The formation of the population of the fern *Matteuccia struthiopteris* in the University Grove of Tomsk University

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Matteuccia struthiopteris (L.) Tod. – Ostrich fern (Onocleaceae) is the circumboreal species widely distributed in the temperate zone of the northern hemisphere. In the paper, we presented the results of the observations on the developing population of *M. struthiopteris* on the man-made lawn situated in the University Grove of Tomsk State University (Tomsk, Russian Federation). In the original making of the lawn, the ground was replaced by new rich soil, which was taken in early summer of 2007 from a native site situated 6.65 km towards south-west from Tomsk University. The formation of the plant community on the new lawn occurred mostly naturally, and sporophytes of this fern appeared naturally. The first sporophytes on the new lawn were detected in 2009, occurring initially in the significant numbers, recorded here. During the time of observation (2009–2018) the number of sporophytes decreased from an original 263 to 96, i.e. 63.5% of sporophytes died. The initial population in 2009 included only juvenile sporophytes. No spore-bearing sporophytes appear during the period of observation. For detection of the source of spores, from which the population on the lawn was formed, we selected three populations having fertile sporophytes, and conducted a molecular-genetic analysis. Two of the analyzed source populations were found to be situated at 160–365 m distance from the developing population and one population occurs near the place where the soil for new lawn making was taken. We used the ISSR method for analyzing the genetic diversity of populations and processed the results by software STRUCTURE Version 2.3.4. The result show, that new population, and population from the soil taking point belong to the same group. The coefficient of Nei's genetic identity between these populations is high (I = 0.931). This confirms the origin of the new population from spores contained in the soil used for the lawn construction.

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Abstract

Matteuccia struthiopteris (L.) Tod. – Ostrich fern (Onocleaceae) is the circumboreal species widely distributed in the temperate zone of the northern hemisphere. In the paper, we presented the results of the observations on the developing population of *M. struthiopteris* on the man-made lawn situated in the University Grove of Tomsk State University (Tomsk, Russian Federation). In the original making of the lawn, the ground was replaced by new rich soil, which was taken in early summer of 2007 from a native site situated 6.65 km towards south-west from Tomsk University. The formation of the plant community on the new lawn occurred mostly naturally, and sporophytes of this fern appeared naturally. The first sporophytes on the new lawn were detected in 2009, occurring initially in the significant numbers, recorded here. During the time of observation (2009–2018) the number of sporophytes decreased from an original 263 to 96, i.e. 63.5% of sporophytes died. The initial population in 2009 included only juvenile sporophytes. No spore-bearing sporophytes appear during the period of observation. For detection of the source of spores, from which the population on the lawn was formed, we selected three populations having fertile sporophytes, and conducted a molecular-genetic analysis. Two of the analyzed source populations were found to be situated at 160–365 m distance from the developing population and one population occurs near the place where the soil for new lawn making was taken. We used the ISSR method for analyzing the genetic diversity of populations and processed the results by software STRUCTURE Version 2.3.4. The result show, that new population, and population from the soil taking point belong to the same group. The coefficient of Nei's genetic identity between these populations is high (I = 0.931). This confirms the origin of the new population from spores contained in the soil used for the lawn construction.

Introduction

Colonization by plants of the free sites and the subsequent formation of vegetation cover on them are important biological problems which, at least for ferns, have received little previous detailed analyses. Ferns quickly colonize free sites in native cenoses, as well as anthropogenically disturbed sites. They can colonize especially primary free areas such as lava fields, dunes, places after deglaciation, as well as newly formed free sites after the fire, flood, etc. (Page, 2002; Walker & Sharpe, 2010; Gureyeva & Timoshok, 2016). Ferns occupy the new area via dispersal of spores or through the vegetative reproduction. Long distance dispersal of spores can likely be highly important in distribution of fern species. Most of the produced spores disperse on a few meters (Wolf, Scheffield & Haufler, 1991), but dispersion to at least thousand kilometers is also possible (Schneller & Liebst, 2007; Kessler, 2010), and dispersal of spores into high-altitude levels is also known (Page, 1979a; Page, 1979b). Most fern species have potentially hermaphroditic gametophytes, therefore the colonization of new habitats and establishment of new fern population is 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 persist in the soil for a long period and are able to form the soil spore bank (Scheffield, 1996; Schneller & Holderegger, 1996b). Potentially spores preserved in the soil have the capacity to germinate when conditions become favorable, for example, in disturbed sites, where competition with other plants is depressed or absent (Scheffield, 1996; Gureyeva, 2002). Some fern groups, such as Onocleaceae, Hymenophyllaceae, Osmundaceae have green chlorophyll-containing spores. These spores have no rest period, typically germinate in less than three days (mean=1.46 days) and have viability lengths of one year or less (mean=48 days); however, periods in darkness at temperature +3 … +5ºC promote viability preservation of chlorophyllous spores for up to two years (Lloyd & Klekowski, 1970; Stecenko & Schevchenko, 1988; Pence, 2000; Kessler, 2002, 2010). Fresh spores of *Matteuccia struthiopteris* (L.) Tod. demonstrate more than 90% germination, with little percentage of germination decrease over a subsequent 2-year period (Gantt & Arnott, 1965). However, another study showed that maximum of germinated spores (95%) is observed in the first days after collecting. Their germination ability decreases under the room temperature approximately to 80% in a month; less than 10% of spores germinated after 2 months of storage, and less than 1% of spores germinated after 7 months of storage. Storage in the low temperature and even in liquid nitrogen prolongs the viability of the spores, so that the cryopreservation is a perspective method for long-term storage of green spores of ferns (Kreshchenok et al., 2014), and spores naturally-reaching the cold temperatures of high-altitude dispersal are claimed to be able to remain viable once returned to normal temperature conditions (Page, 1979a, b).

Klekowski (1984), who investigated of apical meristem of plants, showed that plants having shoot apical meristem based upon single permanent apical cell are more likely to accumulate

unfavorable somatic mutations than plants with stochastic apical meristem. *M. struthiopteris*, which can form extensive clones and seldom reproduce sexually, should have high mutational loads. Such clones which are genetic chimeras are common in *M. struthiopteris*. Cytological investigation of this species showed that in such clones meiosis was normal but post-meiotic maturation of the spores was defective. These clones of *M. struthiopteris* appear to have fixed a dominant mutation that disturbs normal sporogenesis and 94.5% of the 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 so far documented in the ferns (Klekowski, 1988). Nevertheless, the existence of populations of this species in widely separated habitats cannot be explained otherwise than by spore reproduction. According to Aderkas (1983), who studied development and sex expression of gametophytes of *M. struthiopteris* in nature and in culture, in multispore cultures gametophytes demonstrate the most variety sexual-morphological types (male, female, neuter and hermaphroditic); field-found gametophyte showed the least diversity, their populations were contained mainly male or neuter gametophytes, and only two female gametophytes were found among 1299, and no sporelings during the vegetation period. Farrar (1976) did also not observe establishment of gametophyte of *M. struthiopteris* despite the huge number of produced spores (about 1 million per frond).

