

## Reintroduction of the endangered fern species *Woodsia ilvensis* to Estonia: a long-term pilot study

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**Abstract** *Woodsia ilvensis* has become extinct from its last known natural localities in Estonia and has not been rediscovered since 1977. This fern grew in northern and north-western Estonia in areas of suitable habitat. Considering that habitat conditions may have changed in previous localities, an experimental project was started to test if it would be possible to reintroduce *W. ilvensis* into new localities where suitable habitat conditions exist. Two experiments were performed, one on an old stone wall, constructed of stones collected from the surrounding fields, and another on two granite boulders in two localities, one where the surrounding soil was acidic, and the other where the soil was basic. The plants were grown from spores of wild provenance received from Finland via the seed and spore exchange system of botanical gardens. Results confirmed that individual plants can establish and persist for at least 10 years on stone walls without maintenance. The experiment on boulders failed, as plants did not establish there. Young, 2-year old mature individuals proved to be the best stage for planting out onto the stone walls in this case study. The best indicators for selecting suitable habitat were characteristic plant species of the natural community. Here I discuss the experimental methods used and first results of the experiment.

**Keywords** Locally extinct species · Reintroduction experiments

### Abbreviations

- TAA Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, herbarium  
TAM Estonian Museum of Natural History, botanical collections  
TU University of Tartu, Museum of Natural History, herbarium

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## Introduction

Although reintroduction is a relatively new method, it is often used as a conservation tool for achieving the persistence of endangered species in the landscape (Falk et al. 1996). As is known for many reintroduction practitioners, much information and practical knowledge is needed to succeed in reintroducing plants to the wild or new habitats (Gorbunov et al. 2008). Preliminary analysis of historical and current natural distributions, distance to the nearest natural spore/seed source, and availability of sites, indicate whether the reintroduction or recovery of the target species would be possible and feasible. The choices of appropriate methods for propagation, planting out, maintenance and site management are the keys for success. For making these choices, research on life cycle, reproductive biology, population biology and ecological requirements of the particular species or plant group is needed (IUCN/SSC 1995; Falk et al. 1996; Valee et al. 2004). In the case of globally critically endangered species, there is usually too little time and too little information or research available on the target taxa, yet the goal is high—to successfully conserve the species. Species endangered at the local or regional scale, but with a wider global distribution, offer many opportunities for the study and refinement of conservation methodologies.

*Woodsia ilvensis* has been historically rare in Estonia (Åberg 1934; Hein and Puusepp 1962). It was first discovered in 1887, and documented only in four localities at different times (Specimens: 1887 R. Leibert, 1904 H. Hiir, both TAA; 1960 V. Puusepp TU, TAA; 1960, 1969 V. Hein, TAM, TAA), growing as single individuals or small groups of plants. These findings were from the northern and north-western parts of Estonia, where some areas offer suitable habitat, representing thus the south-eastern border of its Scandinavian disjunction (Hultén 1971, p. 13). Despite checking the earlier localities and searching for this species in general, *W. ilvensis* has not been found since 1977. Initially it was assessed as critically endangered (Lilleleht 1998) and until 2004 it belonged to category I of the protected plant species list of Estonia (ERT I 2004). Since 2005, it has been considered an extinct species in Estonia (Kukk and Kull 2005, p. 528). However, worldwide this fern has a wide but disjunct circumpolar distribution (Hultén and Fries 1986, p. 30) and has not been evaluated for IUCN, as its condition and conservation status within the whole distribution range varies. It is, for example, assessed as locally common, locally decreasing, locally endangered or locally extinct (Ingelög et al. 1993; Dyer et al. 2001).

*Woodsia ilvensis* grows in dry and sunny or half shaded rocky habitats, on thin acidic soils and igneous rocks (Hegi 1984). In Scandinavia, this species is locally rather common, usually on sunny and exposed cliffs and rocky mountain slopes, in rock crevices, on boulders and talus (Mossberg and Stenberg 2007, p. 35). In central Europe, it is rather rare, growing as glacial relics in mountain or sub-alpine zones. The closest eastern localities to Estonia are the Ural Mountains where it has also been recorded as growing on calcareous rocks (Tolmatšov 1974). Older findings in Estonia were from coastal cliffs. More recent findings were from old stone walls piled from stones collected from the surrounding fields.

