Aquatic Botany 136 (2017) 186–196

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

# Genetic structure of the submersed *Ranunculus baudotii* (sect. *Batrachium*) population in a lowland stream in Denmark

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A R T I C L E I N F O

*Article history:*
Received 6 July 2016
Received in revised form 14 October 2016
Accepted 22 October 2016
Available online 24 October 2016

*Keywords:*
Hydrochory
Sexual reproduction
Hybridization
Plant fragmentation
ITS
AFLPs

A B S T R A C T

Unidirectional hydrochory, reproduction mode and mating system have different impacts on the ecology of stream plants, and variation in the combination of these processes shapes unique genetic patterns which can have different consequences for the fitness of stream populations.

We studied the genetic structure inferred by AFLPs, ITS SNPs and *matK* sequences of three populations of the common clonal macrophyte *Ranunculus baudotii* in River Aarhus in order to understand the role of vegetative and sexual reproduction in the stream and how genetic variation is distributed along the stream. We also explored the genetic relationships of the *R. baudotii* population in River Aarhus with other species of sect. *Batrachium*, because hybrids have been identified in the stream and they might affect the structure of the populations.

The three studied populations were genetically distinct despite low genetic diversity and plant fragmentation is likely the main form of reproduction in the river system. The establishment of a few seeds, sexual recombination, the occurrence of putative hybrids and genetic drift in the establishment of fragments and seeds dramatically changed the genetic diversity of the populations along the stream and led to differentiation among populations. Further research is needed to understand the conditions favoring sexual reproduction and gene flow distances.



## 1. Introduction

Due to unidirectional flow riverine plant populations tend to approach isolation by distance along the stream. Gene flow distances and directions, reproduction mode and upstream propagule sources (Boedeltje et al., 2003) therefore play a primary role in shaping genetic diversity and structure and, ultimately, fitness of aquatic plant populations. Several hypotheses have been formulated to understand dispersal dynamics along streams, but variation in empirical patterns among species and streams suggests that species-specific dispersal traits and river characteristics are also important. In general, metapopulations are more common than isolated populations among riverine plant populations. Examples of metapopulations are the riparian *Silene tatarica* (Tero et al., 2003), the submersed *Sparganium emersum* (Pollux et al., 2009) and the emergent *Hymenocallis coronaria* (Markwith and Scanlon, 2007). Isolation, when occurring, is over long distances (about 25 km in *Nuphar lutea* – Fer and Hroudova, 2008) and 50 km in *Sparganium emersum* (Pollux et al., 2007), and is often incomplete, as seen for the endemic populations of *Myricaria laxiflora* in the Yangtze River where occasional long-distance dispersal of seeds maintained gene flow among genetically differentiated populations (Liu et al., 2006). Vegetative reproduction is also an important dispersal mode for riverine plants, in particular for submersed macrophytes that are easily fragmented in the stream. As suggested by Pollux et al. (2007), the hydraulic force from water currents has an impact on macrophyte morphology because submerged plants tend to reduce mechanical damage by reducing plant size and achieving a more compact growth habit. This plasticity may have consequences for the ability of the plants to emerge from the water and, thus, for the success of sexual reproduction in the fastest flowing parts of the stream, especially for plants that rely on wind-mediated pollen dispersal (Pollux et al., 2007). Although seeds are believed to travel long distances along the stream and across catchments, and fragments, on average, are not dispersed as far as seeds (Santamaria, 2002), many invasive macrophytes, such as *Elodea canadensis*, *Egeria densa*, *Lagarosiphon major* (Lambertini et al., 2010), and *Alternanthera philoxeroides* (Geng et al., 2007), after their introduc-

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http://dx.doi.org/10.1016/j.aquabot.2016.10.007
0304-3770/

tion to new continents, have been able to found continent-wide metapopulations with plant fragments. Monoclonal populations may therefore also occur among stream populations, and vegetative reproduction may be important for the establishment of new populations and the colonization of new habitats.

Self-fertilized plants are over-represented among stream plants (Barrat-Segretain, 1996), with cases ranging from partial selfing, like the riparian mountain populations of *Mimulus caespitosus* (Ritland, 1989), to cleistogamy (Barret et al., 1993). In different species of *Ranunculus* sect. *Batrachium*, cleistogamous flowers are set under the water surface and pollination can occur in a bubble of air within the closed perianth of the flower bud (Barrat-Segretain, 1996). In additional *Batrachium* species also produce chasmogamous flowers above the water which are pollinated by wind and by beetles (De-yuan, 1991). In combination with clonal growth, self-compatibility can be an advantage for insect pollinated species as the increased floral display of the clonal patch can attract more pollinators (Honnay and Jacquemyn, 2008) and facilitate geitonogamy (pollination among different flowers of the same clone) (Charpentier, 2002). Selfing generates more genetic diversity than vegetative reproduction, although still limited compared to that produced by outcrossing, and may maintain genetic diversity and seed set in isolated populations, increasing fitness and the ability of long-distance dispersal. However, the benefit is often only temporary as selfing is likely to lead to inbreeding depression (Schemske and Lande, 1985).