In reproduction by spores, a combination of certain factors is needed for germination of the spores, formation of gametophytes, fertilization, and formation and establishment of sporelings. Despite that the gametophyte is sporophyte-independent small single-layered thallus growing on the surface of the substrate, it can be more resistant to unfavorable environmental conditions than the sporophyte (Page, 1979a; Page, 1979b; Gureyeva, 2002). Gametophytes of the most fern species are known also to survive through freezing to –20 ºC … – 40ºC, and gametophytes of the some *Dryopteris* and *Polystichum* species dried at room temperature for two weeks to 7–18% of relative humidity, and then immersed in liquid nitrogen, can continue to grow after transfer to normal conditions and can give sporophytes (Sato, 1982).

Such species as *Matteuccia struthiopteris* may be less capable of long-distance dispersal and formation of the viable population via spore way. The proof of the complete absence of spore reproduction in populations of *M. struthiopteris* are our many-years of field investigations of the demographic structure of native populations of this species, growing in the Siberian mountain forests (Gureyeva, 2001, 2003, 2007). Despite accurate searches, we have not found gametophytes of *M. struthiopteris* in any of the studied populations. This circumstance gave us grounds to consider, that self-maintenance of native populations of this species is carried out only by vegetative reproduction of sporophytes. But existence of sporophyte population in different sites, distant from each other, cannot be explained otherwise than by their initial development from spores via initial gametophyte formation.

The evidence of this statement in this study is the emerging of a population of *M. struthiopteris* which has originated from spores. We found the first juvenile sporophytes to appear in 2009 on the new lawn made in the University Grove of Tomsk State University (Tomsk, Russian Federation). As a result, the questions are posed (1) does this population have a chance for a

long-term existence and (2) where is the source of spores from which the population originated? It should be noted that juvenile sporophytes of *Athyrium filix-femina* (L.) Roth were found together with *Matteuccia struthiopteris* on the lawn in the University Grove, but the aims of this study are to detect of the source of the spores, which founded the population of *M. struthiopteris*, and to identify the structure and dynamics of development of that population. The working hypotheses were the following: (1) the population has arisen from spores dispersed to the lawn from neighboring sites where *M. struthiopteris* has been growing, and in this case sporophytes should be genetically polymorphic or genetically identic with those in the one of these populations; (2) spores were introduced with the soil used to fill the lawn, and in this case sporophytes should be genetically identic with those growing in the place where soil was taken.

Materials & Methods

Observations on the developing population of *Matteuccia struthiopteris* were conducted on the new man-made lawn situated along the west wall of the Scientific Library building in the University Grove of Tomsk State University (Tomsk, Russian Federation). For the lawn construction, the ground from this site with the area of 15×3 m was removed and new rich soil was brought in early summer of 2007. This soil had been collected from a natural site near the Kislovka village, 6.65 km (in a straight line) towards the south-west of Tomsk, and prepared for spreading as a lawn. Seeds of cultivated plants were sown on the new lawn surface, but seedlings mostly had died, therefore the formation of the plant community occurred naturally here. The observations on sporophytes were conducted annually from 2009 to 2018. The number of sporophytes, with the definition of their ontogenetic stage, was recorded. Each stage defines the biological age of the sporophyte (a level of its development) at a given moment. Individuals of similar calendar ages can have different biological ages, i.e. they can reach the different ontogenetic stages in the same time period. We divided their progression into ontogenetic stages on the basis of morphological and biological characteristics using the method of discrete description of ontogenesis offered for characteristics of the population of flowering plants (Rabotnov, 1975, Uranov, 1975, Zaugolnova et al., 1988). Later, Shorina (1991a, b) and author of this paper Gureyeva (1990, 1996, 2001, 2003) developed the periodization of ontogenesis for sporophyte and gametophyte stages of ferns. We divided a whole ontogenesis of sporophyte into the following periods and stages: (1) the pre-reproductive period with the stages of embryo, sporeling, juvenile, immature and adult sterile (virginal) sporophyte, (2) the reproductive period with the stages of young fertile, middle-age fertile and old fertile sporophyte; and (3) the post-reproductive period with the stage of the senile sporophyte.

Every year we calculated the number of sporophytes belonging to the certain ontogenetic group. Demographic structure of the population was detected as a proportion of each ontogenetic group (in percent). Ten years of observations made it possible to reveal the dynamics of the number of sporophytes and the demographic structure of the developing population.

We used scanning electron microscopy for the study of spore morphology. Mature spores were coated with gold in the “Quorum Q150R S” sputter-coater, viewed and photographed with the

scanning electron microscope "Mini-SEM SNE-4500M". Spore surface was scanned in a high vacuum at the voltage of 20 kV, through 2000–2500× magnification.

For analysis of the genetic structure of populations, we used ISSR-method (Inter Simple Sequence Repeat) allowing analysis of polymorphism of the genome. For detection of the source population that produced the spores to start the formation of the new population, living material (expanding croziers) was collected in 'experimental' population and four more populations situated at different distances from the 'experimental' one (Tab. 1). Taking into account the clonal organization of the populations of *M. struthiopteris* revealed earlier (Gureyeva, 2003, 2014), we considered possible to select a small number of individuals for analysis (n = 6) in each population.

Total genomic DNA was extracted using "DiamondDNA Genomic DNA Extraction Kit" (Barnaul, Russia) according to the protocol of the manufacturer. For DNA extraction, an additional purification step was included with a mix of phenol, chloroform and isoamyl alcohol (12:12:1). The quality and quantity of DNA were tested on spectrophotometer "Implen P330". Short di- and tri-nucleotide microsatellite repeats were used as primers in polymerase chain reaction (PCR) for ISSR-analysis of DNA polymorphism. Totally, 26 primers were tested, and six primers were selected. Furthermore, the optimal temperature for primers annealing was evaluated, and the reaction was optimized for MgCl2 and Taq-polymerase concentration. In general, the conditions of reaction and primers, which reproduced the greatest number of bands, were selected.

Loci were amplified in reaction mixture with total volume of 15 μL contained 1.5 μL of 10× PCR-buffer, 1.4 μL of $MgCl_2$ (concentration is specific for each primer), 0.12 μL of dNTP (0.6 μM), 0.2 μL of Taq-polymerase (Thermo Scientific, 1 U/μL), 1 μL of primer (10 pmol), 1 μL of DNA (10 ng) and 9.78 μL of ddH_2O. Amplification was conducted in "MJ Mini™ Personal Thermal Cycler" (Bio-Rad, USA). Our thermal cycling program for ISSR consisted of an initial denaturation step (94°C for 3 min), 35 denaturation, annealing, and elongation cycles (94 °C for 30 sec, optimal temperature of annealing of primer for 30 sec, 72°C for 1 min), and a final elongation step (72°C for 10 min). The amplification products were separated in a 2% agarose gel in a single TAE-buffer (0.04 M Tris-acetate, 0.002M EDTA) at a voltage of 80 V, stained with ethidium bromide (1 μg/ml), and visualized in ultraviolet; the result was photo-documented. The visualization was carried out with a transilluminator "Gel Doc™XR+ (Bio-Rad, USA). The presence of amplified DNA fragments in gels was established by the intensity of the color. 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.