Previous localities have been checked repeatedly. The two older localities in Estonia are dominated by a calcareous substrate. These localities may only have been temporarily occupied by this fern, on boulders, sandstone or the shale layer. In the two most recent localities, the habitat was degraded with the stone walls destroyed or removed. Many of the old stone walls within that area still appear to be promising as habitats, but were empty of ferns or overgrown by bushes and mosses. The occurrence of calcicole species such as *Asplenium trichomanes* ssp. *quadrivalens* and *Gymnocarpium robertianum* indicated a possible change in the soil pH.

With this study I tested the possibility of reintroducing the fern *W. ilvensis* into new localities that appear to be suitable habitat, and thus gain knowledge on the dispersal and distribution, availability of suitable habitat conditions and possible establishment of viable populations of this fern species in Estonia.

## Methods

### *Ex situ* propagation

No natural source of local spores exists in Estonia, thus all plants were grown from spores received via the seed and spore exchange system of botanical gardens. Only spores of wild provenance were used for the experiment. These came from Finland (North-Karelia, Jonsuu Botanic Garden, and Uusimaa, Helsinki Botanic Garden). As the distribution area of *W. ilvensis* in Finland is very close to Estonia, and ferns are known by their ability for long-distance dispersal, it was assumed that spores from any local population in Finland could potentially disperse to northern part of Estonia. Sowings were performed in the autumn of 1995, under laboratory conditions, onto a mix of coarse sand and drained peat-land forest soil in Petri dishes (Aguraiuja 2001). In February 1996 the sporelings were replanted into pots with a mix of leaf mould, drained peat-land forest soil and coarse sand, and taken for hardening into shaded sphagnum beds in the spring of 1996, where they were kept and handled with minimal maintenance, until planted into the wild. As the rooting substrate in the crevices of boulders is quite limited, younger plants (at sporeling and premature stages) were chosen for planting onto the boulders in the autumn of 1996. The rest of the experimental individuals over-wintered in the sphagnum bench of the botanical garden and were planted onto a stone wall on Prangli Island in September 1997.

For tracking the duration of developmental stages, the following classification was used: gametophyte, sporeling (juvenile sporophyte with small simple fronds), premature (young pre-reproductive sporophyte with simple pinnate fronds characteristic to the species), mature (reproductive sporophyte).

### Selecting reintroduction sites

Considering that substrate pH may be a limiting factor (Hein and Puusepp 1962) and that changed habitat conditions may not promote species survival in previous localities any longer, I searched for new suitable habitats for testing the success of the planting out of garden-grown *Woodsia* plants. There are only a few almost ‘calcium free’ areas in the northern and north-western parts of Estonia where the vendian layer is denuded (Kala 1995). I evaluated these areas, some northern islands and some siliceous erratic boulders as possibly suitable habitats.

In many regional floras, *W. ilvensis* is associated with *Asplenium septentrionale*. For instance, both species can be found on the easternmost islands of the Gulf of Finland (Glazkova 1996). *W. ilvensis* is also a characteristic species of the Woodsio–Asplenietum septentrionalis (*Androsacion vandellii*) synusia in the foothills of the Alps (Hegi 1984). Furthermore, in its most recently discovered location in Estonia, *W. ilvensis* was documented growing together with *A. septentrionale* (Hein and Puusepp 1962), both species being currently extinct at this site.

On the small Prangli Island there is currently a single indigenous population of *A. septentrionale* with ca. 180 individuals growing on a south-facing old stone wall of an

old churchyard. Thus, *A. septentrionale* was considered an indicator for suitable habitat conditions, and we chose the north-facing side of the same stone wall for our reintroduction experiment on *W. ilvensis*.

