Range-edge effects promote clonal growth in peripheral populations of the one-sided wintergreen *Orthilia secunda*

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**ABSTRACT**

Existing in suboptimal conditions is a frequent occurrence for species inhabiting the cusp of their ecological range. In range-edge populations of plants, the scarcity of suitable habitat may be reflected in small population sizes which may result in increased self-pollination and/or inbreeding and an increase in the incidence of clonal reproduction. These factors may result in a decrease in levels of genetic diversity and a loss of potential adaptive variation that may compromise species’ ability to cope with changes in their environment, an issue that is particularly relevant today with the current concern surrounding global climate change and its effect on species’ distributional ranges. In the present study, we have compared the levels of clonal reproduction in the one-sided wintergreen *Orthilia secunda* (L.) House in (1) populations from its main continuous distribution range, (2) populations occurring on the limits of the continuous range, and (3) peripheral populations outwith the species’ continuous distribution range. Range-edge populations in Scotland and Sweden displayed significantly lower genotypic richness and diversity than those from the main area of the species’ distribution in these countries. Populations from Ireland, which occur in the temperate zone rather than the boreal conditions that are the preferred habitat for the species, and which represent relict populations left over from cooler periods in the Earth’s history, displayed no within-population genetic diversity, suggesting a complete lack of sexual reproduction. Furthermore, the genetic distinctiveness of the Irish populations, which contained alleles not found in either the Scottish or the Swedish populations, highlights the value of ‘trailing edge’ populations and supports the concept of ‘parochial conservation’, namely the conservation of species that are locally rare but globally common.

**Keywords**

Clonal growth, distribution rage, fragmentation, *Orthilia secunda*, range-edge effects.

**INTRODUCTION**

Existing in suboptimal conditions is a frequent occurrence for species inhabiting the cusp of their ecological range (Soulé, 1973; Shumaker & Babble, 1980). Ecologically marginal populations are usually smaller, more isolated and highly fragmented due to lack of suitable habitat in which the species can exist (Brown, 1984; Caughley *et al.*, 1988). The isolated nature of such populations can result in low effective population sizes and a reduction in gene flow (Lawton, 1993; *Young et al.*, 1996), which is often thought to be reflected in plants by factors such as increased self-pollination and/or inbreeding (Arnaud-Haond *et al.*, 2006), and an increase in the incidence of clonal reproduction (Garcia *et al.*, 2000; Dorken & Eckert, 2001; Eckert, 2002; Billingham *et al.*, 2003). These factors, coupled with the increased impact of genetic drift in small populations, will result in a decrease in levels of within-population genetic diversity and such a loss of potential adaptive variation may compromise species’ ability to cope with changes in their environment, increasing the threat of extinction (Hoffman & Blows, 1994; *Young et al.*, 1996; Keller & Waller, 2002). This issue is particularly relevant today, with the current concern surrounding global climate change and its effect on species’ distributional ranges (Thomas *et al.*, 2004).

In plants, clonal reproduction has long been viewed as a mechanism to allow an individual to persist in adverse conditions at the expense of the benefits of sexual reproduction, which include the generation new genotypes via recombination as well as producing seeds that can aid in dispersal (Ouborg *et al.*, 1999). Consequently, factors causing plants to make the switch from sexual to clonal growth are generally correlated with suboptimal conditions...
environmental conditions (Honnay & Bossuyt, 2005). Ecological variables such as availability of light and/or shade (Mandujano et al., 1998; Nabe-Nielsen & Hall, 2002), availability of moisture (Jacquemyn et al., 2005), altitude (Young et al., 2002), and habitat fragmentation (Smith et al., 2003; Lhullier et al., 2006) have all been implicated in the change from sexual to asexual reproduction.

