

Preserving the only endemic vascular plant taxon in Belgium in a highly anthropogenic landscape

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Background and aims – *Sempervivum funckii* var. *aqualiense*, considered the only endemic vascular plant taxon of Belgium still existing in the wild, is threatened by urban development. There is a need for proposing appropriate *in situ* or *ex situ* conservation strategies through a precise evaluation of population demographic and genetic characteristics.

Methods – We investigated demographic dynamics for six years (rosette density, floral production, reproductive success, seed germination and seedling recruitment), pollination (visiting insects and potential pollinators) and genetic diversity using ISSR markers of the unique population of *S. funckii* var. *aqualiense*.

Key results – Visiting insects and potential pollinators, especially common and generalist pollen foraging bees, are abundant in the population of *S. funckii* var. *aqualiense* and the plant seems highly attractive. Pollination limitation does not represent a conservation issue. Despite a good floral production in most years (250–1,558 flowering ramets, comprising 4–36 flowers), viable seed production and germination represent very rare events (0.05% viable seeds and 1 seedling) because of meiosis abnormalities related to the hybrid origin of the taxon. Recruitment by clonal propagation (vegetative rosettes) has been detected. Genetic diversity is very low ($H = 0.0148$), and may have originated from somatic mutations, related to extensive vegetative propagation, but is also compatible with relictual segregating sexual reproduction.

Conclusions – The most cost-effective method for establishing new populations is the transplantation of rosettes. Given the low viable seed production, creating an *ex situ* seed bank requires collecting seeds for many nonconsecutive years. Conserving the rare genotypes can be done by preserving seeds and as many rosettes as possible. The preservation of the only existing population in its entirety appears to be the best option for long-term sustainable conservation of *S. funckii* var. *aqualiense*, and any partial destruction of the population should be avoided.

Key words – Clonal propagation, demography, genetic diversity, *in situ* conservation, pollinators, *Sempervivum funckii* var. *aqualiense*.

INTRODUCTION

Preserving endemic plant taxa in highly anthropogenic landscapes in densely populated countries can be a challenge. In Europe, the remaining wild habitat fragments are usually small and embedded in intensive agricultural and highly urbanized matrices, which have become unsuitable for the survival and dispersal of many species (Essl et al. 2013). Plant populations occurring in these fragments incur many

constraints related to human activities: increased eutrophication and pollution, pesticides and recreational use. These pressures can lead to vegetation trampling, disturbance and degradation (Godefroid & Koedam 2003). Moreover, the preservation of natural remnants often conflicts with the development of human infrastructures, such as roads, buildings, tourist resort development, and mining activities. In the case of endemics, anthropogenic activities may directly threaten the few existing populations (Faucon et al. 2012,

Khan et al. 2012, Tepedino et al. 2012, Ronse et al. 2015), even leading to extinction in the wild (Maunder et al. 2000, Rucińska & Puchalski 2011). However, they may also create secondary habitats, especially for pioneer taxa (Bizoux et al. 2011, Faucon et al. 2012).

Different conservation strategies of endemics can be proposed: (1) protection and management of existing populations, (2) relocation to other sites, and (3) *ex situ* conservation through seed banking or maintenance in living plant collections. Protection of the known populations is the priority, but additional conservation efforts may be needed to recover suitable habitat conditions, especially when habitat quality has been degraded. *In situ* ecological management practices, often replacing traditional agro-pastoral practices, aim at restoring or maintaining habitat quality and increase population sizes of habitat specialists (Miller & Hobbs 2007, Harzé et al. 2015). If restoring habitat quality of existing sites is not possible, relocation to other sites suitable for the target species may be an alternative solution (Volis 2016). However, when populations are genetically depauperate or suffer inbreeding depression because they are too small and spatially isolated, and when the seed rain and soil seed bank fail to provide recruitment of new individuals, the usual ecological management may be not sufficient for recovering viable populations despite restored suitable habitat conditions (Oostermeijer et al. 2003, Volis et al. 2005, Van Geert et al. 2008, Van Rossum 2008, Berjano et al. 2013). *Ex situ* conservation measures complementary to the usual *in situ* conservation methods are increasingly used or recommended (Volis & Blecher 2010, Weeks et al. 2011), provided they follow strict protocols (e.g. Vander Mijnsbrugge et al. 2010, Enßlin et al. 2011, Basey et al. 2015, Godefroid et al. 2016). Seed collections in the wild may be used to create new populations and to constitute an *ex situ* seed bank and living plant collections, which may allow further demographic and genetic restoration and/or genetic rescue of senescent or partially destroyed populations by reinforcement and of extinct populations by reintroduction (e.g. Maunder et al. 2000, Bottin et al. 2007, Colas et al. 2008, Alonso et al. 2014, Zavodna et al. 2015).

