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Distylic *Hottonia palustris* shows high reproductive success in small populations despite low genetic variability

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Abstract

Hottonia palustris L. (Primulaceae) is characterized by a heteromorphic incompatibility system. The strategy of distylic ramets is believed to promote outcrossing, to maintain overall genetic diversity and to prevent inbreeding depression. In spite of this distyly, an extremely low amount of allozymic diversity was observed in 545 individual ramets from 14 populations in Flanders (Belgium). A possible explanation for such low genetic variation is discussed in relation to the vegetative propagating abilities and the ecological niche width of the species. In contrast to the uniformity in allozymes as well as to the feature of single morph populations, there was a high variability in reproductive success between populations such as the number of seeds per ramet (425–2633), the number of flowers per ramet (9–36) and the mean weight of seeds (0.03–0.17 mg). Small populations and even those consisting of only one style morph may show a high reproductive success. As a whole, *H. palustris* showed a negative relationship of reproductive success with the surface area of its populations.

Keywords: *Hottonia palustris*; Isozyme; Heterostylous; Morph bias; Reproductive success

1. Introduction

Water violet (*Hottonia palustris* L., Primulaceae) is known from lowlands throughout Europe, with its largest occurrence in Central and Eastern Europe. It is a circumneutral species from relatively shallow habitats with low to moderately alkaline, meso- to eutrophic, stagnant to slow-flowing freshwater systems with a moderate phosphate and nitrogen content (Haslam, 1978). *H. palustris* is known from soils consisting of sand, river clay or peat with a low carbonate and a high phosphate content. The species cannot use bicarbonates for carbon dioxide assimilation and therefore relies on high carbon dioxide content of the water. In seepage zones these requirements are found especially when the seepage water has passed subsequently through acidic as well as carbonate rich soil layers. In Flanders (Belgium) this species is severely reduced to isolated seepage habitats as a result of drainage and surfacewater pollution.

Hottonia palustris is a perennial herb with a heteromorphic incompatibility system. It produces two floral morphs that differ in the relative positioning of the stigma and anthers in the flowers. Long styled specimens (pins) were reported to be generally more frequent than the short styled ones (thrums) (Ford, 1971; Weeda et al., 1988). Full seed set is achieved if pollen is transferred between different morphs and from another level that corresponds to the receiving stigma (Ford, 1971). We can expect that the effect of a distylic genetic system will be reflected in segregating allozymes as well as in the level of genetic diversity because obligate outcrossers generally maintain high levels of genetic diversity (Hamrick et al., 1979; Hamrick and Godt, 1990). However, clonal growth locally can result in the dominance of a single clone, as was observed for within-lake populations of *Nymhoides peltata* (Uesugi et al., 2004). *H. palustris* also displays clonal growth and the vegetative form can produce independent ramets over time allowing long-lived individuals to generate stable communities that are spatially structured (Weeda et al., 1988). It is known from other species, e.g., *N. peltata* that strongly biased morph ratios, due to clonal growth, may result in shortage of compatible pollen and in reduced fruit set (Wang et al., 2005). Therefore, the mixed reproductive system in *H. palustris* is

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expected to be a major variable that determines the level and distribution of genetic diversity of the species and might ultimately have an effect on reproductive success in populations with only one style morph. In addition, the self-incompatibility between the two floral morphs is rather weak and both cleistogamy through under water self-pollination of unopened flowers (Weeda et al., 1988) and intramorph crosses (geitonogamy through pollination of flowers from the same ramet and crosses between individuals of the same morph) are possible (Darwin, 1877). Consequently, population characteristics and inbreeding also can have an effect on the reproductive success.

The main objective of the study was to evaluate the reproductive success of *H. palustris* populations in relation to population characteristics. Genetic diversity was estimated through enzyme electrophoresis.

2. Materials and methods

For the allozyme analysis, a total of 545 individuals were collected from eight wetlands (14 populations) throughout Flanders (Belgium), ranging from 3°16′ to 4°52′E and 50°55′ to 51°09′ N (Table 1). The distance between the eight wetlands ranged from 6.3–116 km. A population within a wetland was operationally defined as a group of plants separated from their closest conspecifics by more than 200 m. This was applied for wetlands 4 and 8 that each have their populations hydrologically connected. For 11 populations (five wetlands) the ratio of style morphs and samples were taken during the optimum of the flowering period. Three wetlands contained only vegetative ramets during the same period.