In the present study, we have compared the levels of clonal reproduction in central and peripheral populations of the one-sided wintergreen Orthilia secunda (L.) House. The species is a creeping, evergreen perennial with a northern circum-global distribution and is found in Europe, Asia, and North America (Fig. 1). Flowers are hermaphroditic and plants can reproduce both sexually and clonally via rhizomes, with occasional flowering from June to early August. Where seed production does occur, persistence of seedlings is dependent on the presence of mycorrhizal fungi of the genus Tricholoma, as is the case with many members of the Ericaceae (Cairney & Meharg, 2003; Klimešová, 2007). Thompson & Band (1997) also found the seed to be transient in the soil, with little evidence of a persistent seed bank, and these factors may further limit the opportunities for colonization by new founders, particularly under suboptimal conditions at the species’ range-edge. To test the hypothesis that clonal reproduction should be more prevalent at the edge of the range, we compared levels of genetic diversity in populations from the main area of the species’ range in Scotland and Sweden, where populations tend to be large and continuous, with populations inhabiting the limits of the continuous distribution range in both countries. We also compared these with populations from Ireland, where the distribution of O. secunda is highly

Figure 1 Maps showing distribution of Orthilia secunda: (a) Northern hemisphere (Source: Naturhistoriska riksmuseet); (b) UK and Ireland (Source NBN Gateway).
fragmented and where populations tend to be found in exposed scarpland rather than the coniferous or mixed woodlands that represent the optimum habitat for the species and, consequently, clonal growth would be expected to be most predominant. Finally, we examined the genetic distinctiveness of range-edge and peripheral populations compared to central populations to determine whether they are of particular conservation value in the context of climate-induced range shifts.

METHODS

Plant material

Locations of populations sampled and sample sizes are given in Fig. 2 and Table 1. Populations were designated as follows, based on their occurrence relative to the continuous distribution range of *O. secunda* (Fig. 1): ‘main range’ populations were defined as those occurring within the main continuous distribution range of the species, shown by the shaded area in Fig. 1(a); ‘range edge’ populations were defined as those occurring at the edge of the species’ continuous range; and ‘peripheral’ populations were defined as those outwith the continuous distribution range, designated by dots in Fig. 1(a).

The main presence of *O. secunda* within the British Isles is the Scottish Highlands (Fig. 1b). Six sites of *O. secunda* were sampled in the Scottish collection. Four of these were from the main area of the range (i.e. ‘main range’): Glen Glass (three patches sampled from pine woodland); Black Isle (two patches sampled from pine woodland); Little Scatwell (two patches sampled from birch woodland); and Tor Achilty (three patches sampled from birch woodland). The remaining two populations were located at the southern edge of the species’ distribution range: Glen Mhor (two patches sampled from a river valley in mixed woodland) and Glen Banvie (three patches sampled from pine woodland). Two populations were also sampled from Sweden, where the species is abundant: Lomselenäs is in the centre of the range, and a patch was sampled from spruce/birch woodland; Flurkmark is on the eastern edge of the range and a patch was sampled from spruce/birch/alder woodland. In all cases, one leaf sample was collected from individual rosettes at semiregular intervals across the area of each patch. Where multiple patches were sampled within a location (‘Population’), they were separated by between 20 m and 3000 m.

In Ireland, *O. secunda* is only known to occur in two locations, namely the Lough Navar scarplands in County Fermanagh, and Cranny Burn in County Antrim. The Lough Navar scarplands, where almost all extant Irish populations of *O. secunda* are found, have been carved out of a sandstone plateau between 150 m and 300 m in elevation. Patches were generally small and irregular, although some (e.g. F6A and F6B) were among the largest sampled in the study, and samples were collected from individual rosettes at intervals across the area of each patch. As the species is protected under Schedule 8 of the Wildlife (Northern Ireland) Order 1985, it is an offence to pick, uproot, or destroy the plant and, consequently, a single leaf was taken from large rosettes only where it was possible to remove the leaf without potentially damaging the whole plant.

