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Rubus humulifolius rescued by narrowest possible margin, conserved *ex situ*, and reintroduced in the wild

Marko-Tapio Hyvärinen

Botany Unit, Finnish Museum of Natural History LUOMUS, P.O. Box 7 (Unioninkatu 44), FI-00014, University of Helsinki, Finland

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ABSTRACT

Rubus humulifolius is a Eurasian species that has got wide geographic distribution from western parts of Russia to Manchuria. The westernmost and separate population of *R. humulifolius* was found in Central Finland in 1917. The population was assumed to be formed *via* anthropogenic dispersal either in early nineteenth or early eighteenth century. In 20's the population was regarded as viable as it covered an area of a hectare almost as a monoculture in the field layer and it was protected by law in 1933. However, the state of the population started to decline in the same year as the area was ditched. In 1957 there were only fifteen rosettes left and five rosettes of were transplanted from the site to a private cottage garden just before the whole area was turned into a construction area and remaining population destroyed.

The rescued population thrived but attempts to reintroduce plants from it to other sites considered to be suitable for the species were unsuccessful. Therefore, a research project initiated in 1986 for finding a suitable *in vitro* method for vegetative propagation of the plant. Eventually, the right formula for a substrate was found and 1500 new plants were produced in a couple of months. Now the *in vitro* propagated descendants of the five rescued ramets grow in several Finnish botanic gardens as part of their living collections and they also have been reintroduced to a natural site close to the original one in Central Finland.

One can assume that a plant population that has gone through two bottlenecks – *i.e.* establishment of new population by presumably few long-distance dispersed genetic individuals and population decline to near extirpation – has got very narrow genetic diversity. Whether this is the case and whether inbreeding depression could explain *e.g.* the observed poor seed production in the population remains as a challenge for future research.

Owing to *ex situ* conservation and *in vitro* technique applied for the first time to reintroduce an endangered species *R. humulifolius* is no more in immediate danger of extirpation. However, it is still classified as Critically Endangered (CR) as there is still only one population in the wild and it may be genetically depauperate.

1. Introduction

Historically environmental degradation including destruction of habitats has been the main reason for the demise of native plants (Rejmánek, 2018). Nowadays, climate change has an increasing role in accelerating the rate of species decline and extinction (Urban, 2015) and, regarding the conservation of genetic diversity, the threshold for a safe operating zone has already been passed (Steffen et al., 2015). As we can only guess how many species are already in the state of extinction debt (*sensu* Tilman, May, Lehman, & Nowak, 1994), urgent measures are necessary to ensure the plant biodiversity on earth.

In recent decades we have seen an increase in the numbers of carefully designed, assessed, implemented and monitored plant reintroductions (IUCN/SSC, 2013). Unplanned operations targeted for rescuing a single native plant population from an immediate danger of local extinction are relatively rare but there are some historical records of those with variable rate of success (*e.g.* Lesouef, 1988). Undoubtedly there have been a great many unreported *bona fide* attempts to alleviate immediate threats faced by plant populations by reintroductions but due to legislative reasons, poor operative success or to protect the new populations (*e.g.* in case of collectable ornamental plants) they are not made public.

In the face of climate change more predictive and proactive measures such as assisted migration *sensu* Hällfors et al. (2014) have been developed and widely discussed in recent years. However, these kind methods are still dependent on the genetic material of the original taxon concerned has been either banked and propagated *ex situ* or are available in the wild in such quantities that collection and translocation is possible without adverse impact on the original populations.

In turn, the idea of de-extinction, defined as the introduction of

E-mail address: marko.hyvarinen@helsinki.fi.