For testing the occasional establishment on erratic boulders, two were selected in two different locations on mainland: one in an area with acidic soil (Harjumaa, Viimsi, Pringi), the other in an area with basic soils (Raplamaa, Jalase, Matsi). As some of the historically documented locations were from areas dominated by calcareous soils and limestone rock, an attempt was made to test if *W. ilvensis* could still occasionally establish on erratic boulders.

### Reintroduction experiment

The whole experiment was planned at a small scale with the aim to test the possibility of reintroduction without losing too many individuals. For this reason 12 individuals were planted per site. Since the primary root of a fern emerges directly from the embryo, does not grow very long and is quickly replaced by adventitious roots, it was decided initially to use mainly younger stages of sporophyte (sporeling and premature individuals) to study the establishment of this fern on boulders. In 1996, 24 one-year old sporophyte individuals were planted into north-facing crevices and cavities of two boulders, in places where a thin layer of decayed plant material and mosses were present.

In 1997, 12 two-year old sporophytes, at a mature stage but still small in size and with a single rhizome tip, were planted in sandy soil between the stones of an old north-facing stone fence on Prangli Island. Planting took place in September according to more rainy local weather conditions and giving plants enough time to get rooted before winter. The plants were watered only immediately after planting and then left under natural conditions.

4 years later, in 2001, a group of 4 six-year old individuals were added as a separate patch to the stone wall site. The aim was to check if the age and size of individuals at out-planting, and the amount of years grown under artificial conditions would make a difference to survival in their natural habitat.

### Monitoring of experimental populations

Monitoring took place on a yearly basis. During years of extreme dry and hot summers (for example in 2001, 2002, 2006) the experimental site on the stone wall was visited twice, the second time after the beginning of the autumn rains. The condition and vitality of individuals were assessed and the following data were documented: the height and diameter of the clump, number of fronds and rhizome tips. The developmental stages of the sori were also described with the aim to learn more about the timing of spore dispersal of the species under natural conditions. During each visit the whole habitat patch was carefully searched for evidence of regeneration.

## Results

### *Ex situ* propagation

Spores germinated well. Spore germination and the formation of gametophytes took 24–52 days with the mean germination time of 8 replicates was 46.29 days (SE 15.7). The

formation of new sporelings was continuous, with the longest duration from spore germination to sporeling formation up to 104 days. There was no clear size difference between plants at the premature and at the beginning of the mature stages. The transition from premature to mature seemed to be gradual for this species and it was somewhat difficult to determine exactly without damaging the plants. Though still small in size, most 2-year old individuals developed sori.

The plants went through moderate shock when light conditions changed, and became pale green when taken from laboratory conditions to the shaded sphagnum bench, but recovered well.

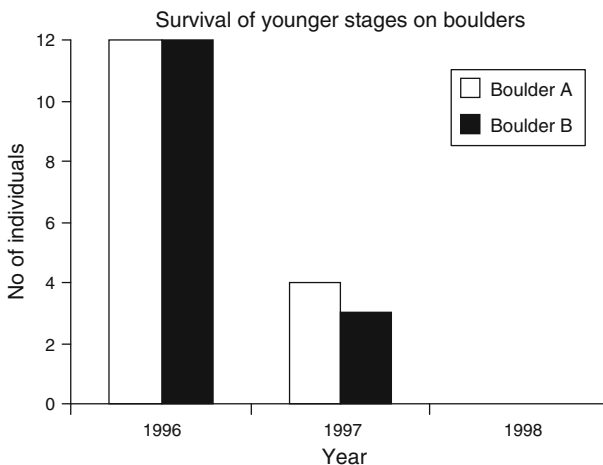
#### Experiment on boulders

The plants did not establish on boulders. Young sporophytes, which were planted into the crevices of boulders at the stages of sporeling and small premature individuals, survived only for 1 year, and gradually died out throughout the summer of the second year (Fig. 1). New fronds were observed, but the size of the individuals was the same or decreased. In only one case was the transition from sporeling to premature stage observed. The formation of reproductive fronds was not found on those plants that were planted as premature individuals, or were missed as the fronds were too small to see the details of developing reproductive structures.