DNA extraction

Samples were stored at −20 °C, and DNA was extracted from individuals using the Qiagen DNeasy Plant Mini Kit (Qiagen, West Sussex, UK), after an initial 8 min grinding at 30 Hz using a Retsch MM300 mixer mill (Qiagen). DNA was quantified visually on 1% agarose gels stained with ethidium bromide and diluted to a concentration of 50 ng/µL for subsequent polymerase chain reaction (PCR).

Nuclear microsatellite isolation

Nuclear microsatellite markers were developed by sequencing clones from a library of intersimple sequence repeat (ISSR) PCR products as described in Provan & Wilson (2007). A total of 96 positive (white) colonies were screened and primers were designed to amplify 19 microsatellite motifs detected using the Primer program (version 5.0). Of these, only five gave polymorphic, clearly scorable, single-locus products (Table 2).

Microsatellite genotyping

Microsatellite PCR was carried out using one of the following two sets of parameters: (1) initial denaturation at 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 30 s and a final extension at 72 °C for 5 min; (2) initial denaturation at 94 °C for 3 min followed by 10 touchdown cycles of denaturation at 94 °C for 30 s,
annealing at 68 °C for 30 s (−1 °C per cycle), extension at 72 °C for 30 s followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. PCR was carried out in a total volume of 10 µL containing 100 ng genomic DNA, 10 pmol of 32P-end labelled forward primer, 10 pmol of unlabelled reverse primer, 1× PCR buffer (5 mM Tris-HCl [pH 9.1], 1.6 mM [NH₄]₂SO₄, 15 µg/mL BSA), 200 µM each dNTP, 2.5 mM MgCl₂,
and 0.5 U Taq polymerase (Genetix, New Milton, Hampshire, UK). Products were resolved on 6% denaturing polyacrylamide gels containing 1x TBE and 8 m urea after addition of 10 μL of 95% formamide loading buffer. Product sizes were determined by comparison with a 10-bp ladder (Invitrogen, Carlsbad, CA, USA). Gels were run at 70 W constant power for 2 h, transferred to 3 mm Whatman blotting paper (Whatman, Kent, UK), and exposed to X-ray film overnight at −20 °C. All PCRs were carried out on a MWG Primus thermal cycler (MWG Biotech, Ebersberg, Germany).

**Data analysis**

Levels of within-population diversity were estimated using G, the number of distinct multilocus genotypes (MLGs), genotypic richness (R), defined as \((G – 1)/(N – 1)\), and Simpson’s diversity index modified for finite sample sizes \((D^*)\), which was calculated as:

\[
D^* = 1 – \sum \left[ N_j(N_j – 1)/N(N – 1) \right]
\]

where \(N_j\) is the number of individuals displaying the \(j\)th MLG. All calculations were carried out using the genclonem software package (V1.1; Arnaud-Haond & Belkhir, 2007).

Levels of genetic differentiation between range-edge and central populations were calculated within the analysis of molecular variance (AMOVA) framework (Excoffier et al., 1992) using ARLEQUIN software package (version 3.01; Excoffier et al., 2005). As many of the multilocus genotypes for *O. secunda* were represented more than once within a given population, we calculated the probability \(P_{GEN}\) of each multilocus genotype arising through sexual as opposed to clonal reproduction following the method of Parks & Werth (1993):

\[
P_{GEN} = \left[ 2^{\Pi x_i} \times x_s \right]^{-1}
\]

where \(h\) is the number of loci at which the genotype is heterozygous, \(x_i\) is the allele frequency of the first allele in the genotype at locus \(i\), and \(x_s\) is the allele frequency of the second allele in the genotype at locus \(i\). The observation of heterozygosity allows the differentiation between genetic identity due to clonal propagation and identity due to inbreeding or selfing, which would lead to an increase in homozygosity. Samples that shared multilocus genotypes with \(P_{GEN} < 0.05\) were considered clonemates and duplicate genotypes were removed from the analysis. \(P_{GEN}\) values were calculated using the genclonem software package.