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proxies of extinct or extirpated species (see Abeli et al., 2019) to reinstate its former the ecological functions, is not based on genetic material of the original taxon that has been conserved but rather based on either on extant ecological similarity or methods such as genetic engineering that may allow creation of proxies. In this context the term proxy acknowledges that the resurrected individuals are materially different from the focal species of the attempted de-extinction (Abeli et al., 2019) and, according to Shapiro (2017) the precise replica is not essential as the main aim is in ecology. However, it has not been yet defined how far from either genetic or ecological space should the proxies come from. Therefore, introductions with proxies can be viewed as extreme cases of conservation reintroductions and the knowledge gained from the latter may well be valuable for de-extinction attempts. This is a report of a historical emergency rescue of one endangered plant species in Finland, *Rubus humulifolius* C. A. Mey in the brink of extirpation and subsequent establishment of its *ex situ* and *in situ* conservation using several methods and lessons learned from it.

R. humulifolius is a small (10 − 30 cm tall) perennial herb with reniform 3 − 5-lobed doubly serrate leaves resembling those of hop (hence the species name *humulifolius,* hop-like). It spreads vegetatively by subterranean rhizomes and typically occupies mesic swampy boreal heath forests characterized by Norway spruce. *R. humulifolius* flowers in June with small modest-looking white flowers but produces berries relatively rarely and often berries remain underdeveloped. Moreover, the viability of seeds is not known, there are no reports of seedlings and, hence, it seems likely that the individuals mostly spread by vegetative means (Kypärä, 2012; Raatikainen, Puska, & Törmälä, 1987). Therefore, the species is assumed to form locally relatively large clones rather than genetically diverse small-scaled mosaics.

The area of distribution of *R. humulifolius* spreads from eastern Carelia through Russia all way to the coast of Pacific Ocean mainly following the Southern and Mid-Boreal vegetation zones (Kypärä, 2012). The species is also found in Northern Manchuria. This is a distribution typical for a group of approx. 30 plant species that have their main area in eastern Asia and a narrow wedge-shaped area that barely reaches N. Europe (Raatikainen, Valovirta, Puska, & Törmälä, 1990). The distribution area of *R. humilifolius* is generally rather uniform but occurrences in the west are scattered by nature and the closest appearances to the one in Finland are, according to present GBIF records and historical sources (Mikkola, 1927), around Lake Onega, Russia, c. 500 km east. Hence the extant isolated Finnish population is the only one in the area of EU. In Russia the species is classified as endangered in the regions at the southern border of the distribution (*e.g.* Geltman, 2018). In Finland the species has been classified as either extinct or endangered since the systematic evaluations of the status of Finnish flora started in mid-eighties (*e.g.* Rassi, Alanen, Kemppainen, Vickholm, & Väisänen, 1985).

2. History of the Finnish population: from extirpation to *ex* and *in situ* conservation

The original population of *Rubus humulifolius* in Kypärämäki, Jyväskylä, Central Finland (62°15'N, 25°42'E) was first discovered in 1917 and nine years later the population was thoroughly mapped and measured by Mikkola (1927). According to him the population covered approximately one-hectare area and was dominating the field layer occasionally up to 100 % cover. Moreover, he clearly indicated the population was viable in 1920′s and rather spreading than diminishing.

The origin of the odd Finnish *R. humulifolius* population so far from the relatively uniform distribution area in Russia is not known. Mikkola (1927) states that if the population has started from a single or few ramets it must be at least a couple hundred years old. It has been speculated that the site was close to maintenance route of Russian military during the so-called Finnish War in 1808–1809 or the origin may be even older since there were Russian military camps nearby the site during the end of the Great Northern War (1700–1721) between Russia and Sweden (Kypärä, 2012). Nevertheless, the species has been regarded as a neophyte (*i.e.* arrived after the year 1600) rather than an archeophyte in Finland and it seems likely that the population has started from a single dispersal event.

Since 1920′s the population suffered several setbacks. Even though the species was officially protected in 1933 the habitat was diched in the same year. According to Raatikainen et al. (1987) the uniform population cover was fragmented into three patches already in 1933. In 1937 the situation was largely unaltered but there were alarming signs of building activity in the area. Therefore, some plants were reintroduced to two new sites c. 1 km from the original site but those attempts failed. The population further declined and in 1953 it covered only 150 m^2 area until in 1957 there were only 15 ramets left growing scattered in banks of ditches (Valovirta, 1958).

