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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.

fruit and the number of fruits per ramet) was done with product moment correlation (*r*) and Spearman rank correlation coefficients (r_s).

3. Results

3.1. Genetic variation and morph bias

Eight enzyme systems were analyzed and revealed 15 monomorph loci (gdh-2, gdh-1, lap-2, lap-1, pgd-2, pgd-1, got-3, got-2, got-1, pgm-2, pgm-1, aco-2, aco-1, me-1, mdh-1). The frequencies of the mating types rarely were equally distributed (Table 1). This was observed only in population 6 (Krankenhoeve), 8C (Walenbos C) and 8F (Walenbos F). Deviations from the 1:1 morph ratio with a larger frequency of short-styled specimens was found in population 7 (Dunbergbroek) and 8A (Walenbos A) whereas population 4 A (Blaasveldbroek A) and 8D (Walenbos D) showed a dominance of long-styled specimens. In four populations, only thrum types were present (Table 1).

3.2. Reproductive success

Within populations, a significant difference was observed in the number of seeds per ramet between the short styled and long styled types (Table 2). These results at population level reject the null hypothesis of equality between the two style types. In every population the number of seeds per ramet was higher for the ramets with long styled types (Table 2). Considering all individuals, the number of seeds per ramet was significantly higher in the long styled than in the short styled ramets (Mann–Whitney *U*-test, $Z = -11.22^{***}$, Table 2).

A highly significant positive relationship between the number of seeds per ramet and the number of flowers per ramet was observed within all studied populations (Table 2). The Spearman Rank correlation coefficient (between individuals from the same population) varied between $r_s = 0.666$ and $r_s = 0.868$ ($p < 0.001$). A significant negative relationship between the number of seeds per ramet and the mean weight of seeds per ramet was observed for most populations. The significant results varied between $r_s = -0.418$ ($p < 0.01$) and $r_s = -0.762$ ($p < 0.001$) except for population 8A (Walenbos A) with a positive relationship ($r_s = 0.563$, $p < 0.001$). The smallest populations showed a high number of seeds per ramet ranging from 835 to 2387 at individual level and averaged at 1710 and 1638 at the population level for 8C and 8D (Walenbos C and D), respectively.

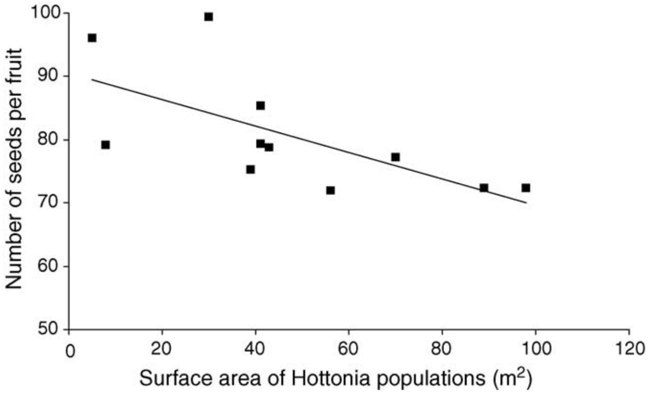

Fig. 1. Relationship between number of seeds per fruit and surface area of *Hottonia palustris* populations.

At population level, the number of fruits per ramet was not significantly influenced by the population area ($r = -0.29$, $p = 0.55$), whereas the number of seeds per fruit was significantly negatively correlated with the population area ($r = -0.63$, $p < 0.05$) (Fig. 1) and with the number of seeds per ramet to the population area ($r = -0.62$, $p < 0.05$) (Fig. 2).

4. Discussion

4.1. Extreme low allozyme diversity

Though a few studies have revealed low levels of allozyme variation in clonal species, most investigation of terrestrial clonal plants report high levels of genetic variation within populations (Jonsson et al., 1996). Terrestrial clonal plants show a greater tendency for multiclonality (as identified by allozyme variation) with widespread clones (Ellstrand and Roose, 1987). Isozymes in *H. palustris* revealed no variation within the studied region (maximum distance between

Table 2
Reproductive success measured as seeds per ramet (overall and for each flower type), flowers per ramet and weight of seeds per ramet (mean ± S.D.)