The success of these conservation methods depends on species life-history traits, such as the reproductive system, seed production, viability and dispersal abilities, clonal propagation ability, genetic variation patterns and ecological niche requirements (Montalvo et al. 1997, Godefroid et al. 2016, Reiter et al. 2016). For instance, knowledge of population genetic diversity and structure can significantly contribute to define appropriate seed sampling for *ex situ* conservation and subsequent rescue (Gray 1996, Volis & Blecher 2010, Alonso et al. 2014, Van Geert et al. 2015). Successful reintroductions or relocations require transplanting populations large enough to be viable on the long term. The minimum viable population size needed depends on species reproductive biology, e.g. mating system, pollination processes and seed production, and on population demographic dynamics, especially rejuvenation through seedling recruitment, but also clonal ability (Menges 2008, Godefroid et al. 2011, Weeks et al. 2011). For instance, to optimize seed production and avoid genetic drift and S-Allee effects, conserving or rescuing populations of insect-pollinated plant species with a self-incompatible reproductive system requires increasing

mate availability by using several seed sources and transplanting large and dense flowering populations (Bottin et al. 2007, Colas et al. 2008, Fant et al. 2013, Melen et al. 2016). Also, as they are obligate outcrossers, it necessitates conserving or restoring plant-pollinator interactions (Aguilar et al. 2006, Menz et al. 2011). Clonal propagation can contribute to population persistence by increasing genet longevity, and allow somatic mutations propagation and accumulation of somatic mutations on the long term (de Witte & Stöcklin 2010, Gross et al. 2012). The role of clonal propagation is particularly important for population persistence if sexual reproduction and seedling recruitment are reduced, in case of extreme ecological conditions such as fire-prone or metaliferous habitats or as a result of sterility or of pollination disruption in highly fragmented habitats (Wolf et al. 2000, Gross et al. 2012, James & McDougall 2014).

Sempervivum funckii F.Braun ex Koch var. *aqualiense* E.Morren (Crassulaceae) is considered the only endemic vascular plant taxon of Belgium still existing in the wild (Lambinon & Verloove 2012). This succulent plant taxon is typical of rupicolous calcareous or basophilic grasslands (*Alyso alyssoidis*-*Sedion albi* Müller 1961), a EU priority habitat according to the Directive 92/42/EEC (code 6110). It currently occurs as a single population, located on the side of a cliff, above a road, close to the town of Aywaille, along the Amblève river, a tributary of the river Meuse, in southern Belgium (Beaujean 1997). The taxon was mentioned for the first time in a floristic report in 1813 (as *S. montanum minus*). In 1873 it was described based on morphological characters as a variety of *S. funckii* F.Braun ex Koch, however differing from the originally described *S. funckii* by longer flower stalks, longer (4–7 cm) and leafy stolons and ligulate leaves (Morren 1873a, 1873b, Beaujean 1997). *Sempervivum funckii* is considered a hybrid (*S. arachnoideum* × *montanum* × *tectorum*) of horticultural origin, naturalized in Central Europe (originally in Austria, since extinct, but also reported in Germany), but there is no recent verification of its taxonomical status (Lloyd Praeger 1932, Kühn & Klotz 2002, 't Hart et al. 2005).