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). Dataset was analyzed using statistical methods. Genetic identity (I) and standard genetic distance (D) between populations were calculated using the method by Nei (1972). The genetic structure of *M. struthiopteris* populations was estimated on the basis of the ISSR data set using the software

STRUCTURE Version 2.3.4 (Pritchard, Stephens & Donnelly, 2000). It analyzes the distribution of genetic patterns inside the population and among the populations and assigning samples to the groups that have similar patterns of variation. STRUCTURE uses a Bayesian clustering approach with Markov Chain Monte Carlo (MCMC) estimation. The MCMC process starts from randomly assigning samples to a number of groups that are defined by the user (which is represented as K value and usually taken as the probable number of populations plus 2–3). The number of K was from 2 to 8. The program was run for 100000 of burn-in repetitions and 500000 of MCMC simulations for each K. The optimal value of K, which indicates the number of genetically different clusters in the data set, was determined from 10 replicates for each K value (Evanno, Regnaut & Goudet, 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 Evanno et al. (2005) method. Method STRUCTURE is used in the study of genetic variation of some plant species to unravel the origin and the phylogeographic patterns of its populations (Shiposha et al., 2016).

SEM-observation of spores and DNA analyses were conducted in the Laboratory of Structural and Molecular Analysis of Plants (Tomsk State University, Tomsk, Russia).

Results & Discussion

Ecological and biological features of *Matteuccia struthiopteris*

Matteuccia struthiopteris (L.) Tod. – Ostrich fern (Onocleaceae) is circumboreal species widely distributed in the temperate zone of the northern hemisphere, growing in the boreal forests. Distribution of the species as a whole coincides with the forest zone and mountain forest belt. The species prefers well-aerated gray and brown forest soils, where it is an indicator of high permanent moisture associated with soil-groundwater. *M. struthiopteris* is widely distributed in humid regions of Southern Siberia (Altai-Sayan mountainous country), often dominates here in 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 grows also along valleys of rivers and streams, forms thickets, pure or often mixed with *Athyrium filix-femina* (L.) Roth (Gureyeva, 2001).

Sporophytes of *M. struthiopteris* are perennial plants with an average lifetime in native phytocenoses 40–58 years (Gureyeva, 2001). Perennial part of 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 (to 115–125 cm length) fronds, and long plagiotropic underground rhizomes (stolons), formed mainly at the base of orthotropic one (Fig. 1A). Fronds are trimorphic: cataphylls (abortive fronds without stipe and blade), trophophylls (green photosynthetic fronds) and sporophylls (fertile fronds bearing sporangia). They surround a large, dense, upward directed terminal bud, which consists of the croziers of varying degrees of maturity (Fig. 1B). In the center of the terminal bud, there is a multicellular structured apex with a single apical initial described in detail by Romanova and Shalisko (2004). Apex includes three zones: zone of surface initials, the zone of sub-surface

initials and cup-shaped zone. All the cells of apex are derivatives of the single tetrahedral apical initial, which is located on the top of the apex. Formation of fronds starts with increasing one of the cells in the organogenic zone of apical meristem (surface initial zone). Primarily, the base of the frond (phyllopodium) is formed then the crozier grows apically and twists spirally on the upper end (Romanova & Shalisko, 2004). The frond primordia, which develop into a young frond crozier, are in the center of the terminal bud. Mature croziers are disposed at the periphery of the bud. In fact, all types of fronds are perennial, because its development from the primordium to mature crozier lasts up to 5 years. The most perennial part of the frond is phyllopodium, which becomes as part of the rhizome and remains in its composition for many years so long as the rhizome is alive. Green parts of trophophyll (stipe and blade) are summer-green, i.e. they live one vegetation season.

Plagiotropic underground rhizomes (stolons) are formed on the orthotropic rhizome. Stolons are perennial, they develop from "detached meristem" (by Wardlaw, 1946) located in internodes of the orthotropic rhizome. Commonly, the good-developed stolons are formed at the base of orthotropic rhizome and they can reach 1.5–3 m in length. Stolons' apex bear cataphylls, each of which consists of phyllopodium only; stipe and blade do not initialize. This distinguishes the cataphyll of the stolon from the cataphyll of the orthotropic rhizome. Stolons grow underground for a prolonged period, then change the direction of growth and form the over-ground orthotropic rhizome bearing fronds (Fig. 1C). When the direction of the stolon growth is changed from plagiotropic to orthotropic, phyllotaxis varies from 1/3 to 2/5 or, in large specimens, – 3/8 (Nekhlyudova & Filin, 1993). The new plagiotropic stolons are formed at the basal part of the new orthotropic rhizomes. Plagiotropic stolons pass to orthotropic growth in places with a lower density of other rosette rhizomes bearing the fronds, as a result, they are located at a more or less equal distance from each other. Whole *M. struthiopteris* populations represent the peculiar "net" of interwoven plagiotropic parts of shoots, in knots of which the orthotropic parts are disposed. In general, such type of organization of fern populations has been described by the author of this paper and named as *Matteuccia*-type (Gureyeva, 2007). Thus, the native populations of *M. struthiopteris* represent the clones containing genetically identic individuals formed mainly by vegetative reproduction (Fig. 1C).

The terminal bud of adult sporophyte consists from the significant number of croziers (30–57), the number of fronds forming funnel-shaped rosette may be 7–13(18). Sporophylls, which can develop not annually, are located vertically in the center of the rosette. They appear much later than the trophophylls, in the middle of summer, become green-brown by autumn and retain their vertical arrangement until next autumn (Fig. 2A). In Siberia, spores mature in autumn, but pinnae remain with wrapped frond margins, which tightly cover the sori and prevent sporangia from opening (Fig. 2B, C). Spore dispersal mostly inside unopened sporangia can starts in winter if sporophylls protrude under the snow cover. In this time, margins of sporophylls become unrolled under the drying effect of frost (Fig. 2D), sporangia and spores fall into the snow, and then, after its melting – on the soil. If the sporophylls are completely covered with snow, these processes occur in the spring. Nevertheless, a lot of spores remain on the sporophylls and dissipate in the

spring and even in summer of the next year. Low winter and spring temperatures in Siberia promote retention of green spore viability, so they can germinate in late spring or in early summer of the next year following their maturation. Thus, the long spore dispersal period in *M. struthiopteris* can be considered as an adaptation to prevention of spore germination in autumn, which can lead to the death of gametophytes during overwintering. As the result, spores germinate in a more favorable ecological condition of the next vegetation period, and the developing gametophytes have opportunity to fertilization and formation of sporelings. Farrar (1976) also reported about late maturation and shed of spores in *M. struthiopteris* growing in Michigan (USA): its late-maturing fronds bear > 90% unopened sporangia in December and > 80% – in March.

Spores of *M. struthiopteris* are monolete, ellipsoidal to nearly spheroidal, large, 53–63.7 × 36.5–54.6 µm in size with perispore, and 44.3–49.4 × 34.1–37.9 µm without perispore (Fig. 2E, F). The perispore is fragile, with wing-like folds that form the closed luminae of the different shape and size. The surface of perispore is echinulate with perforations.