#### Experiment on the stone wall

The reintroduction experiment on the stone wall was more successful. All individuals originated from the same sowing made in 1995 and thus belonged to the same age class. 12 two-year old individuals were transplanted to the wild in 1997. 4 six-year old individuals were added as a separate patch in 2001.

Three plants of those transplanted in 1997 died during the two first years at this site. The other nine individuals survived and continued growing (Table 1; Fig. 2). The size of



**Fig. 1** Survival of experimental individuals of *W. ilvensis* planted: *Boulder A* on acidic soil; *Boulder B* on basic soil

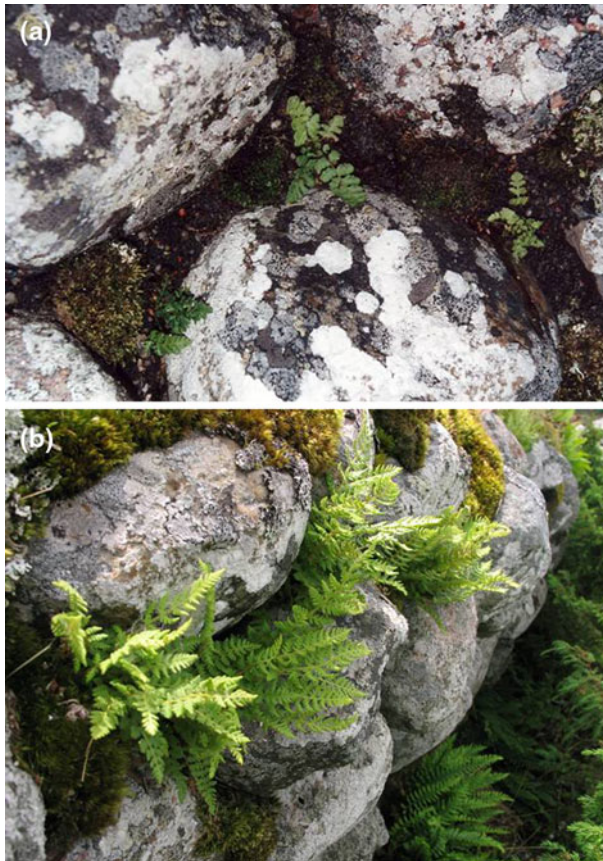
**Table 1** Characteristics of the individuals of *Woodsia ilvensis* in experimental population on Prangli Island in Estonia

Year	Mean vertical diameter		Mean horizontal diameter		Mean height of the clump		Mean number of the fronds in the clump			
	cm	SD	cm	SD	cm	SD	Mean	Min	Max	SD
Planted as 2-year old individuals in 1997										
2002	2.8	1.4	2.2	0.8	2.3	0.6	8	6	12	2.0
2003	4.5	1.3	5.7	2.4	3.4	1.2	12	4	23	6.4
2004	9.1	3.5	5.5	3.7	5.0	1.3	16	8	25	4.0
2006	9.3	4.1	8.7	2.8	7.7	2.4	20	10	36	9.1
2007	12.9	5.0	12.4	4.3	8.2	2.8	28	8	53	15.0
2008	11.4	4.7	10.0	2.4	8.8	3.3	37	11	72	19.9
2009	12.6	3.8	12.8	3.6	10.5	2.5	35	9	64	16.1
2010	12.1	3.5	10.8	4.2	10.0	2.4	31	4	54	14.5
Planted as 6-year old individuals in 2001										
2003	12.8	3.0	13.9	2.32	11.0	1.4	23	14	30	6.7
2004	12.9	2.3	12.1	1.05	9.8	2.0	21	17	36	9.8
2006	11.0	1.1	11.0	1.63	8.0	2.0	31	22	41	8.7
2007	14.0	1.4	11.3	1.19	8.6	3.1	32	19	38	8.8
2008	8.3	4.1	9.8	3.62	8.9	4.9	19	8	32	9.9
2009	9.4	1.1	10.8	4.80	8.6	2.1	21	12	35	9.9
2010	9.3	3.3	11.5	3.87	10.9	4.5	26	19	41	10.2

individuals increased gradually until 2007, after which size stayed relatively constant; mean vertical diameter ranged from 11.4 cm (in 2008) to 12.9 cm (in 2007), mean horizontal diameter from 10.0 cm (in 2008) to 12.8 cm (in 2009) and mean height from 8.2 cm (in 2007) to 10.5 cm (in 2009).