The taxonomical status of the population ascribed to *S. funckii* var. *aqualiense* also remains obscure. A karyological study has shown that the taxon present in Aywaille is of hybrid origin (Van Rossum et al., unpubl. res.). Phylogenetic analyses (Raspé et al., unpubl. res.) based on ITS and four plastid markers (*rps16* intron, *rpl16* intron, *trnT-trnE* spacer, and *ycf6-trnC* spacer), including the potential parental *Sempervivum* species and naturally occurring hybrid specimens, such as *S. × fauconnetii* from the Jura Mountains (no sample could be obtained from naturalized populations of *S. funckii* from Germany), gave no clear differentiation patterns among species and hybrids (see also Klein & Kadereit 2015). They also did not allow elucidating the taxonomical status of the Aywaille population and whether the population is of horticultural origin or is a Medio-European relict as reported for other rare species present in the site (Saintenoy-Simon 2005). Indeed, many thermophilous Medio-European or Mediterranean species (e.g. *Artemisia campestris* L. subsp. *campestris*, *Galatella linoisyris* (L.) Rechb.f., *Campanula patula* L., *Helianthemum apenninum* (L.) Mill., *Silene viscaria* (L.) Jess.) reach the western or the northern margin, often disjunct, of

their distribution range on the rocky cliffs along the Meuse and its tributaries (Vanden Berghen 1955, Lawalrée 1956, De Sloover & Dufrêne 1998). Nevertheless, the taxon named *S. funckii* var. *aqualiense* is legally protected in Belgium and has a patrimonial value for the Belgian botanists and conservationists (Beaujean 1997, Saintenoy-Simon et al. 2006), becoming an umbrella taxon for its habitat and for the other rare and protected species co-occurring in the site (e.g. *Artemisia campestris*, *Galatella linosyris*, *Campanula patula*, *Silene viscaria*; Saintenoy-Simon 2005).

Although the taxon is protected and the site consists of a protected and well-managed natural reserve, the proximity of urbanized and human activities (road traffic) might compromise its long-term persistence. Indeed, the cliff is quite unstable, leading to a risk of stones falling onto the road or traffic, which has led to the closure of the road, displacing traffic through the town of Aywaille. Several projects for stabilizing the cliff are under consideration, which could harm the vegetation, including the only existing and protected population of *S. funckii* var. *aqualiense*. In the frame of these projects several strategies for preserving this unique endemic taxon have been proposed to compensate a partial or total destruction of the population, such as creating new patches by plant translocation or by moving the topsoil containing the soil seed bank (Guillitte 2004a, 2004b). However, nothing is known about its reproductive biology and genetic diversity, which are key factors to evaluate whether or not the population can recover after disturbance (Montalvo et al. 1997), and whether reconstituting the population afterwards might be an option. To answer to these conservation issues, we investigated the demographic dynamics for several years (rosette density, floral production, reproductive success, seed germination and seedling recruitment), pollination (visiting insects and potential pollinators) and genetic diversity of the population of this unique taxon. We discuss the implications of our results for the preservation of this taxon and propose practical conservation recommendations.

MATERIAL AND METHODS

Study taxon and population

Sempervivum funckii var. *aqualiense* is an insect-pollinated perennial herb forming dense basal rosettes of succulent leaves (Morren 1873a, 1873b, electronic appendix 1). It flowers in June and July, one rosette (ramet) producing one inflorescence. Its pink flowers produce nectar. The fruit consists of a group of follicles, producing very small, dust-like, seeds. The species propagates clonally by forming lateral vegetative rosettes. *Sempervivum funckii* as well as *S. arachnoideum* L., *S. montanum* L. and *S. tectorum* L. are considered self-compatible. Flowers are protandrous, but autogamy can reportedly occur (Knuth 1908, Kühn & Klotz 2002). The taxon named *S. funckii* var. *aqualiense* only occurs in the natural reserve of Heid des Gattes (Aywaille, Belgium; 50°28.74'N, 5°41.35'E), which comprises one of the last natural sandstone cliffs of the region (Beaujean 1997). It is typically restricted to thin beds of clayey calcareous sandstone. A second site close to the first one was lost when exploited as a quarry. The population extends along 35 m of the cliff, and covers about 400 m², albeit in patches. Management of Heid

des Gattes consists of preventing forest recolonization of the cliff by tree cutting. The site has also been grazed by goats for decades.