During ontogenesis, the establishment of vegetative organs of the sporophyte takes place in the pre-reproductive period; the reproductive period is characterized by the ability of the sporophytes to produce spores and by full development of all its organs. During the subsequent post-reproductive period the sporophytes lose the ability to spore production, and the process of rhizome death progresses intensively. The characters of stages and their symbols are the following.

Embryo (*em*) is the initial stage of the sporophyte development on the gametophyte. This stage starts after the fertilization of the ovule and ends with the beginning of the formation of the first root and frond. The embryo looks like a meristematic prominence situated on the ventral surface of the gametophyte.

Sporeling (*sl*) has the first twice- to four- or eight-lobed frond (fronds) and the first root (roots). The gametophyte is still attached to the base of the sporeling. The first frond of the sporeling is 08–1.5(2) cm length.

Juvenile sporophyte (*j*) (Fig. 3A–C). This stage begins after the gametophyte died off. Sporophytes are rosette plants with 1–4 fronds of the juvenile type. During this stage, the gradual complication of frond structure takes place. In contrast with the adult sporophyte, which rosette is formed from several simultaneously expanded fronds, in juvenile sporophytes, fronds expand gradually, with each subsequent frond becoming more complicated (the number and dissection of pinna pair increases) and larger than the previous one. The rhizome is short, orthotropic with a low number of phyllopodia on it and has no dying-off parts. This stage may be divided on the base of frond shape and size into three sub-stages (*j1, j2, j3*). Fronds of the juvenile sporophyte increase from 1–2 to 10–12 cm length during the stage.

Immature sporophyte (*im*) (Fig. 3D) is the transition stage from a juvenile sporophyte to an adult one. The sporophyte dimensions increase and dissection of fronds is getting more complicated during this stage but differs from the dissection of the adult sporophyte. The rhizome does not

have dying-off parts and includes a greater number of phyllopodia. The fronds of the immature sporophyte increase during the stage from 10–15 to 20–30 cm length.

Adult sterile (virginal) sporophyte (*v)* (Fig. 3E) has adult features: fronds acquire the dissection, shape, and size peculiar to the species. The rhizome has a small dying part, its living part consists of many phyllopodia. The first stolons start to form at the base of the rhizome. They are short and do not form the orthotropic part. The fronds of the adult sterile sporophyte increase to 35–50 cm length.

Young fertile sporophyte (*sp1*). This stage starts with the appearance of the first sporophylls bearing sporangia containing spores. The number of the trophophylls is 3–4 with a length of 50–85 cm; sporophylls start to form in this stage in the number 1–2. The maternal rhizome is large, orthotropic, 5–10 cm high, with well-developed stolons, some of which are long and have orthotropic part with new fronds (daughter sporophyte). So that, the vegetative reproduction is started on this ontogenetic stage. At the basal part of the rhizome, the parts that were formed during juvenile and immature stages die off.

Middle-age fertile sporophyte (*sp2*) (Fig. 3F) has the maximal number of sporophylls (3–5) and trophophylls (5–14) with a length of 70–125 cm. All organs of the sporophyte reach maximal dimensions. The rhizome (10–25 cm in high) has a maximal number of stolons 1.5–3 m long, most of which bear daughter rosette sporophyte. The separation of the daughter rhizomes through the dying off and destruction of stolons starts. The processes of growing and dying of the maternal orthotropic rhizome are balanced. The parts of the rhizome that have developed during immature and adult sterile stages die off.

Old fertile sporophyte (*sp3*) develops a low number of fronds (2–4), especially sporophylls (0–1). Trophophylls are 43–79 cm length. The process of dying-off in the maternal rhizomes prevails over the process of new grows. The rhizome, which has been orthotropic in previous stages, becomes ascending, because of dying-off of the bottom part. Length of the living part of the rhizome is 4–10 cm. The ability to form of the new stolons is decreased.

Senile sporophyte (*s*) loses the ability to the formation of sporophylls and spores and form only trophophylls (2–3), which have the size (28–45 cm length) and dissection similar to immature or even juvenile sporophyte. In contrast with the decrease of dimensions of fronds, the rhizome is large, but the number of dying phyllopodia in its composition prevail the living ones. Because of the death and destruction of the basal part, the rhizome lays on the soil horizontally. All stolons formed during previous stages are separated or died.

Demographic structure of the developing population of *Matteuccia struthiopteris*

The first sporophytes of *Matteuccia struthiopteris* were discovered in developing population (LW) in the new lawn in 2009. Taking into account the results presented in the available publications on *M. struthiopteris* spore breeding (Aderkas, 1983; Klekowski, 1985, 1988), it can be said that we have a unique spontaneous experiment on the formation of a population of this species from spores. Considering the time required for the development of gametophytes from the spores, and then 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 in the same year

(2007). August and September 2007 were warm, an average temperature in the period was 15ºC (daytime 17.7ºC, night 12.4ºC), therefore gametophytes had the possibility for development, and formation of sporelings, because gametophytes from green spores develop more rapidly than those from non-green spores (Lloyd & Klekowski, 1970). Spores could be contained in the soil used to lawn making and in this case, those that were on the soil surface may have then germinated because a light is required for their germination (Gantt & Arnott, 1965). Spores could also have become dispersed to the lawn by wind, from populations in which spore-bearing sporophytes grew. Such spore-bearing sporophytes were present in three of populations studied – RV, ST, and KS. Populations RV and ST are situated closer to experimental place (160 and 365 m) than population KS (6.65 km). Consequently, dispersion of spores by wind was more likely from nearest places than from distant one. The population GR did not include adult spore-bearing sporophytes in the period of observation.

To date, the population in the experimental site (LW) has been existing for 11 years, although it constantly experiencing a negative impact: grass-cutting took place annually 2–3 times per vegetation period. In the first year of observation (2009) the population in the experimental site (LW) includes 263 sporophytes. The number of sporophytes decreased gradually and reduced to 96 in 2018, i.e. 63.5% of sporophytes died during 10 years. Perhaps, the death of sporophytes was associated with increased competition from flowering plants that diversity increased from 7 species in 2009 to 25 species in 2018. Abundance expressed as a percentage of the projective coverage of aerial part of plants increases gradually from 5–7% at the beginning of observation to 100% in 2016–2018. Furthermore, the great negative impact is exerted by grass-cutting. It is especially important for ferns because sporophytes are forced to develop new fronds after each mowing. But under this regime, every next generation of fronds is thus developed from immatured croziers, and the rhizome does not accumulate sufficient reserves and is gradually depleted. This leads to the death of the sporophyte.