**RESULTS**

The five microsatellite loci developed for this study displayed between two (OSNSSR169) and 12 (OSNSSR181) alleles in the 35 patches studied. The number of genets (\(G\)) within patches from Scotland and Sweden ranged from one (BI4) to 17 (SW1) in the main range populations (mean = 8.455) and from two (GB15) to six (GM6) in the range edge populations (mean = 4.333). All peripheral populations from Ireland were each fixed for a single genet, with a total of 15 genets detected in the 18 patches analysed. Fixed heterozygosity occurred in 14 of the 15 genets, precluding inbreeding or selfing as the cause of the observed uniformity. Genotypic richness (\(R\)) within patches from Scotland and Sweden ranged from zero (BI4) to 0.733 (BI1) in the main range populations (mean = 0.364) and from 0.043 (GB15) to 0.250 (GM6) in range edge populations (mean = 0.154). Values for Simpson’s diversity index \((D^*)\) within patches from Scotland and Sweden ranged from zero (BI4) to 0.967 (SW1) in the main range populations (mean = 0.751), and from 0.177 (GM1) to 0.733 (SW2) in range edge populations (mean = 0.545). Values for both \(R\) and \(D^*\) were zero for all peripheral populations from Ireland. All diversity values are given in Table 3. Values for \(G\), \(R\), and \(D^*\) were significantly higher in main range populations compared to range edge populations (Mann-Whitney U-test: \(P = 0.035\), \(P = 0.028\), and \(P = 0.020\), respectively).

An analysis of molecular variance (AMOVA; Table 4) revealed a small but significant degree of genetic differentiation between main range and range edge populations from the continuous range (i.e. Scotland and Sweden) of the species \(\Phi_{CT} = 0.0309; \ P = 0.025\). A higher degree of genetic differentiation was observed between peripheral populations from Ireland and the populations from the continuous range \(\Phi_{ST} = 0.0721; \ P < 0.001\), suggesting genetic distinctness of Irish populations, since Scottish and Swedish populations were not significantly differentiated from each other \(\Phi_{ST} = 0.0452; \ P = 0.843\). In addition, three private alleles (i.e. not found in Scotland or Sweden) were detected in the Irish populations: the 133 bp allele at locus OSNSSR137 and the 143 bp and 145 bp alleles at locus OSNSSR181 (Fig. 3).

<table>
<thead>
<tr>
<th>Location</th>
<th>Population</th>
<th>(n)</th>
<th>(G)</th>
<th>(R)</th>
<th>(D^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main range</td>
<td>GG1</td>
<td>24</td>
<td>2</td>
<td>0.043</td>
<td>0.464</td>
</tr>
<tr>
<td></td>
<td>GG2</td>
<td>23</td>
<td>8</td>
<td>0.318</td>
<td>0.842</td>
</tr>
<tr>
<td></td>
<td>GG3</td>
<td>22</td>
<td>5</td>
<td>0.190</td>
<td>0.732</td>
</tr>
<tr>
<td></td>
<td>BI1</td>
<td>24</td>
<td>12</td>
<td>0.733</td>
<td>0.942</td>
</tr>
<tr>
<td></td>
<td>BI4</td>
<td>24</td>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>LS1</td>
<td>23</td>
<td>10</td>
<td>0.409</td>
<td>0.874</td>
</tr>
<tr>
<td></td>
<td>LS4</td>
<td>24</td>
<td>6</td>
<td>0.217</td>
<td>0.746</td>
</tr>
<tr>
<td></td>
<td>TA1</td>
<td>24</td>
<td>14</td>
<td>0.565</td>
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</tr>
<tr>
<td></td>
<td>TA2T</td>
<td>22</td>
<td>8</td>
<td>0.333</td>
<td>0.853</td>
</tr>
<tr>
<td></td>
<td>TA2B</td>
<td>19</td>
<td>10</td>
<td>0.500</td>
<td>0.883</td>
</tr>
<tr>
<td></td>
<td>SW1</td>
<td>24</td>
<td>17</td>
<td>0.696</td>
<td>0.967</td>
</tr>
<tr>
<td>Range edge</td>
<td>GM1</td>
<td>22</td>
<td>3</td>
<td>0.095</td>
<td>0.177</td>
</tr>
<tr>
<td></td>
<td>GM6</td>
<td>21</td>
<td>6</td>
<td>0.250</td>
<td>0.610</td>
</tr>
<tr>
<td></td>
<td>GB4</td>
<td>24</td>
<td>5</td>
<td>0.174</td>
<td>0.634</td>
</tr>
<tr>
<td></td>
<td>GB12</td>
<td>23</td>
<td>5</td>
<td>0.182</td>
<td>0.723</td>
</tr>
<tr>
<td></td>
<td>GB15</td>
<td>24</td>
<td>2</td>
<td>0.043</td>
<td>0.342</td>
</tr>
<tr>
<td></td>
<td>SW2</td>
<td>23</td>
<td>5</td>
<td>0.182</td>
<td>0.779</td>
</tr>
</tbody>
</table>
DISCUSSION