Demography

Vegetative rosette density was measured in September 2011 as the number of rosettes in 25 randomly selected 10 cm × 10 cm plots covering the whole population area. Floral production was measured during six consecutive years (from 2007 to 2012), at the end of the flowering peak (end of June–beginning of July), based on two variables: (i) flowering population size was estimated by counting the number of flowering ramets (flowering rosettes) over the whole population, and (ii) ramet floral display by counting the number of flowers on 30–64 inflorescences randomly distributed across the population. A one-way ANOVA and Tukey HSD tests were performed using Statistica 12.6 (Statsoft 2015) to test for differences in ramet floral display between years (after testing for normality). To estimate reproductive success, two fruits with closed follicles were sampled from each of 15 randomly selected fruiting ramets at the end of July 2012 and kept in a 70% ethanol solution. For each fruit the number of follicles was counted, and for five follicles per fruit, the number of mature (filled) seeds, aborted (filled but not completely developed) seeds and unfertilised ovules (empty seeds) were counted using a stereo microscope (electronic appendix 1). Climatic data (e.g. precipitation) for the period 2007–2012 were available for the Bierset meteorological station, 25 km from Aywaille (provided by the Royal Meteorological Institute of Belgium).

Ex situ and in situ germination experiments

The population was visited every year (from 2007 to 2012) in March–April to search for seedlings. Seeds were collected from ripe fruits of 12 randomly selected infructescences in October 2007 and 2008 and August 2009. Seeds were too small to conduct a tetrazolium chloride viability test. When not conserved in an ethanol solution it was also difficult to distinguish between mature, aborted and empty seeds. We tested germination in Meise and Kew Botanic Gardens, using treatments known to be successful for other *Sempervivum* species (Royal Botanic Gardens Kew 2015). In Meise, two replications of 50 seeds (unsorted seeds) were sown for each of 11 infructescences in October of the same year under different controlled conditions: (i) on 1% agar (10 g/l) poured into Petri dishes (following Davies et al. 2015) left for three months in germination incubators at 22°C and 12/12 photoperiods with or without a cold stratification at 5°C for 56 days; (ii) in 2008 and 2009: on soil in separate cold (min. 6°C) and warm (min. 18°C) greenhouses, with or without a cold stratification for 56 days, left for one year. In Kew, two replications of 16–25 seeds from each of six infructescences were placed on agar poured into Petri dishes and imbibed slowly in high humidity conditions overnight and then placed into four conditions at 8/16 (light/dark) photoperiods for 56–84 days: (i) at 15°C; (ii) at 15°C with gibberellic acid (GA₃; 250 mg/L); (iii) at 15°C with KNO₃ (101 mg/L); (iv) at 5°C for 84 days, then 15°C.

An *in situ* germination experiment was carried out, by sowing seeds from a bulk (unsorted seeds) sampled from 30 infructescences (~10,000 seeds per plot) in August 2010 in three 1.60 m² plots located within the population, in seemingly suitable habitat conditions (i.e. presence of a mix of rock outcrops and shallow soil, corresponding to thin beds of clayey calcareous sandstone), but where the species was absent. The plots were examined for seedlings in October 2010 and in several periods of the year in 2011 and 2012.

Visiting insects and potential pollinators

We evaluated potential problems with flower visitation and pollination of *S. funckii* var. *aqualiense* by testing the attractiveness of this plant to potential pollinators. Visual observations of visiting insects were made on two groups of flowering ramets, at the flowering peak in June 2011 during two time periods (10:30–12:00 on 15 Jun. 2011 and 14:55–16:50 on 27 Jun. 2011). Visual determinations were based on morphology and behaviour. After visual observations, 21 specimens of dominant insect visitors were collected by net for further species identification in the laboratory (Laboratory of Zoology, University of Mons). Determination was based on their morphology following various keys (e.g. Patiny & Terzo 2010 for the bee genus *Andrena*). Pollen was collected

from the body of pollen foragers to determine which taxa could be considered pollinators of *S. funckii* var. *aqualiense*.

Genetic diversity

Leaf material was collected in April 2007 from 46 rosettes along a transect covering the whole population, and at least 1 m apart to reduce the clonality effect and obtain an accurate estimate of genetic diversity. All leaves were conserved at -80°C until DNA isolation, which was performed using a CTAB protocol (Doyle & Doyle 1990).

