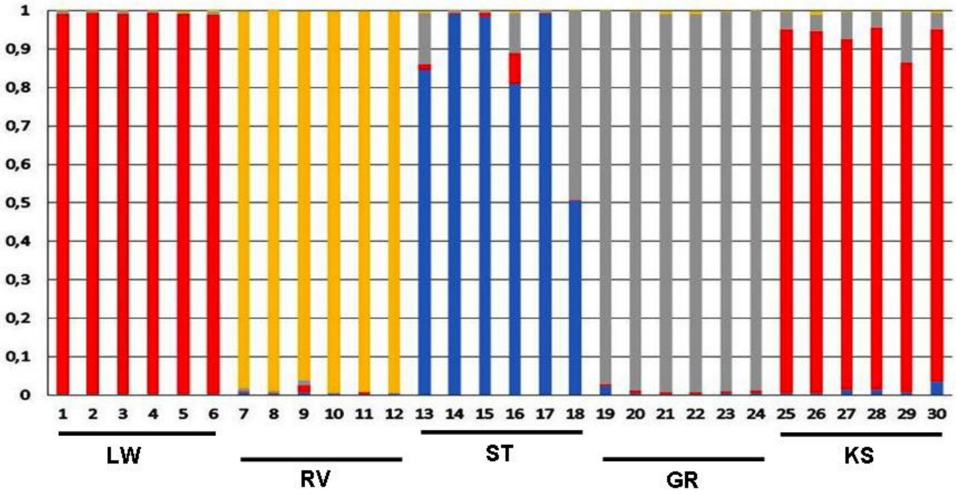

Figure 7. The structure of five populations of *Matteuccia struthiopteris* prepared using the STRUCTURE program (Pritchard et al. 2000). Each individual is represented by a vertical line divided into colored segments that represent the individual's proportion in K clusters. LW, RV, ST, GR, KS are the population codes (Table 1).

Conclusions

The fern *Matteuccia struthiopteris* produces chlorophyllous spores that can germinate within several days after their maturing. However, this species has several adaptations to prevent spore dispersal after their maturation. These include: (1) late-summer formation of sporophylls; (2) revolute frond margins, which prevent sporangia from opening; (3) long period of spore dispersal, including late autumn, winter, and spring; (4) low temperatures in this period, which preserve green spore viability. As a result, spores germinate and form gametophytes under favorable environmental conditions in the year following their maturation period.

Our study conducted on the man-made lawn, demonstrate a "natural experiment" when *M. struthiopteris* population spontaneously established from spores, even though the clonal structure of populations is characteristic of this fern species in natural conditions. During the observation period (2009–2025) we observed two wave of colonization of the lawn by spores. The first wave of colonization coincided with the construction of the lawn and covering it with fresh soil, the second wave coincided with severe damage of the lawn surface and subsequent refilling it with the new soil layer. The number of sexually generated sporophytes was significant: 263 in the first wave of colonization and 315 in the second one.

Contrary to the existing data indicating that *M. struthiopteris* has a large genetic load, and therefore sexually generated sporophytes are incapable of long-term survival, our study demonstrates that such sporophytes are capable of long-term existence (17 years of our observation) even under adverse conditions (mowing, a single but intense trampling, covering by the soil layer). The proven genetic identity of sporophytes from the lawn population to sporophytes growing at the site, where the soil was taken for the lawn construction, indicates that the spores were introduced to the lawn with this soil and that green spores of *M. struthiopteris* are capable of the formation of the spore bank, albeit a short-lived one. Under favorable environmental conditions, in the absence of competition from other plants, spores germinate quickly, forming gametophytes, where fertilization and sporophyte formation occur.

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