In 1957 in spite of the well-known location of the population of this endangered species that was also protected by law the officials of the municipality of the city of Jyväskylä allowed building in the area. According to Kypärä (2012) five shoots of *R. humulifolius* were rescued by a naturalist, E.J. Valovirta, only two hours before bulldozers destroyed the whole habitat and the remaining population. He then transplanted these five shoots in his cottage garden in Laukaa Central Finland where they formed a new population that covered c. 4 m^2. This population was then used to re-introduce the species on several wild sites but unfortunately these attempts failed. Therefore, three *ex situ* populations were founded in Finnish botanic gardens in early 1980′s (Raatikanen, 1997).

New *ex situ* conservation methods came to help in 1986 when micropropagation was applied to *R. humulifolius* (Puska & Törmälä, 1987). The method was first developed with a common cogeneric species *R. saxatilis* but it became soon obvious that the species did not share quite the same requirements (Raatikainen et al., 1987). The greatest obstacle was the lack of root formation as only 5 % of individuals rooted when planted in soil after the *in vitro* phase (Raatikainen et al., 1990). Therefore, it was essential to find a way to induce root formation already while in micropropagation. After lowering the nutrient content in Agar by half and adding 2.5 mg/l Indole-3-butyric acid (IBA) 50 % of individuals produced roots which was regarded as major breakthrough (Raatikainen et al., 1990). Today the 1/2 Murashige and Skoog solid medium together with 6-Benzylaminopurine (BAP) 0.1 mg/l and 1-Naphthaleneacetic acid (NAA) 0.05 mg/l are successfully used in micropropagation of *R. saxatilis* (A. Hämäläinen, pers. comm.).

After this tedious fine-tuning the method started to work also for *R. humulifolius* and a total of 1500 plants were produced *in vitro* by 1988. These were then re-introduced in the wild in Sallaajärvi, Jyväskylä (62°12'N, 25°36'E) *c.* 5.5. km from the original site. This site was obviously carefully chosen to match the niche of the species and the population still exists in the location. In order to ensure its survival, the area was made a nature reserve in 1989 and the population is monitored in regular intervals. According to the census in 2009 there are 125 individual plants growing in the area (Kypärä, 2012). However, recent observations in the area seem to indicate that the population has declined since then (Johanna Hallman, pers. comm.). Nevertheless, this is the first time in history that an endangered plant population has been saved by micropropagation and successfully reintroduced in the wild (Raatikainen et al., 1990).

As stated above *R. humilufolius* originating from the Finnish wild population is also *ex situ* conserved in the botanic gardens of Helsinki, Oulu and Jyväskylä Universities (Fig. 1). During a 2012–2017 EU-Life +-funded project ESCAPE *ex situ* conservation of Finnish endangered plant species was improved in a multitude of ways (Hyvärinen, 2017). During the course of the project a method for cryopreservation of *R. humulifolius* was developed (Edesi, Tolonen, Ruotsalainen, Aspi, & Häggman, 2020) and material from the *ex situ* conserved plants has been cryopreserved ever since. Hence, the existence of the genetic material from the westernmost population of the species is secured by several conservation methods and therefore its extirpation looks very

Fig. 1. A flowering individual of *Rubus humulifolius* growing in near natural conditions in Kumpula Botanic Garden in Helsinki, Finland.

unlikely. However, the future of the extant wild population of *R. humulifolius* seems still uncertain.

3. Discussion and lesson learned

The history of the Finnish *Rubus humulifolius* population is a lucid example of conservation actions that may take place in circumstances where conservation may not be in accordance to laws and regulations or legislation covering emergency procedures may be completely absent. In this case local authorities ignored the protected status of the species, which clearly made rescue operation ethically sound if not necessarily legal. One should also note that *ex situ* conservation has only recently become recognized in legislation in many countries and it is still not a standard procedure in plant conservation toolbox everywhere.