Inter Simple Sequence Repeats (ISSRs) were amplified first using 9 primers with only one selective nucleotide. In a second step, 6 primer combinations (electronic appendix 2) were used to increase the number of amplified bands. Cycling conditions were the same for all primers and primer combinations: 3 min at 94°C, followed by 35 cycles consisting of 30 s at 94°C, 30 s at 50°C, and 90 s at 72°C, and by 7 min at 72°C. The 25 µL amplification mixture contained 12.88 µL ultrapure water, 2.5 µL 10 X reaction buffer, 200 µM dNTPs (Fermentas), 200 µg mL⁻¹ Bovine Serum Albumine (Fermentas), and 0.625 U DreamTaq® polymerase (Fermentas). A positive and a negative control were added to each PCR. ISSRs were separated by electrophoresis on 1.5% agarose gels in 0.5 X TBE buffer, at 100 V for 3 h, and visualized under UV light with ethidium bromide. The PCR positive control was loaded along with the other samples in each of row of wells and used as a reference sample for scoring the bands.

Measures of genetic diversity (PLP, the proportion of polymorphic loci and *H*, Nei's (1973) gene diversity) were estimated using Popgene 1.32 (Yeh & Boyle 1997).

RESULTS AND DISCUSSION

Demography

The mean number of vegetative rosettes per 10x10 cm plot was 29.1 (SE = 1.4; range 20–57). So, on average, a density of 2,910 rosettes can be found per m², which means that the population consists of more than 400,000 rosettes. The flowering population size (number of flowering ramets) ranged from 250 (in 2008) to 1,558 (in 2010; fig. 1). Ramet floral display, i.e. the number of flowers per flowering ramet (fig. 1), ranged from 14.8 (in 2009) to 16.8 (in 2012) on average (SE = 0.9–1.1; ranging 4–36 flowers), except in 2008 (mean ± SE = 3.3 ± 0.5, ranging 1–16 flowers), when it was significantly lower (ANOVA: $F_{(5,260)} = 36.09$, $P < 0.001$); Tukey HSD test: $P < 0.001$ than in the other years (Tukey HSD tests between the other years: $P > 0.05$). This difference might be related to a particularly rainy spring in 2008 (precipitation from March to May: 308 mm in 2008 against 80 to 213 mm in the other years). Fruits had 10–13 follicles (mean ± SE = 11.1 ± 0.2), each of which initially contained 25–58 ovules (mean ± SE = 38.6 ± 0.6). From the 5,590 seeds counted from 30 fruits (323 follicles), only 3 (0.05%) were fully developed and 4 (0.07%) were aborted, the other seeds were all empty (unfertilised). This means that the annual viable seed production varied from 85 (in 2008) to 2,579 (in 2010). Despite a good floral production in most years, reproductive success in the Aywaille population is thus extremely low. This is not

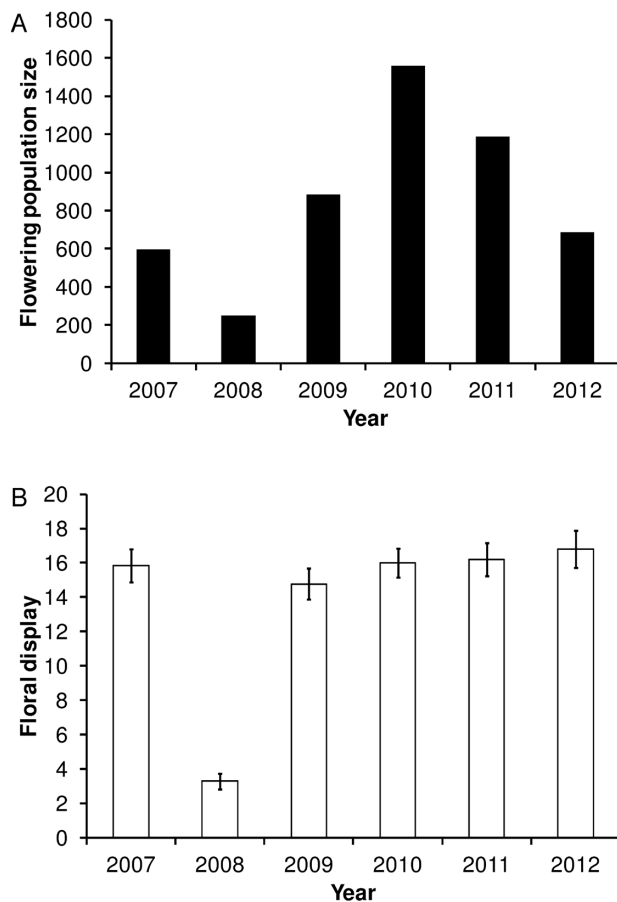


Figure 1 – Floral production in the Aywaille population of *Sempervivum funckii* var. *aqualiense* from 2007 to 2012: A, flowering population size; B, ramet floral display (mean ± SE).