Range-edge effects and clonal growth in Orthilia secunda

Orthilia secunda utilizes clonal growth throughout its range, with evidence of vegetatively propagated ramets in all of the patches analysed in this study. Range edge populations, however, displayed significantly lower levels of genotypic richness and diversity compared to main range populations, with extreme levels of clonality in peripheral populations from Ireland, where all populations were each comprised of a single MLG (see below). This phenomenon was first reported in a study on the tristyous clonal aquatic herb Decodon verticillatus by Eckert & Barrett (1993), who found that populations at the northern margin of the species’ range were fixed for both a single allozyme MLG and a single style morph. Since then, relatively few studies on the population genetics of range-edge populations of clonal plants have been published (Lammi et al., 1999;Billingham et al., 2003;Jump et al., 2003;Alberto et al., 2006;Eckstein et al., 2006), but the majority of these have also reported a decrease in clonal diversity. Jump et al. (2003) noted a decrease in genotypic diversity in range-edge populations of the dwarf thistle Cirsium acaule but not in the melancholy thistle C. heterophyllum. This result was surprising, since both species had displayed identical decreases in population density and seed production from centre to range edge. A reduction in seed production for plant species at the edge of their range has also been reported in other studies (Pigott, 1968; Pigott & Huntley, 1981;Reinartz, 1984;Eckert & Barrett, 1993;Garcia et al., 2000;Dorken & Eckert, 2001), although studies by Lammi et al. (1999) and Yakimowski & Eckert (2007) found range-edge populations to

Table 4 Partitioning of genetic variation using AMOVA.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Partition</th>
<th>d.f.</th>
<th>Fixation indices</th>
<th>% variation</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main range vs. range edge</td>
<td>Among groups</td>
<td>1</td>
<td>Φ&lt;sub&gt;CT&lt;/sub&gt; = 0.0309</td>
<td>3.09</td>
<td>P = 0.025</td>
</tr>
<tr>
<td></td>
<td>Among population within groups</td>
<td>15</td>
<td>Φ&lt;sub&gt;ST&lt;/sub&gt; = 0.2239</td>
<td>21.70</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>277</td>
<td>Φ&lt;sub&gt;ST&lt;/sub&gt; = 0.2479</td>
<td>75.21</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Main range/range edge vs. peripheral</td>
<td>Among groups</td>
<td>1</td>
<td>Φ&lt;sub&gt;CT&lt;/sub&gt; = 0.0721</td>
<td>7.21</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Among population within groups</td>
<td>33</td>
<td>Φ&lt;sub&gt;CT&lt;/sub&gt; = 0.2348</td>
<td>21.79</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>295</td>
<td>Φ&lt;sub&gt;CT&lt;/sub&gt; = 0.2899</td>
<td>71.01</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Scotland vs. Sweden</td>
<td>Among groups</td>
<td>1</td>
<td>Φ&lt;sub&gt;CT&lt;/sub&gt; = −0.0452</td>
<td>−4.52</td>
<td>P = 0.843</td>
</tr>
<tr>
<td></td>
<td>Among population within groups</td>
<td>15</td>
<td>Φ&lt;sub&gt;ST&lt;/sub&gt; = 0.2430</td>
<td>25.40</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Within populations</td>
<td>277</td>
<td>Φ&lt;sub&gt;ST&lt;/sub&gt; = 0.2087</td>
<td>79.12</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