The observation on the dynamics of the number of sporophyte of different ontogenetic stages in the studied population in the lawn (LW) during 2009–2018 showed the following (Fig. 4A). In the first year of observation, the population includes only juvenile sporophytes of the early sub-stages (j1 + j2). The number of both these groups was maximal in the year of their discovering then decreased gradually from 2009 to 2018. The number of j1-sporophytes was minimal from 2015 to 2017, in 2018 this group disappeared. Group j3 appeared in 2010, its number reached the maximum value in 2011, then decreased to 2018. Immature sporophytes appeared in 2012, reached the maximal number in 2014, then their quantity started to decrease. Increase in the number juvenile (j3) and immature sporophytes is associated with the transition of some individual from the previous ontogenetic stage to the next one. The decrease of all sporophyte ontogenetic groups is associated with both the dying off and the transition from one ontogenetic stage to another. The first adult sterile sporophytes were discovered in 2013 and their number reminded at the same level from 2014 to 2018. No fertile sporophytes appeared during the period of observation.

The proportion of the different ontogenetic groups in the population varied in different ways (Fig. 4B). Participation of j1 and j2 sporophytes decreased gradually from 36% and 64% in 2009 to 0 and 7% respectively. The proportion of j3-sporophytes reached the maximum value in 2011 (about a half of the sporophytes survived in that year), then it remained on the same level (30–35%) from 2012 to 2018. From 2014 to 2018, more than 50% sporophytes survived in each year of observation, were immature, and their proportion remained the greatest from 2013. Participation of adult sterile sporophytes was low from the moment of their appearance in 2013 to the present time (3–7% from the number of sporophytes survivor in these years).

Thus, all survived sporophytes have the calendar age of 11 years, but they have reached different ontogenetic stages during that period. Some of them developed gradually from the first sub-stages of the juvenile stage and reached the adult sterile one, the majority of sporophytes reached the immature stage, but some sporophytes stayed in the juvenile stage.

On the whole, the observed population is young, it is composed of pre-reproductive sporophytes and is not capable to self-supporting either by spores or by vegetative means, because of the absence of both spore-bearing sporophytes and stolons. Therefore such a population may be determined as an invasive one (Rabotnov, 1950).

Genetic structure and differentiation of *Matteuccia struthiopteris* populations

Genetic structure was studied in five populations of *Matteuccia struthiopteris* listed in Table 1. Total genomic DNA of 30 samples of *M. struthiopteris* sporophytes was analyzed. They generated 102 discernible bands using six selected ISSR primers (Tab. 2). 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 the populations of *M. struthiopteris*.

Genetic differentiation was significantly high (AMOVA, $F_{ST} = 0.672$, $P < 0.0001$). The analysis performed for 5 populations of *M. struthiopteris* shows that variation within the population is 33%, among populations – 67%. This confirms that all of the studied populations are separate (Tab. 3).

The smallest Nei's genetic distance between *M. struthiopteris* populations is characteristic for LW and KS (0.072) and GR and KS (0.074), the greatest – for LW and RV (0.275). Respectively, genetic identity is greatest between LW and KS (0.931) and GR and KS (0.929), the smallest between LW and RV (0.760) (Tab. 4). This suggests that the spores, from which have been started to develop both LW and GR populations came to these places from KS. Since sporophytes appeared immediately in large numbers, it is more logical to suppose that they were not carried by the wind but were contained in the soil that was used to the lawn construction.

In STRUCTURE processing a clear peak in the value of ΔK (382.6) is at $K = 4$ (Fig. 5), therefore the optimal number of groups best fit our data set 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 the frequencies of alleles, while the populations RV, ST, and GR differ from each other and belong to three groups (Fig. 6). This diagram clearly shows that sporophytes from the population in the experimental site (LW) and sporophytes from the

population near Kislovka village (KS) are genetically identic, and it may be only in case of development of the population in LW from spores brought from KS with the soil. Some individuals collected in three populations (GR, ST, KS) showed genetic admixture between three genetic groups, indicated in gray, blue and red (Fig. 6). Probably some spore-bearing individuals from KS and ST are the source of spore for initiation of population GR, which is young to date. Spores from ST could be dispersed by wind, spores from KS – with soil, which partly was added to GR area. Nei's genetic identity is high for two pairs of populations GR–ST (0.905) and GR–KS (0.929). This confirms the previous suggestion. But in general belonging of each population to the separate genetic group is the result of the clonal organization of populations.

Conclusions

Matteuccia struthiopteris has chlorophyllous spores, which can germinate during several days after their maturing. But this species has several adaptations for prevention of dissipation of spores just after their maturation. These are the following: (1) the late-summer formation of sporophylls; (2) wrapped frond margins, which prevent the opening of sporangia; (3) the long period of the disperse of spores, including late autumn, winter, and spring; (4) low temperatures in this period, which promote retention of green spore viability. As a result, spores can opportunity to germinate and form gametophytes under the favorable environmental conditions in the year following their maturation period. In our study gametophytes and then sporophytes appeared simultaneously, but in 11 years after appearance, sporophytes reached the different stages due to the different rate of their ontogenetic development. Currently, the population developing in the man-made lawn includes juvenile, immature and adult sterile sporophytes, with predominance of immature ones. No fertile sporophytes appeared during the period of observations. The number of sporophytes decreased from 263 to 96 during the 10 years of observations.

Our study proof, that *M. struthiopteris* is able both to form the short-term (to ten months) soil spore bank and to develop the populations from spores. Sexually generated sporophytes have the ability to long-term existence even under the periodical unfavorable impact (grass-cutting). Observed population on the man-made lawn is the most genetically similar with the population located in 6.65 km from it, where the soil was taken for the lawn construction. It could be only in case the development of the studied population from spores containing in that soil. All other studied populations of *M. struthiopteris* having spore-produced sporophytes despite their territorial proximity (160–365 m from the experimental site) turned out to be genetically different to each other. Nevertheless, there is neither absolute similarity nor absolute difference between the studied populations. This means that they exchange hereditary information (DNA) through spore dispersal. But in general, the clonal structure is characteristic for the populations of *M. struthiopteris*.

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Figure 1

Structure of the sporophyte and scheme of vegetative reproduction of *Matteuccia struthiopteris*.

(A) Transition of the stolon to the formation of an orthotropic rhizome and development of the 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 *Matteuccia struthiopteris*: sporophyte with orthotropic rhizomes and stolons, and formation of sporophytes of vegetative origin after their separation. (1) orthotropic rhizome; (2) stolon; (3) separated sporophytes of vegetative origin; (4) stipes of the fronds. A and C by Gureyeva (2003).