Table 1 – Floral visitors of *Sempervivum funckii* var. *aqualiense*.
n, number of observed individuals.

Family / Species	<i>n</i>	Sex	Pollen collection of <i>Sempervivum</i>
Andrenidae / <i>Andrena bicolor</i> (Fabricius, 1775)	3	Female	Yes
Apidae / <i>Apis mellifera</i> (Linnaeus, 1758)	5	Female (worker)	No
Apidae / <i>Bombus lapidarius</i> (Linnaeus, 1758)	2	Female (worker)	No
Apidae / <i>Bombus terrestris</i> (Linnaeus, 1758)	1	Female (worker)	No
Apidae / <i>Nomada</i> sp.	1	Female	No
Halictidae/ <i>Lasioglossum</i> sp. [<i>L. leucopus</i> (Kirby, 1802), <i>L. nitidulum</i> (Fabricius, 1804), <i>L. punctatissimum</i> (Schenck, 1853)]	18/25	Female/male	Yes/No
Megachilidae / <i>Anthidium oblogatum</i> (Illiger, 1806)	1	Male	No
Megachilidae / <i>Anthidiellum strigatum</i> (Panzer, 1805)	2	Female	Yes
Megachilidae / <i>Megachile</i> sp.	1	Female	Yes
Coleoptera	8	?	No
Lepidoptera / <i>Macroglossum stellatarum</i> (Linnaeus, 1758), <i>Pieris rapae</i> (Linnaeus, 1758)	2	?	No

surprising, given the hybrid origin of the taxon. Indeed, abnormalities during meiosis of pollen mother cells have been observed, leading to the production of a highly variable proportion (0.17–0.63) of viable pollen grains (Van Rossum et al., unpubl. res.).

Ex situ and *in situ* germination experiments

No seedlings were found in either the whole population during the six years of the study or the three *in situ* sown plots in 2011 and 2012. However, three rooted rosettes were found in plot 2 and one in plot 3, indicating dispersal by clonal propagation. Indeed, detached rosettes can re-root where they have fallen if the habitat conditions are suitable. From the different germination experiments in controlled conditions, only one seed sown on soil in October 2009 (cold stratification, warm greenhouse) germinated in the spring 2010, developing into a healthy rosette (electronic appendix 1). Such extremely low germination rate is in agreement with the very low viable seed set. However, this finding also means that the very few viable seeds produced can lead to viable progeny, although our results indicate that clonal propagation rather than sexual reproduction by seeds is the main mechanism for population persistence, as reported for the potential parental species *S. tectorum* and *S. arachnoideum* (Kühn & Klotz 2002). Other narrow endemic taxa were also found to propagate mainly, or exclusively, asexually, such as the clonal *Calystegia collina* (Greene) Brummitt (Wolf et al. 2000), the apomictic *Limonium barceloi* Gil & L.Llorens (Khan et al. 2012) and the sterile shrub *Grevillea renwickiana* F.Muell. (James & McDougall 2014).

Visiting insects and potential pollinators

Sempervivum funckii var. *aqualiense* has a large, simple, and open flower, and so is expected to attract a wide diver-

sity of non-specialized pollinators. Our observations confirm that *S. funckii* var. *aqualiense* is pollinated by insects known to have generalist foraging behaviour (e.g. bumble bees; Somme et al. 2015). Its attractiveness to potential pollinators was also very high. After only 3.5 h of observations we recorded a high diversity of floral visitors in the orders of Coleoptera, Lepidoptera and Hymenoptera. Pollen foragers belonged to Hymenoptera including four families and 11 species, especially small solitary bees belonging to *Lasioglossum*, *Andrena* and *Halictus* genera, but also larger bees (*Anthidium* and *Megachile*) and the social bees *Apis mellifera* and *Bombus lapidarius* (table 1). We found *S. funckii* var. *aqualiense* pollen on the bodies of all pollen foragers, which therefore could be considered as pollinators. *Macroglossum stellatarum* and *Pieris rapae* were not pollinators as they only collected nectar without touching the anthers.