Interspecific hybridization is also common among stream plants with floating flowers, and some genera like, for instance, *Potamogeton* and *Ranunculus*, have several interbreeding species. These genera include groups of very closely related species between which hybrids may form in many combinations (Hylander, 1982). *Ranunculus* sect. *Batrachium* (Emadzade et al., 2010, 2011) is one of such species complex and one of the most difficult groups of aquatic species from a taxonomic point of view. The taxonomic challenge is due to the fact that these species may be distinct in some parts of the distribution range whereas limits can be blurred in others because either of intraspecific phenotypic variation or interspecific hybridization which may occur wherever two or more species occur together. This has brought to different taxonomic treatments and floristic traditions in various countries. A systematic revision of *Ranunculus* sect. *Batrachium*, including all species of the section and specimens from the whole distribution range, is needed to link taxonomic and phylogenetic traits in light of the recent genetic research (Hoerandl et al., 2005; Telford et al., 2011; Zalewska-Gałosz et al., 2015; Bobrov et al., 2015).

*Ranunculus baudotii* (Godron) F. Shultz (sect. *Batrachium*) is largely a coastal species that occurs throughout Europe with a few localities in North Africa (Hulten and Fries, 1986). It is found mostly in brackish waters (Tutin et al., 1980), but in Denmark it is common also in lakes and streams (Moeslund et al., 1990). Previous studies showed that *R. baudotii* beds in the Danish lowland streams are primarily established by drifting fragments. The study of Riis (2008) showed, in fact, that in River Aarhus about 80% of macrophyte colonization events, including *R. baudotii*, were from drifting shoots within a 300 m reach and only 20% from drifting seeds, and that 90% of the released fragments were retained within 4 km (Riis and Sand-Jensen, 2006). Despite the frequent occurrence of vegetative reproduction, *R. baudotii* flowers and sets seed abundantly in River Aarhus.

In this study we investigated the genetic structure of the *R. baudotii* population in River Aarhus. The objectives of the study were to elucidate i) the relative importance of vegetative *vs.* sexual reproduction and ii) how genetic diversity is shaped and distributed within and among beds and at various distances along the stream. Given the occasional occurrence of *R. baudotii x pseudofluitans* hybrids in the stream (Riis, 2008; Riis and Baattrup-Pedersen, 2011), we also investigated iii) the occurrence of potential hybrids in our sample set, which could confound the interpretation of the genetic structure, because hybrid populations would show similar genetic patterns to those shaped by isolation by distance by unidirectional hydrochory.

**Table 1**
Sampling plan. Site and bed IDs, geographic distances and bed size.

| Site name | Site ID | Bed ID | Distance from source (km) | Bed area ($m^2$) |
|---|---|---|---|---|
| Bodil Mølle | BM | BM1 | 10.000 | 0.91 |
| | | BM2 | 10.010 | 0.56 |
| | | BM3 | 10.020 | 0.91 |
| | | BM4 | 10.030 | 1.26 |
| | | BM5 | 10.040 | 0.70 |
| Pinds Mølle | PM | PM1 | 11.000 | 0.91 |
| | | PM2 | 11.010 | 0.77 |
| | | PM3 | 11.020 | 0.63 |
| | | PM4 | 11.030 | 0.84 |
| | | PM5 | 11.040 | 1.33 |
| Skibby | SK | SK1 | 20.000 | 0.94 |
| | | SK2 | 20.008 | 0.77 |
| | | SK3 | 20.016 | 0.70 |
| | | SK4 | 20.028 | 1.40 |
| | | SK5 | 20.038 | 0.60 |

## 2. Methods

### 2.1. Sampling

Seventy five samples of *R. baudotii* were collected from three sites (BM, PM and SK) along a 10 Km stretch of River Aarhus (Table 1). Five beds were sampled from each site at a distance of about 10 m downstream from each other, and five samples were collected from each bed along two perpendicular transects across the beds. PM was one Km downstream of BM and SK was nine Km downstream of PM. The number of samples represent a significant portion of the within-site and within-stream variation in this small stream. All samples fit the description of *R. baudotii* Godron (Moeslund et al., 1990) in having whitish stems and lacking floating leaves as opposed to the similar species *R. peltatus* Schrank and *R. aquatilis* L. Leaves were shorter than internodes in which our samples differed also from *R. pseudofluitans* (Syme) Newbold ex Baker & Foggitt and *R. fluitans* Lam. (Tutin et al., 1980). Additional three species of *Ranunculus* sect. *Batrachium* occur in Denmark, i.e. *R. hederaceum* L., *R. circinatum* Sibth. and *R. trichophyllum* Chaix in Vill., but these are all morphologically very distinct (Moeslund et al., 1990).

### 2.2. DNA extraction

DNA was extracted from dry apical shoots and leaves (from about 1 square cm of plant material) conserved in silica gel with the Qiagenís DNeasy Plant Mini Kit. DNA quality was checked on a 0.8% agarose gel run at 120 V for one hour and was quantified with the Nano Drop Spectrophotometer ND-1000 (Saveen Werner) at 280 nm wavelength.

### 2.3. AFLPs

100 ng/μl of genomic DNA were restricted with 2.5 units each of *Eco*RI and *Mse*I enzymes and ligated to 5 pmol *Eco*Ri and 50 pmol *Mse*I double stranded adapters, with one unit of T4 DNA ligase. Restriction and adapter ligation occurred simultaneously in a Peltier Thermal Cycler PTC-200 (MJ-Research) programmed for