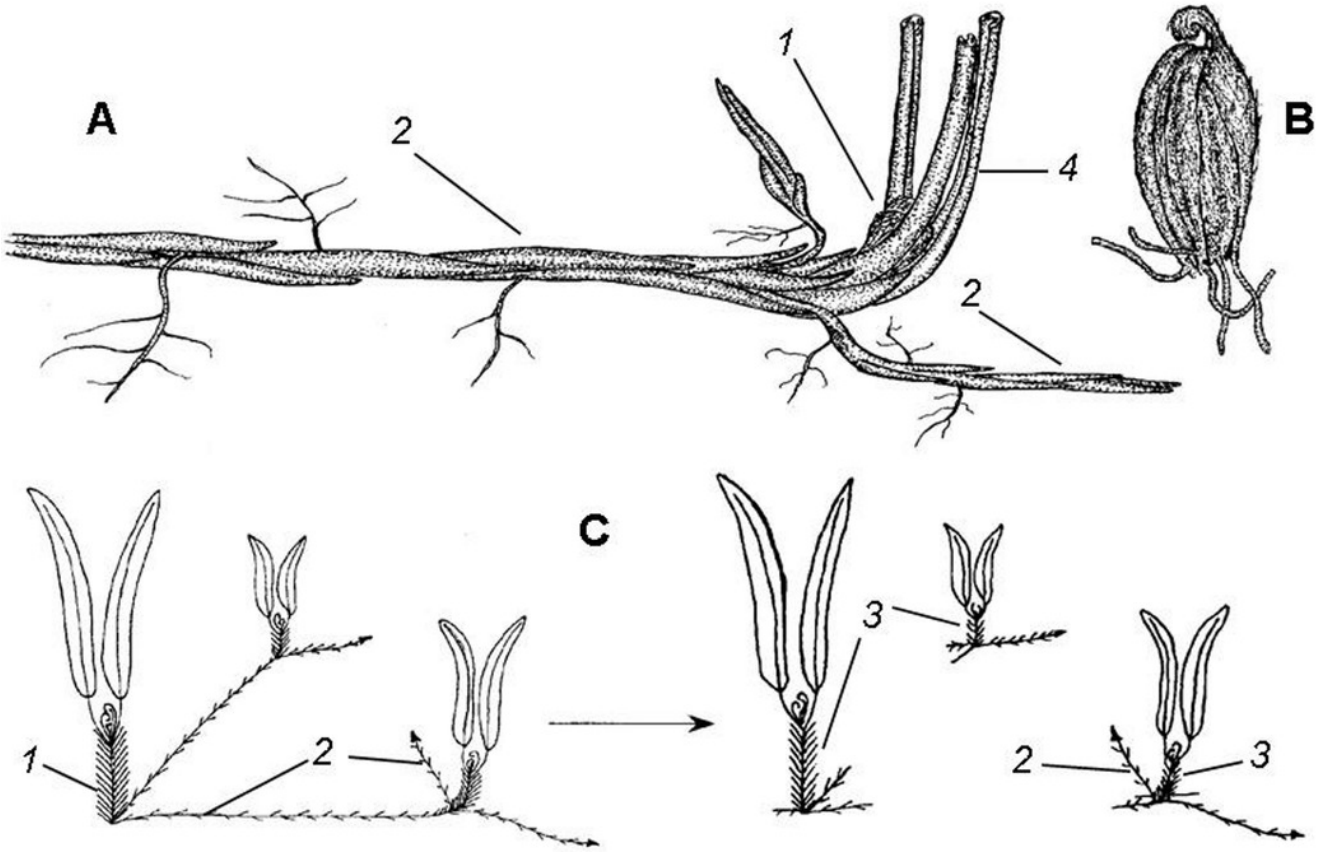

Figure 2

Sporophylls and SEM-micrograph of the spores of *Matteuccia struthiopteris*.

(A) vertically arranged sporophylls formed in 2017 and 2018 in the same rosette, trophophylls are died (September 27, 2018). (B) The lower side of the sporophyll. (C) The upper side of the sporophyll. (D) Sporophyll matured in the previous year. (E) Mature spore with perispore. (F) Mature spore without perispore, laesura in the center. Scale bars 20 µm (E, F).

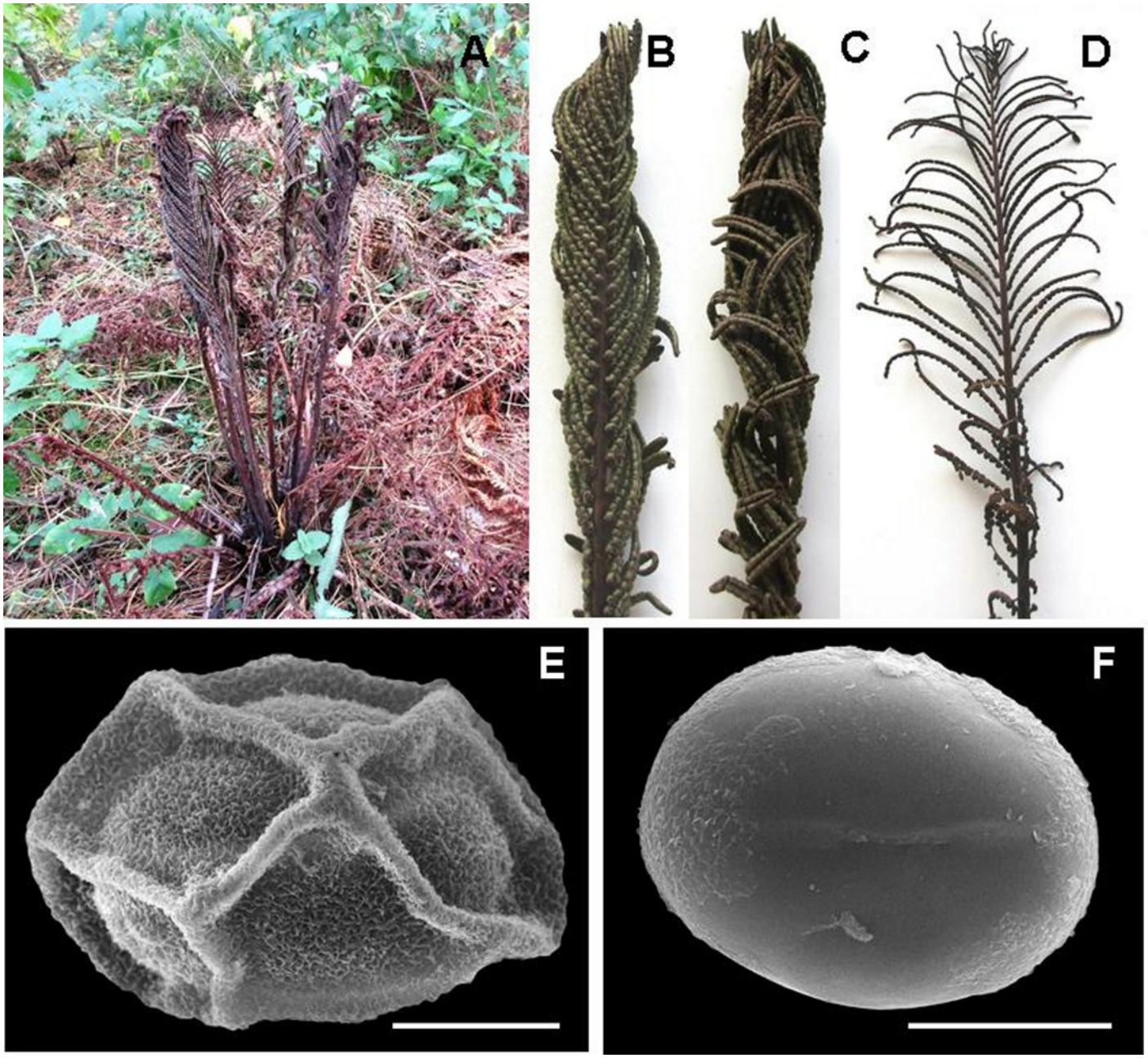

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Figure 3

Ontogenetic stages of *Matteuccia struthiopteris* sporophytes growing on the lawn in the University Grove (A–E) and in native habitat (E).