In Finland *R. humulifolius* has been red-listed since the assessment of the conservation status of species started in 1986. However, its classification has changed in two decades from extinct in wild (EW) in 2000, and regionally extinct (RE) in 2010 to critically endangered (CR) in 2019 (Ryttäri et al., 2019). In spite of having a growing introduced population in the wild since 1988 the species was long regarded as extinct in Finland because of its "cultivated origin" (Raatikanen, 1997). However, in the most recent evaluation (Ryttäri et al., 2019) the species was regarded as a fully naturalized in its present habitat, and hence, classified as Critically Endangered (CR).

The above change in status reflects a change in the premise how benign conservation introductions are viewed. Reintroduction is defined as "*intentional movement and release of an organism inside its indigenous range from which it has disappeared*" (IUCN/SSC, 2013). Moreover, according to the most recent guidelines by IUCN (IUCN Standards & Petitions Subcommittee, 2019) the IUCN categorization process designed for the assessment of the conservation status of wild taxa should apply to introduced populations if a) the intent of the introduction is was to reduce the extinction risk of the taxon being introduced, b) the introduced subpopulation is geographically close to the natural range of the taxon, c) the introduced subpopulation has produced viable offspring, and d) at least five years have passed since the introduction. It is evident that all categories apply to the extant *R. humulifolius* population in Finland.

Nevertheless, the questions of genetic variability and representativeness of the extant *in situ* population and *ex situ* populations in Finnish botanic gardens in relation to the ones located in the main area of distribution still remain. As these Finnish populations have passed through two genetic bottlenecks, the first one in dispersal and the second one in population decline and the subsequent rescue operation the genetic variation in them may be diminished. Moreover, *ex situ* conservation in living collections of botanic gardens representing three divergent climates may entail different selection pressures compared to the ones in wild, and that might have imposed microevolutionary changes. There has been discussion whether *in situ* conservation allowing continued evolution of wild biota in rapidly changing environments should be preferred over *ex situ* conservation that is often more secure but may either halt microevolution (*e.g.* cryopreservation) or in some forms impose maladaptation (*e.g.* living collections) to conditions in nature (*e.g.* Nagel, Durka, Bossdorf, & Bucharova, 2019).

Based on the historical records the population of *R. humulifolius* in Finland was associated with similar plant community than in the main distribution area in Siberia (Mikkola, 1927). This community reflects specific circumstances such as relatively high humidity and nutrient-rich soil. Moreover, within its narrow niche the species seems to be a good competitor largely owing to its ability to spread by rhizomes (Mikkola, 1927). Apparently, individuals of R. *humulifolius* function as part of the ecosystem also altering its structure and adding to its functional and genetic diversity as well as to the beta diversity of plant communities.

Even though the reintroduced population has undergone several genetic bottlenecks and potentially maladapted to natural conditions during the long *ex situ* conservation period, the reintroduction has so far turned out to be a successful attempt for de-extinction of this locally extinct species. One can speculate that the *ex situ* site in the cottage garden (see Kypärä 2020) provided an environment that resembled the original one and thus hindered maladapatation or "domestication". Similarly, any attempt for plant de-extinction using proxies may benefit from the use of near-natural conditions in *e.g.* botanic gardens for testing the adaptivity of the chosen proxy/proxies and conditioning the for the wild.

The question of the origin of the Finnish population of *R. humulifolius* should also be addressed. For that and the genetic variability question it would be advisable to carry out thorough molecular studies and phylogeographic analyses combined with a general estimate on how much genetic variation does the Finnish population harbour compared to the whole population. This would, however, require extensive sampling throughout the distribution area which may not be always feasible. However, a comparative genetic or even genomic study between the Finnish population and Western Russian ones that are better connected to the main distribution area would also shed much light on this issue.

In order to unveil the reasons for the demise of the extant population not only census data but demographic data should be collected potentially combined with some management of the environment. These data could then be analysed, for instance, as a life-table response experiment (Caswell, 2001) or some species-based approach for plant conservation such as SHARP (Systematic Hazard Analysis of Rare-Endangered Plants) put forward by Aronne (2017). These approaches could potentially highlight what are the demographic bottlenecks and could these be alleviated by management actions or more refined selection of the habitat for potential new re-introductions of the species.

Declaration of Competing Interest

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

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