Nr	Seeds/ramet (a)	Flowers/ramet (b)	Seed weight/ramet (10^{-5} g) (c)	r_s (a) with (b)	r_s (a) with (c)	Seeds/ramet Long styled	Seeds/ramet Short styled	Mann–Whitney *U*-test
1	1144 (±284)	17.7 (±4.7)	11.1 (±2.2)	0.768***	−0.575**		1144 (±284)	/
4A	1357 (±265)	24.0 (±6.4)	9.9 (±3.7)	0.746***	−0.665***	1457 (±172)	958 (±177)	−3.74
4B	1725 (±334)	25.1 (±4.2)	7.2 (±2.2)	0.677**	−0.472 n.s		1725 (±334)	/
6	1569 (±349)	19.7 (±5.7)	10.2 (±2.0)	0.868***	−0.762***	1787 (±240)	1255 (±217)	−5.61
7	1354 (±522)	22.3 (±4.7)	9.6 (±3.2)	0.774***	−0.418**	1901 (±582)	1181 (±363)	−4.15
8A	1385 (±354)	22.9 (±7.5)	9.3 (±3.0)	0.772***	0.563***	1821 (±213)	1216 (±232)	−4.96
8B	1204 (±355)	19.2 (±7.5)	8.3 (±3.0)	0.788***	−0.644***		1204 (±355)	/
8C	1710 (±166)	21.7 (±3.5)	12.0 (±2.2)	0.807***	0.541 n.s	1781 (±119)	1548 (±149)	−2.15
8D	1638 (±453)	23.5 (±6.2)	10.5 (±2.5)	0.745***	0.279 n.s	1757 (±423)	1186 (±234)	−2.45
8E	1153 (±360)	17.8 (±2.5)	7.3 (±2.6)	0.666***	−0.566***		1153 (±360)	/
8F	1240 (± 431)	21.6 (± 6.4)	9.2 (±1.2)	0.745***	−0.748***	1484 (±347)	954 (±335)	−4.43

Mean with S.D. (r_s with $^{***}p < 0.001$; $^{**}p < 0.01$;$^{*}p < 0.05$); n.s. non significant; Mann–Whitney *U*; Z = ±1.96 for the 95% confidence interval.

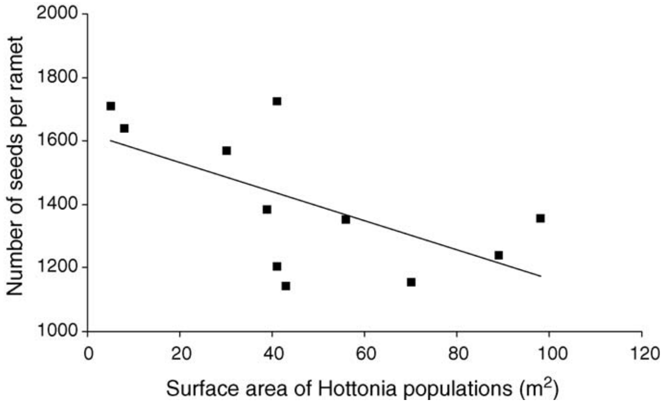

Fig. 2. Relationship between number of seeds per ramet and surface area of *Hottonia palustris* populations.

populations was 116 km), despite the existence of genetically based different flower morphs that are expected to promote outcrossing and maintain substantial levels of genetic divesity. Both allozymic and flower type monomorphisms suggest clonal growth, large clonal sizes but low clonal diversity. Different assumptions can be made to explain this monomorphic nature. Either isozymes could have low resolving power to detect variability in this particular species or the ecological niche width of the species is too small.