(A) Juvenile sporophyte, substage *j1*. (B) Juvenile sporophyte, substage *j2*. (C) Juvenile sporophyte, substage *j3*. (D) Immature sporophyte. (E) Adult sterile (virginal) sporophyte. (F) Adult fertile sporophytes in the native cenosis near Kislovka village. Scale bars: (A–D) 5 cm; (E) – 10 cm.

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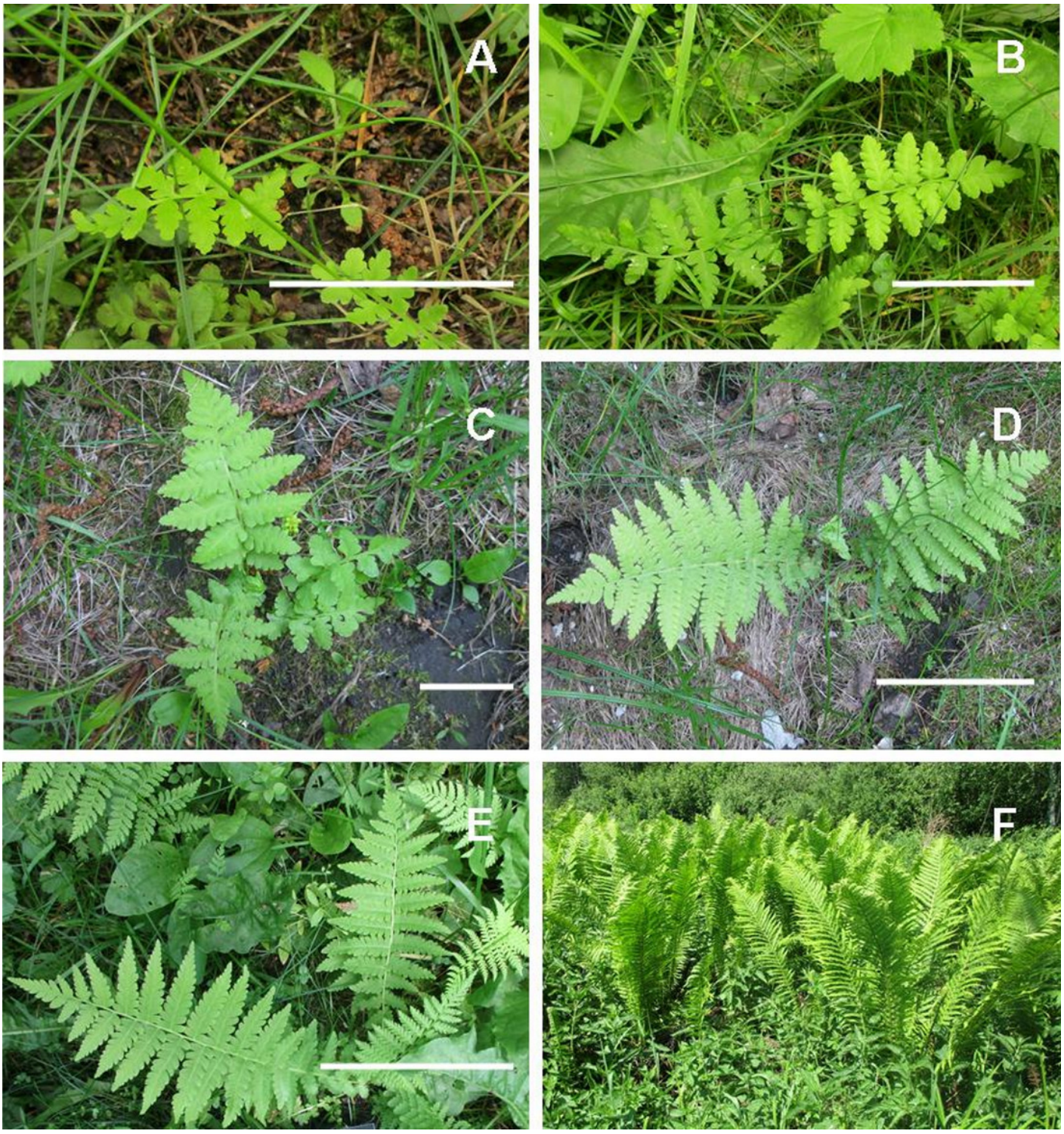

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Figure 4(on next page)

Demographic structure of the *Matteuccia struthiopteris* population developing on the new lawn in the University Grove (2009–2018).

(A) Dynamics of the number of sporophytes of the different ontogenetic stages. (B) Dynamics of the demographical structure of the population. Vertical axes: the number of sporophytes of each ontogenetic stage (A) and proportion of each ontogenetic group in population (B); horizontal axes – years of observations.

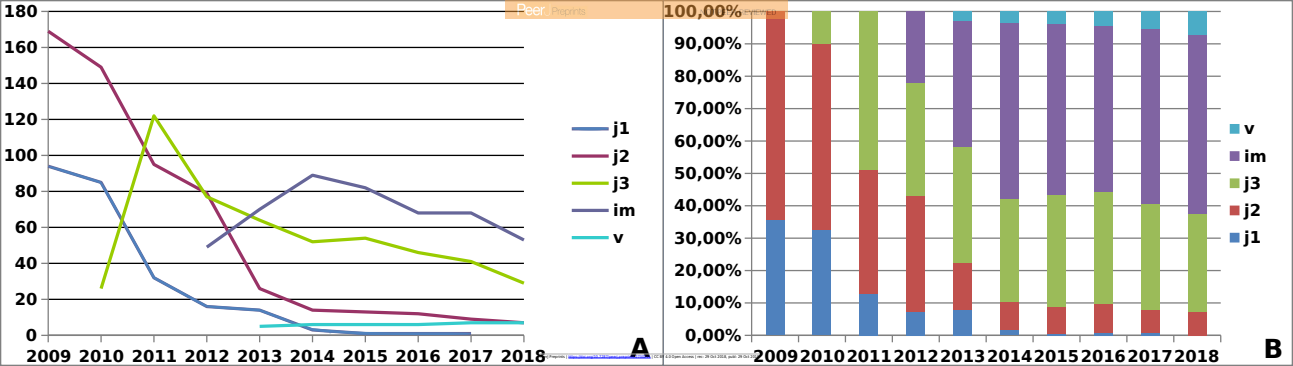
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Figure 5(on next page)