In large populations, 50 ramets per population were collected in such a way that the distance between the individuals was maximized to reduce the possibility of collecting clonal shoots (1- Leiemeersen, 2-Honegem, 4A-Blaasveldbroek A, 4B-Blaasveldbroek B, 6-Krankenhoeve, 7-Dunbergbroek, 8A-Walenbos A, 8B-Walenbos B, 8E-Walenbos E, 8F-Walenbos F). In smaller populations all visibly different ramets were collected unless there was direct evidence for their same origin (3-Hingene, 5-Antitank, 8C-Walenbos C, 8D-Walenbos D). The area of each population was given by the surface (m^2) of the total *H. palustris* cover and ranged from 30–98 m^2 for the larger populations and from 2–5 m^2 for the smallest ones.

The leaves were frozen in liquid nitrogen and stored at −70 °C, prior to crushing in 1.0 mL of extraction buffer (0.5 M Tris–HCl pH 6.8, 20% glycerol, 1% β-mercapto-ethanol, 0.5% Nonidet and 10% phosphate-polyvinylpyrrolidone of MW 10,000). Extracts were centrifuged for 15 min at 10,000 g. Electrophoresis was carried out on vertical 7.5% polyacrylamide gels (1.5 M Tris–HCl pH 8.8) with a Tris–HCl glycine, pH 8.0 electrode buffer system. The gels were run at 25 mA for a period of 3–4 h. The enzyme-specific staining procedures followed Vallejos (1983). Eight enzyme systems were resolved and clearly scored: 6-PGD (6-phospho-D-gluconate dehydrogenase; E.C.1.1.1.44), LAP (leucine aminopeptidase; E.C.3.4.1.1.1), GDH (glutamate dehydrogenase; E.C.1.4.1.2), GOT (glutamate–oxaloacetate transaminase; E.C.2.6.1.1), PGM (phosphoglucomutase; E.C.2.7.5.1), ACO (aconitase; E.C.4.2.1.3), ME (malic enzyme; E.C.1.1.1.40), MDH (malate dehydrogenase; E.C.1.1.1.37). The following enzymes gave no clear banding patterns: IDH (isocitrate dehydrogenase; E.C.1.1.1.42), PEP (peptidase; E.C.3.4.-.-), XDH (xanthine dehydrogenase; E.C.1.2.1.37), ALD (aldolase; E.C.4.1.2.13).

The reproductive success was measured in 402 ramets from five wetlands (11 populations, with sample sizes ranging from 13–56 fruiting individuals). The number of seeds per fruit was counted for each ramet, and the mean number of seeds was calculated per fruit as well as per ramet. The seed weight was measured for each ramet.

Significant differences in fitness traits between amounts of short styled and long styled types were tested using a Chi square. Because the global within group variation did not met a normal distribution, Mann–Whitney *U*-tests were used to analyze the deviation scores derived from the number of seeds per ramet in relation to the style morph. This test was performed within and between populations.

The relationship of the population area with reproductive success (the number of seeds per ramet; the number of seeds per

Table 1
Location of 14 *Hottonia palustris* populations in Flanders (Belgium) and deviations of the long styled (LS) and short styled (SS) types

Nr	Locality	Longitude	Latitude	Number of LS types	Number of SS types	χ^2 (*p*)
1	Leiemeersen	3°16′05″	51°09′16″	0	24	/(Only thrum types)
2	Honegem	4°00′13″	50°57′11″	Only vegetative	Only vegetative	Only vegetative
3	Hingene	4°16′10″	51°06′58″	Only vegetative	Only vegetative	Only vegetative
4A	Blaasveldbroek A	4°23′33″	51°03′32″	24	6	0.001
4B	Blaasveldbroek B	4°23′24″	51°03′24″	0	14	/(Only thrum types)
5	Antitank	4°39′44″	50°58′23″	Only vegetative	Only vegetative	Only vegetative
6	Krankenhoeve	4°34′15″	51°02′21″	33	23	0.18
7	Dunbergbroek	4°43′07″	50°56′04″	13	37	0.0007
8A	Walenbos A	4°52′02″	50°55′39″	12	31	0.002
8B	Walenbos B	4°52′38″	50°56′10″	0	49	/(Only thrum types)
8C	Walenbos C	4°52′30″	50°56′03″	9	4	0.25
8D	Walenbos D	4°51′98″	50°55′89″	19	5	0.043
8E	Walenbos E	4°52′48″	50°55′56″	0	49	/(Only thrum types)
8F	Walenbos F	4°51′57″	50°55′50″	27	23	0.57

χ^2-test; $p < 0.05$ results in a significant deviation of 1:1 ratio.