Differences between isozymic and morphological information do not necessarily mean that different genets cannot be present. Isozymes are only a small sampling of the genome. The number of multilocus genotypes identified in a population gives only a minimum estimate of the number of genets (Jonsson et al., 1996). However, isozymes are expected to be sufficiently informative about basic genetic variability, particularly in heterostylous species. Isozymes proved to be very convenient in detecting allelic variability and heterozygosity of heterostylous *Primula* species (Van Rossum et al., 2002, 2004). Clonal diversity was also detected with isozymes in heterostylous *Eichhornia paniculata* but monomorphic loci were found in several populations containing only a single flower morph type (Husband and Barrett, 1991). Because isozymes revealed variability in other studies on heterostylous plants, we consider that the allozymic monomorphism in *H. palustris* is equally informative and not a drawback of a technique.

The degree of the genetic variation for a broad range of different macrophyte genera has been low (Triest, 1991; Harris et al., 1992). Compared to terrestrial plants, only a few aquatic plants exhibit significant levels of genetic variability within and between populations (Les, 1991; Laushman, 1993) and examples are known from *Hydrilla verticillata* (Verkleij and Pieterse, 1991), *Potamogeton pectinatus* (Hettiarachchi and Triest, 1991), many seagrass species, (Mc Millan, 1991), *Typha* (Sharitz et al., 1980), where significant differences were found for physiological, biochemical, morphological and phenological traits from plant-to-plant and population-to-population. Such findings contrasted with isozyme studies and implies that genetic variability is not necessarily correlated to other categories of polymorphism. Accordingly for the studied *Hottonia* populations, there must be at least two different genotypes (the long styled and the short styled genet) in the mixed populations. In the populations that have only the short styled morph ramets, there is at least one clone but there can be more.

4.2. Niche width

The amount of genetic variation within species has also been related to niche widths. Species or populations with wider niches are expected to have higher levels of genetic variability than those with narrow niches (Van Oostrum et al., 1985). *H. palustris* is not a good competitor and grows in seepage habitats with a sparse vegetation. As is the case for *Alisma* species (Triest and Roelandt, 1991), the allozymic monomorphism of *H. palustris* is no disadvantage for their reproduction and their successful colonization of the, mostly isolated, seepage habitats. *H. palustris* reaches higher biomass when overlying water is absent than when submerged in shallow waters of up to one meter depth. Seeds remain viable after dessication and germinate in light and aerobic conditions at a wide range of temperatures (Brock et al., 1989). The latter case-study of the species in The Netherlands showed that germination percentages were higher on moist substrate than when submerged and might indicate that yearly water level fluctuations may account for much of the variation between seedling recruitment and clonal regrowth in a population. Despite the characteristics of a self-incompatible plant, *H. palustris* seems to thrive on apomixis to develop its populations.

Self-incompatible perennial plant species such as *Arnica montana*, *Rutidosis leptorrhynchoides*, *Primula veris*, *Digitalis lutea* and *Primula elatior* revealed reduced fruit and seed set as a result of a reduced reproductive success (Young et al., 1996; Kéry et al., 2000; Luijten et al., 2000; Van Rossum et al., 2002), whereas *H. palustris* populations from this study revealed a negative relationship of seeds per fruit and per ramet, indicating a sufficient pollination success in those small populations with a low morph bias or with excess of long styles types. No consideration was taken of the germinable seeds. This parameter might be considered as important as the number of seeds produced, especially in populations and species where sexual reproduction is rare or non-existent. The loss of power of sexual reproduction can result from the accumulation of mutations affecting seed and pollen fertility (Barrett, 1980a, 1980b). Most vegetative apomicts remain facultative so they can set seed, although where only one compatible genotype occurs, the apomixis may become essentially obligate (Richards, 2003).