Results of the Bayesian assignment analysis using the STRUCTURE Harvester

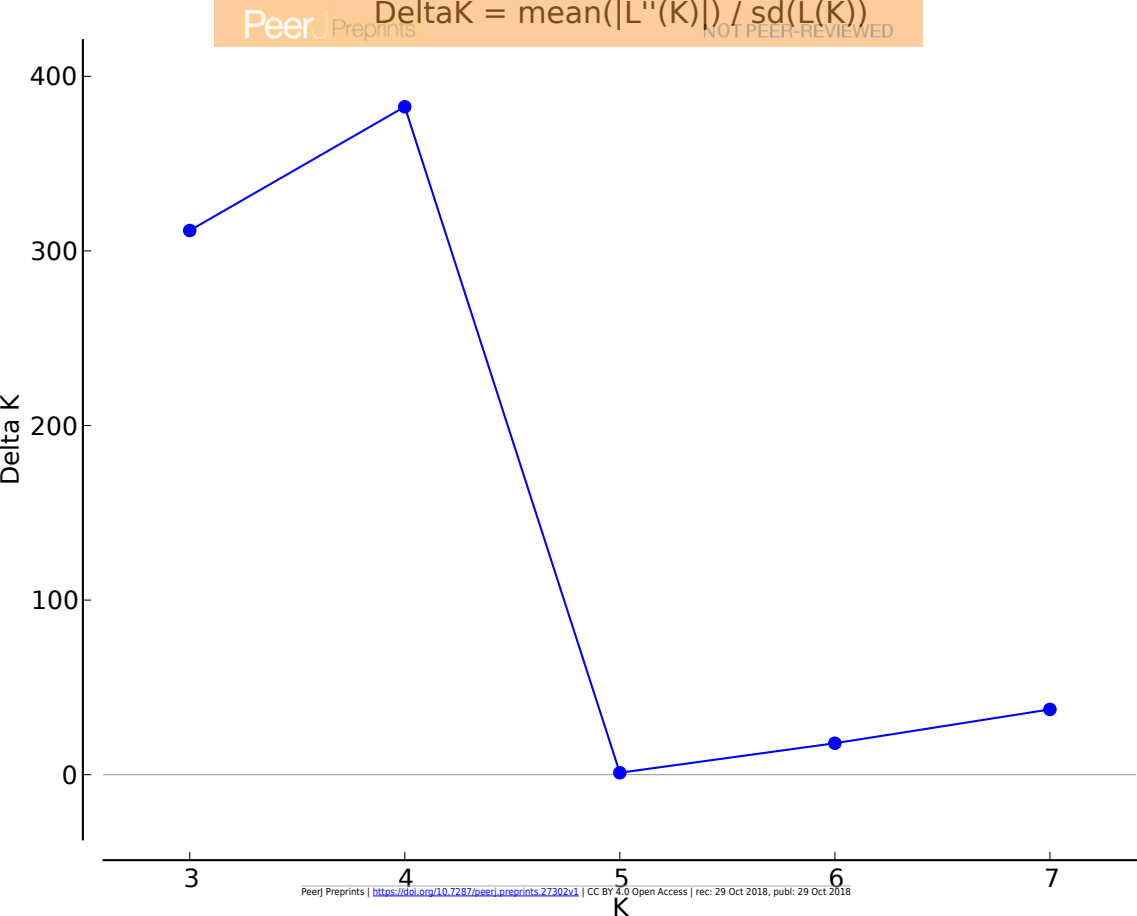

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Figure 6(on next page)

Population structure of *Matteuccia struthiopteris.*

Population structure of 30 individuals of *Matteuccia struthiopteris* based on six ISSR markers of total genomic DNA using the best assignment result (K = 4) retrieved by STRUCTURE. Each individual is represented by a vertical line divided into colored segments that represent the individual's proportion in K clusters. Abbreviations of populations follow those indicated in Table 1.

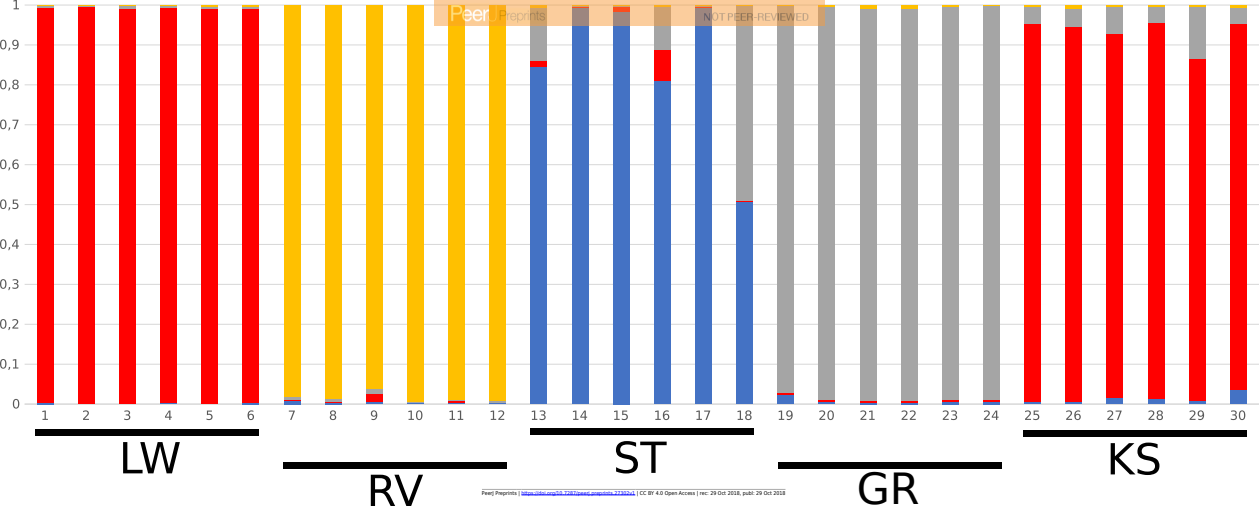

Table 1(on next page)

Sampled populations of *Matteuccia struthiopteris*

Population code	Locality	Latitude (N) Longitude (E)	Additional information
LW	Russia, Tomsk, Tomsk State University, University Grove, new man-made lawn along the west wall of the Scientific Library building.	56º28'05.4" N 84º56'57.1" E	An 'experimental' population appeared presumably in 2007; includes young sporophytes only.
GR	Russia, Tomsk, Tomsk State University, University Grove, old man-made lawn near the north wall of the Scientific Library building.	56º28'06.4" N 84º56'57.9" E	Population grows in 20 m from LW; includes young sporophytes only.
RV	Russia, Tomsk, Tomsk State University, Siberian Botanical Garden, in the ravine.	56º28'01.3" N 84º56'48.3" E	Population grows in 160 m from LW in ravine with small-leaved forest; includes many well-developed spore-bearing sporophytes.
ST	Russia, Tomsk, Tomsk State University, Siberian Botanical Garden, the stream near the west boundary of the territory.	56º28'00.9" N 84º56'34.1" E	Population grows in 365 m from LW in the wet site of the stream valley overgrown with shrubs; includes the separate spore-bearing sporophytes.
KS	Russia, Tomsk Region, vicinity of the village Kislovka towards south-west from Tomsk.	56º25'37.3" N 84º53'16.5" E	Population grows in 6.65 km from LW in the open wet site in the valley of the Kislovka river, where soil was taken in 2007 for making of lawn; includes mainly well-developed spore-bearing sporophytes.

Note:

The distances between experimental (LW) and other population localities were measured in a straight line.

Table 2(on next page)

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

1

Table 3(on next page)

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

Source of variance	d.f.	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

1

Table 4(on next page)

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.