Figure 3 Bubble plots showing allele frequencies in Scottish, Swedish, and Irish populations. Circle sizes are proportional to allele frequency. Allele sizes are given in base pairs.
be equally viable as those in the centre of the range, suggesting local adaptation.

There may be several possible explanations for the apparent increase levels of clonal reproduction observed in range-edge populations of *Orthilia secunda* and these may not be mutually exclusive. Foremost among these is the fact that range-edge populations of a species tend to occur in suboptimal conditions (Abrahamson, 1980; Eriksson, 1996; Peck et al., 1998; Eckert et al., 1999; Eckert, 2002). Consequently, range-edge effects and availability of suitable habitat may be a factor in the predominance of clonal growth in the range edge and peripheral populations of *O. secunda* analysed in this study. The southern Scottish populations are located on the cusp between suboptimal temperate habitats and the boreal conditions that represent the preferred habitat for the species and the completely clonal populations from Ireland are located well into the temperate zone. Another, more subtle ecological factor may be the interactions between seedlings and ericoid mycorrhizal fungal endophytes (*Tricholoma spp.*) These fungi allow the establishment and persistence of plant species in the Ericaceae family under particularly harsh edaphic conditions (Cairney & Meharg, 2003) and it is believed that seedlings of *O. secunda* may not be able to germinate successfully in the absence of these mycorrhizae (Klimešová, 2007).

**Extreme clonality in Irish populations of Orthilia secunda**

The complete lack of genetic diversity within populations of *O. secunda* from Ireland is quite remarkable since the majority of studies on clonal plants have found at least some degree of intrapopulation variation (see Widén et al., 1994 for review). Several population genetic studies have previously revealed a complete lack of genetic variation within clonal plant populations, but these tend to reflect species-level or range-level uniformity (e.g. Grant et al., 1994; Hollingsworth & Bailey, 2000). In this study, the Irish patches are mostly genetically distinct from one another but entirely clonal within a patch. Examples of extremely low levels of within-population variation but high levels of between-population or between-region variation are not unknown (e.g. Bauert et al., 1998; Cariaga et al., 2005; Kameyama & Ohara, 2006). Many of these studies, however, have relied on limited numbers of individuals and it has been demonstrated that estimates of genotypic diversity in clonal organisms are sensitive to sample size; as sample sizes increase, the chances of detecting multiple genotypes also increase (Aspinwall & Christian, 1992; Widén et al., 1994; Eckert, 2002). In the present study, a third of the Irish patches studied were represented by more than 20 samples, most notably patch F6A where 140 plants were sampled but all appeared to be ramets of a single genet. Furthermore, although clone sizes were not estimated in the present study thus precluding a comparison of average MLG sizes in peripheral vs. main range/ range edge populations, two of the largest patches in the study (Patches F6A and F6B) were unicolonial, whereas the other large patches (100 m² or more) studied from Scotland and Sweden contained more (between five and 17) and presumably smaller MLGs.