A single clone may end up by dominating a large area and occupying a range of microhabitats (Jonsson, 1995; Barrett et al., 1993) what means that the clonal diversity in established populations could be much lower than in colonizing populations. There could be several causes for this structuring of clonal diversity or the restriction of pollen transfer between genets as a direct result of this clonal growth. A strong interaction between clonal growth, sexual reproduction and reproductive success is more likely to be observed in heterostylous species with a strong incompatibility system such as known for *N. peltata* (Wang et al., 2005). Population structuring, as observed for seagrasses, may also result from bottleneck effects associated with stochastic events leaving only a few isolated pockets of individuals which have survived to recolonize to the distribution observed today (Waycott et al., 1996). However, inbreeding would merely promote local homogeneity and could be expected to promote between-population divergence. *H. palustris* in Flanders shows a genetic homogeneity within as well as between populations. The spatial structuring of the distylic ramets however revealed that small populations and even populations consisting of only one style morph are not suffering from lowered reproductive success. For *H. palustris*, it can be suggested that the weak incompatibility system, allowing intramorph crosses, selfing through geitonogamy and selfing through cleistogamy is more advantageous for the survival of this species with a narrow ecological niche, than would be a strong or complete self-incompatibility system.

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References

Barrett, S.C.H., 1980a. Sexual reproduction in *Eichhornia crassipes* (Water hyacinth). I. Fertility of clones from diverse regions. J. Appl. Ecol. 17, 101–112.

Barrett, S.C.H., 1980b. Sexual reproduction in *Eichhornia crassipes* (Water hyacinth). II. Seed production in natural populations. J. Appl. Ecol. 17, 113–124.

Barrett, S.C.H., Eckert, C.G., Husband, B.C., 1993. Evolutionary processes in aquatic plant populations. Aquat. Bot. 44, 105–145.

Brock, T.C.M., Mielo, H., Oostermeijer, G., 1989. On the life cycle and germination of *Hottonia palustris* L. in a wetland forest. Aquat. Bot. 35, 153–166.

Darwin, C., 1877. The different forms of flowers on plants of the same species. John Murray, London.

Ellstrand, N.C., Roose, M., 1987. Patterns of genotypic diversity in clonal plant species. Am. J. Bot. 74, 123–131.

Ford, E.B., 1971. Ecological Genetics, 3rd ed. Chapman and Hall Ltd, London, p. 410.

Hamrick, J.L., Godt, M.J.W., 1990. Allozyme diversity in plant species. In: Brown, A.H.D., Clegg, M.T., Kahler, A.L., Weir, B.S. (Eds.), Plant Population Genetics, Breeding and Genetic Resources. Sinauer Associates, Sunderland, Massachussets.

Hamrick, J.L., Linhart, Y.B., Mitton, J.B., 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Annu. Rev. Ecol. Syst. 10, 173–200.

Harris, S.A., Maberly, S.C., Abbott, R.J., 1992. Genetic variation within and between populations of *Myriophyllum alterniflorum* DC. Aquat. Bot. 44, 1–21.

Haslam, S.M., 1978. River Plants. Cambridge University Press, Cambridge, p. 396.

Hettiarachchi, P., Triest, L., 1991. Isozyme polymorphism in the genus *Potamogeton* (Potamogetonaceae). In: Triest, L. (Ed.), Isozymes in Water Plants. Opera Bot. Belg. National Botanic Garden, Meise, pp. 87–114.

Husband, B.C., Barrett, S.C., 1991. Colonization history and population gnetic structure of *Eichhornia paniculata* in Jamaica. Heredity 66, 287–296.

Jonsson, O., 1995. Old populations of the rhizomatous sedge *Carex bigelowii* show little intermingling of clones (genets). Abstr. Bot. 19, 105–113.

Jonsson, B., Jonsdottir, I.S., Cronberg, N., 1996. Clonal diversity and allozyme variation in populations of the arctic sedge *Carex bigelowii*. J. Ecol. 84, 449–459.

Kéry, M., Matthies, D., Spillmann, H., 2000. Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. J. Ecol. 88, 17–30.