Plants grown under garden conditions until 2001 were roughly three times larger than those planted out in 1997. These plants also survived the shock of out-planting, but slowly decreased in size during consecutive years (Table 1).

Repeated observations showed that *W. ilvensis* adapted well to dry and open habitat conditions. The ferns survived long droughts by curling up the fronds during the dry months of July–August, while turning back green and continue to grow after the late summer or autumn rains in August–September. Increase in the number of rhizome tips seemed to be a good indicator for growth and vitality of individuals in the beginning, but became difficult to assess when plants grew larger in size. The sori developed yearly, though spore production may have been aborted during the droughts. The spores originating from experimental plants were viable and germinated well in the laboratory. Usually sori began to develop in June and during more favourable weather conditions the spores were ready to disperse from the middle of July onwards. In case of droughts, spore formation and ripening was interrupted or delayed, yet whitish underdeveloped sori were still observable on curled and dry looking fronds. No regeneration or natural recruitment was observed before 2008. The first newly established young sporophytes were found close to the parent plants and were recognized by their small juvenile type of fronds.



**Fig. 2** Individuals of *W. ilvensis* on Prangli Island in Estonia: **a** in 2001, **b** in 2008

## Discussion

The reintroduction experiment confirmed that individual plants of *W. ilvensis* can establish in Estonia and persist in nature for at least 10 years without maintenance. We experimentally recreated a single colonisation event. The success in this indicates that the reason for rarity and decline of *W. ilvensis* in Estonia is most likely the limited distribution of suitable habitat conditions rather than a change of local climatic conditions. It seems that *W. ilvensis* may not be a permanent component of Estonian flora, but rather an occasional inhabitant of suitable habitat at the south-eastern limit of its natural distribution range (Hultén 1971).

The experimental individuals have been in the wild since 1997, with clumps still growing and increasing in size. *W. ilvensis* rhizomes are short-creeping and branching, and are known to have up to 20 rhizome tips (Hegi 1984). Furthermore, newly established young sporophytes were observed next to parent plants. This will make it difficult to distinguish between transplanted and newly established individuals in the future, as newly established plants are recognizable only temporarily while having the fronds of juvenile sporophyte.

Recruitment of new individuals is a critical measure of reintroduction success (IUCN/SSC 1995). It may take a long time until a structured population evolves, and in case of such a small number of founder individuals, the probability of this event happening is as high as that of extinction (Falk et al. 1996). Also in general, there is little known about the population ecology of ferns and baseline data about establishment, recruitment, growth rates and survivorship in natural populations of different species are lacking. Emulating known or inferred/estimated natural processes of dispersal and establishment could be one way to accelerate the process of establishment via multiple reintroductions (Primack 1996). The initial results and experience gained from the current experiment with *W. ilvensis* is encouraging, and calls for the gradual out-planting of individuals during several consecutive years, thus simulating repeated colonization, recruitment and population growth. As the number of individuals and amount of spores/seeds are limiting in endangered species, it would be more sustainable to start studies with smaller experimental populations, gradually increasing the size and number of patches in accordance with the results from ongoing survival analyses. Further research is planned to learn more about the population establishment of two species of the same growth form and similar habitat requirements—*W. ilvensis* (naturally extinct, one ‘reintroduced’ population) and *A. septentrionale* (category I protected plant species in Estonia with a single currently known population) together—and extend the project area over three small northern islands of Estonia in the Gulf of Finland, where patches of habitat similar to the one on island Prangli are still available.