Recently, the majority of studies on clonal plant populations have utilized dominant, anonymous marker methodologies such as randomly amplified polymorphic DNA (RAPD), intersimple sequence repeat (ISSR), and amplified fragment length polymorphism (AFLP). Codominant markers, such as microsatellites and allozymes, can discriminate heterozygotes from homozygotes, and the ability to reveal fixed heterozygosity means that they are more efficient markers for studies of clonal organisms, since relatively few loci are required to discriminate clonal growth from selfing and excessive inbreeding (Piquot et al., 1996; Dorken & Eckert, 2001). Many species in the Pyrolaceae are self-compatible (Knudsen & Olesen, 1993), but geitonogamous fertilization (i.e. between different ramets of the same genet) would lead to loss of heterozygosity, and fixed heterozygotes were detected in 14 of the 15 genotypes identified in the Irish patches using five microsatellite loci. When one considers the higher resolving power of microsatellites relative to allozymes, this makes the levels of clonality revealed in *O. secunda* even more striking, since theoretical and empirical studies have shown that even limited seedling recruitment via sexual reproduction can result in high levels of within-population genotypic diversity (Watkinson & Powell, 1993; Stehlik & Holderegger, 2000; Bengtson, 2003).

As well as their occurrence in the suboptimal temperate zone, there may be several other factors associated with the highly fragmented nature of the Irish populations which may further explain the extreme clonality observed. It has been suggested that habitat fragmentation may result in a predominance of vegetative reproduction in plant populations (Rossetto et al., 2004; Honnay & Bossuyt, 2005), although there is little direct empirical evidence to support this. While the spatial limitations imposed by population fragmentation may not be a causal factor in the switch to asexual reproduction, they may be associated with the extreme or unsuitable environmental conditions encountered at the margins of a species’ range. Extant populations of *O. secunda* in Ireland are thought to be relict populations left over from cooler periods in the Earth’s history and it is likely that they have existed in their present fragmented distribution throughout the majority of the Holocene. The observed distribution of genotypic diversity is consistent with low levels of seedling recruitment due to the lack of availability of suitable habitat, with new populations being founded by rare, long-distance dispersal events followed by persistence via clonal reproduction. Studies have also suggested the role of pollen and pollinator limitation in fragmented habitats leading to a decrease in the occurrence of sexual reproduction (Rathcke & Jules, 1993; Wilcock & Neiland, 2002), although this is expected to be more of a factor in obligately outcrossing species rather than potentially self-compatible hermaphrodites such as *O. secunda*. Additionally, grazing has also been shown to promote clonal growth (Klein & Steinger, 2002), and grazing by feral sheep and goats has been highlighted as one of the major threats to the species in this region.

**Conservation value of range-edge populations**

*Orthilia secunda* is a common species throughout many parts of its range (e.g. Scotland and Sweden), but it is rare in Ireland and thus has been granted Priority Species status. This is not an
uncommon scenario, and previous studies on the population genetics of range-edge populations have highlighted the potential conservation value of such populations (e.g. Yakimowski & Eckert, 2007). One of the fundamental reasons in support of so-called ‘parochial conservation’ (Hunter & Hutchinson, 1994) is the lack of cohesion between species’ ecological boundaries and anthropogenically imposed political boundaries. It has also been suggested that populations inhabiting the edge of a species’ range may contain unique genotypes essential to maintaining overall intraspecific diversity since they may harbour some degree of adaptive potential necessary to survive changing climatic conditions (Booy et al., 2000; Hampe & Petit, 2005). In the present study, the ‘trailing edge’ populations of O. secunda from Ireland were genetically divergent from main range and range edge populations of the species, highlighting their potential importance. In addition, despite the lower effective sample sizes from the Irish populations, particularly compared to numbers of samples from Scotland, alleles were found at two of the five loci studied that were unique to the Irish populations. This further demonstrates the worth of parochial conservation, and with current concerns surrounding the effects of global climate change, conservation strategies that target trailing edge populations may play a key role in the future survival of many species.

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REFERENCES


genetic data analysis. Evolutionary Bioinformatics Online, 1, 47–50.


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