Genetic diversity

The number of consistently scorable ISSR bands per primer or per primer combination ranged from 2 to 6 (electronic appendix 2) and the proportion of polymorphic loci was very low (PPL = 0.059 for single primers and 0.039 for primer combinations). In total, only two polymorphisms were observed over the 60 bands scored. Each polymorphism was observed in only one of the sampled rosettes. Nei's gene diversity estimate *H* was 0.0148 (SD = 0.0600), with the population assumed to be at Hardy-Weinberg equilibrium. In case of inbreeding, *H* would be even lower (when $F_{is} = 0.5$, $H \pm SD = 0.0047 \pm 0.0191$). The extremely low level of polymorphism in the population may have originated from somatic mutations, related to extensive vegetative propagation, but it is also compatible with relictual sexually segregating genetic diversity (Ellstrand & Roose 1987, Klekowski 1997). Somatic mutations can be a source of genetic diversity, especially when sexual reproduction is disrupted (Gross et al.

2012), as reported for the rare sterile clonal shrub *Grevillea renwickiana* (James & McDougall 2014) and for the clonal serpentine endemic *Calystegia collina* (Wolf et al. 2000).

Conclusions

Visiting insects and potential pollinators are diverse and abundant in the population of *Sempervivum funckii* var. *aqualiense*. The flowers seem highly attractive for common and generalist bees like *Lasioglossum* species and honey bee workers. These pollinators are not under any particular threat in this region (Nieto et al. 2015). Pollination limitation, therefore, does not represent an issue for the conservation of the plant taxon *in situ*, unlike other narrow endemic species, such as the insect-pollinated self-incompatible *Convolvulus lineatus* L. (Berjano et al. 2013) and the moth-pollinated self-compatible *Silene sennenii* Pau (Martinell et al. 2010). No pollination limitation was found for the bee-pollinated self-incompatible *Physaria obcordata* Rollins (Tepedino et al. 2012).

Viable seed production and seedling recruitment represent very rare events, and establishment and dispersal of new rosettes within the population mainly occur by clonal propagation. Creating new patches or populations can thus better be achieved by vegetative propagation and transplantation of rosettes. Given the low number of viable seeds produced each year, creating an *ex situ* seed bank that could be used for *ex situ* plant propagation and further (re)introduction will require collecting viable seeds (max. 20% the total production to avoid to negatively impact the population) over at least ten (non-consecutive) years to reach the minimal required number of 5,000 viable seeds (ENSCONET 2009). Using removed topsoil for population restoration after its destruction as proposed by Guillitte (2004a, 2004b) is not pertinent for *S. funckii* var. *aqualiense* as the presence of a soil seed bank is unlikely. The cliff wall on which this taxon occurs is one of the last areas of clayey calcareous sandstone that have not been exploited by mining activities (Saintenoy-Simon 2005). Whether other sites might provide suitable habitats still needs to be tested. Given the very low genetic diversity, however estimated from a restricted part of the genome, conserving the rare genotypes can be done by preserving as many rosettes as possible, but also by preserving seeds. Further genetic analyses on a larger number of rosettes and using molecular markers known to be highly polymorphic such as nuclear microsatellites might help to better capture the genetic diversity present in the population. Nevertheless, identifying very rare genotypes in the field might be difficult given the high number of rosettes and low seed production, and enough lateral vegetative rosettes need to be present to allow sampling. Therefore, the preservation of the only existing population in its entirety appears to be the best option for long-term sustainable conservation of *S. funckii* var. *aqualiense*, and projects that might partially destroy the population should be halted. This study exemplifies the need for prioritizing the preservation of *in situ* populations of endemic taxa, even in highly anthropogenic landscapes.

SUPPLEMENTARY DATA

Supplementary data are available in pdf at *Plant Ecology and Evolution*, Supplementary Data Site (<http://www.ingentaconnect.com/content/botbel/plecevo/supp-data>), and consist of the following: (1) photographs of *Sempervivum funckii* var. *aqualiense*; and (2) number of scorable bands and polymorphism observed for each ISSR primer and primer combination among 46 rosettes of *Sempervivum funckii* var. *aqualiense*.

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