The entire growing process of a plant relies on adaptation to environmental conditions, thus *ex situ* propagation should involve the hardening of plants for future planting into natural habitats, decreasing somewhat the stress of the planting out process and increasing on-site survival (Jacobs et al. 2004; Davis and Jacobs 2005). The *ex situ* conditions of conservation collections should emulate natural substrates and conditions, and the plants should be handled with minimal interference and maintenance.

Both the stage-class and age-class of founder individuals may affect establishment, subsequent population growth and extinction probabilities. This may be specific, depending on life history characteristics of the species (Guerrant 1996). Ferns are a difficult group to reintroduce into the wild. Fern spores are small unicellular reproductive structures. Fertilization takes place on a very small one cell layer thick gametophyte and the success of sporophytes in a community is limited both by the successful establishment of free-living gametophytes and the survival of sporophytes (Cousens 1988; Werth and Cousens 1990).

Sowing the spores of *W. ilvensis* into the reintroduction site was not tested here. Experience from sowing trials of *Diellia pallida* into its habitat (Aguraiuja 2005) confirmed that environmental changes (like soil erosion, smothering effect of leaf litter, out wash by rain etc.) in the natural habitat are too unpredictable for successful establishment of gametophytes. Further observational studies on natural populations are needed to evaluate the feasibility of this type of study.

The out-planting of gametophytes could have been another interesting experiment. The preconditions would be rather persistent microhabitat conditions, as for example moist rock crevices (McHaffie 2004), but these features were not available at the current study site. Knowledge on the ecology of gametophyte generation of most ferns is also lacking.

Many fern species are disturbed by repeated plantings, requiring longer periods of time to recover and needing more after-care (Jones 1987). For this reason it is important to consider which developmental stage of the sporophyte could be best for successful establishment in the new habitat. Theoretically, it would be good to plant out individuals at an early stage of development, enabling a longer period for adaptation to natural conditions



and the rhythms of natural changes in the habitat. Individuals at the sporeling and pre-mature stages failed to establish on boulders, but small younger in age mature individuals established successfully on the stone wall. Ferns that were smaller and younger (2-year old) when planted out, developed more compact cushion-type clumps with more fronds and increased continuously in size. Those that were planted out as 6-year old individuals decreased in size during several consecutive years.

Recent germination tests under laboratory conditions have shown that spores produced by experimental plants were viable and germinated. As *W. ilvensis* is a fern adapted to dry and open habitats, it may be that only a series of wetter years with more suitable conditions for germination and gametophyte generation would favour the establishment of new individuals (Dyer et al. 2001; Aguraiuja et al. 2004; McHaffie 2004). The timing of germination in natural conditions (in the autumn or spring, or both) needs further study.

Ferns in general are shallow-rooted plants. Rather thin and wiry adventitious roots grow out of the stem, and get their water and nutrition from a relatively small area of the upper layers of the soil (Jones 1987). It can be assumed that the establishment of ferns on boulders is very occasional, depending on the thickness of the soil layer, the size of the crevice, canopy cover above the boulder, shade and moisture conditions of the habitat patch and local climate during the summer months. Even though the experiment on the stone wall was more successful, and most of the individuals survived in places where they were planted, more research on microhabitat requirements and conditions may be needed to identify the 'right' micro-sites for planting the individuals of endangered species. Not only moisture content in general, but also the moisture regime in the soil of the micro-habitat could be vital for long-term survival of fern individuals, particularly in case of the ferns of dry habitats.

In the current case study, the habitat conditions of previous locations had changed and thus finding the most appropriate habitat patch with characteristic plant communities was a key in the successful reintroduction of this fern species. The best indicators for selecting suitable habitat were characteristic plant species. If appropriate habitat with a characteristic plant community and habitat conditions could still be found, then any management of the site, like watering the plants or reducing the competition with other species (weeding), will not be needed.

The experience gained from this project showed that the reintroduction of this fern is a long-term process that requires long-term planning of different activities during different stages, and long-term monitoring of survival and establishment.

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