Laushman, R.H., 1993. Population genetics of hydrophilous angiosperms. Aquat. Bot. 44, 147–158.

Les, D.H., 1991. Genetic diversity in monoecious hydrophile *Ceratophyllum* (Ceratophyllaceae). Am. J. Bot. 78, 1070–1082.

Luijten, S.H., Dierick, A., Oostermeijer, J.G.B., Raijmann, L.E.L., den Nijs, H.C.M., 2000. Population size, genetic variation, and reproductive success in a rapidly declining, self-incompatible perrenial (*Arnica Montana*) in the Netherlands. Conserv. Biol. 14, 1776–1787.

Mc Millan, C., 1991. Isozyme patterning in marine spermatophytes. In: Triest, L. (Ed.), Isozymes in Water Plants. Opera Bot. Belg. National Botanic Garden, Meise, pp. 193–200.

Richards, A.J., 2003. Apomixis in flowering plants: an overview. Phil. Trans. R. Soc. Lond. B 358, 1085–1093.

Sharitz, R.R., Wineriter, S.A., Smith, M.H., Liu, E.H., 1980. Comparison of isozymes among *Typha* species in eastern United States. Am. J. Bot. 67, 1297–1303.

Triest, L. (Ed.), 1991. Isozymes in water plants. Opera Bot. Belg., 4. National Botanic Garden, Meise, pp. 269.

Triest, L., Roelandt, B., 1991. Isozymes in diploid and polyploid *Alisma* species (Alismataceae). In: Triest, L. (Ed.), Isozymes in water plants. Opera Bot. Belg., 4. National Botanic Garden, Meise, pp. 167–192.

Uesugi, R., Goka, K., Nishihiro, J., Washitani, I., 2004. Allozyme polymorphism and conservation of the Lake Kasumigaura population of *Nymphoides peltata*. Aquat. Bot. 79, 203–210.

Vallejos, E., 1983. Enzyme activity staining. In: Tanksley, S.D., Orton, J.J. (Eds.), Isozymes in plant genetics and breeding. Part A. Elsevier, Amsterdam, pp. 469–516.

Van Oostrum, H., Sterk, A.A., Wijsman, H.W.J., 1985. Genetic variation in agamospermous micro-species of *Taraxacum sect. erythrosperma* and *sect. obliqua*. Heredity 55, 223–228.

Van Rossum, F., Echchgadda, G., Szabadi, I., Triest, L., 2002. Commonness and long-term survival in fragmented habitats: *Primula elatior* as a study case. Conserv. Biol. 16, 1286–1295.

Van Rossum, F., Campos de Sousa, S., Triest, L., 2004. Genetic consequences of habitat fragmentation in an agricultural landscape on the common *Primula veris*, and comparison with its rare congener. *P. vulgaris*. Conserv. Genet. 5, 231–245.

Verkleij, J.A.C., Pieterse, A.H., 1991. Isoenzyme patterns in leaves of *Hydrilla verticillata* (Hydrocharitaceae). In: Triest, L. (Ed.), Isozymes in Water Plants. Opera Bot. Belg, 4. National Botanic Garden, Meise, pp. 49–57.

Wang, Y., Wang, Q., Guo, Y., Barrett, S.C.H., 2005. Reproductive consequences of interactions between clonal growth and sexual reproduction in *Nymphoides peltata*: a distylous aquatic plant. New Phytologist 165, 329–336.

Waycott, M., Walker, D.I., James, S.H., 1996. Genetic uniformity in *Amphibolis antarctica*, a dioecious seagrass. Heredity 76, 578–585.

Weeda, E.J., Westra, R., Westra, C., Westra, T., 1988. Nederlandse ecologische flora. IVN (Ed.) 302.

Young, A., Boyle, T., Brown, T., 1996. The population genetic consequences of habitat fragmentation for plants. Trends Ecol. E 11, 413–418.