Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae)

MARCEL E. DORKEN* and CHRISTOPHER G. ECKERT
Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6

Summary

1 In flowering plants the balance between sexual and clonal, asexual reproduction can vary widely. We quantified variation in sexual reproduction in a tristylos, clonal, aquatic plant, *Decodon verticillatus*, and investigated the role of ecological and genetic factors in causing this variation.

2 We surveyed components of sexual fertility and vegetative growth in 28 populations distributed along a 500-km latitudinal transect in New England, USA. Northerly populations tend to be monomorphic (M) for style length, and probably therefore have reduced sexual reproduction compared with southerly, trimorphic (T) populations.

3 Compared with T populations (*n* = 10), M populations (*n* = 18) exhibited large reductions for all components of sexual reproduction, including flower production, pollen deposition, pollen tube growth, fertilization, fruit set and seeds per fruit. Seven M populations produced no seed at all, and the other 11 very little (mean = 24 vs. 1139 seeds per plant in trimorphic populations). Clonal propagation was also greatly reduced in M populations.

4 A survey of three polymorphic allozyme loci detected only single, usually heterozygous, genotypes in 15 M populations, whereas all T populations were genotypically diverse. The other three M populations contained three or fewer genotypes and one always predominated. Sexual recruitment is therefore extremely rare.

5 Comparison of the sexual fertility of M and T populations in a concurrent common glasshouse experiment with our field data revealed that reduced sexual performance in northern M populations is principally due to genetic factors, but is also caused by ecological factors that covary with latitude.

6 This abrupt shift away from sexual reproduction in populations at the northern periphery of the geographical range in *D. verticillatus* may greatly limit their evolutionary potential and restrict further northward expansion.

Key-words: clonal reproduction, genotypic diversity, peripheral populations, pollination, sexual sterility

Introduction

Most perennial plants combine sexual reproduction with some form of clonal propagation (Richards 1986). The balance between these forms of reproduction affects propagule size, establishment dynamics and the trans-
greatly reduces pollen tube growth (Eckert 1999). The consistent infertility of the fourth popu-
lation (mean = 176) combined with a thorough search of the populations where the sample was mono-
D. verticillatus (L.) Ell. (Lythraceae) is a diploid, tristylos perennial common to a variety of wetland
habitats throughout eastern-central North America (Graham et al. 1985; Eckert & Barrett 1994a). The showy
magenta flowers are visited primarily by bumble bees (Bombus spp.), honey bees (Apis mellifera) and, in the
southern portion of its range, various butterflies (C.G. Eckert, unpublished data). Unlike most tristylos
species, D. verticillata is highly self-compatible, though not autogamous (Eckert & Barrett 1994a). On aver-
age, 30% of progeny are produced by self-fertilization (Eckert & Barrett 1994b; C.G. Eckert, unpublished
data), which occurs primarily through geitonogamy (Eckert 2000). Inbreeding depression is nevertheless
strong (Eckert & Barrett 1994c).

Clonal reproduction occurs when, upon contacting water or moist soil, branch tips greatly thicken and
produce much buoyant, aerenchymous stem tissue as well as adventitious roots. Although clonal progeny may
disperse widely on the water surface when the above ground shoots die over winter, most probably remain
rooted in the substrate within 2 m of the parent plant, to which they are no longer connected.

STUDY POPULATIONS
We sampled 18 monomorphic and 10 trimorphic popu-
lations, distributed along a 500-km transect running parallel to the Atlantic coast in New England (Fig. 1).
The entire transect is within the northern portion of the species’ range, but there is a marked S–N transition
from trimorphic to florally monomorphic populations (Eckert & Barrett 1992). Reported style morph ratios
were based on a random sample of 23–596 plants per population (mean = 176) combined with a thorough
search of the populations where the sample was mono-

© 2001 British Ecological Society.
1 km on the same river, were considered part of the same population (following Eckert & Barrett 1992). We used 1990 estimates for population size (N = the number of ramets, both flowering and non-flowering; Eckert & Barrett 1992) since neither size or morph structure vary significantly between years (Eckert & Barrett 1995). Overall, N ranged from 47 to 10,000 ramets (median = 312), with trimorphic populations significantly larger than monomorphic ones (mean N – SE = 2972 ± 1257 vs. 1312 ± 626, median = 700 vs. 200, range = 47–10,000 vs. 75–10,000; Wilcoxon test P = 0.036).

SEXUAL REPRODUCTION IN NATURAL POPULATIONS

Flower production and fruit development
In September, we haphazardly sampled 20 plants from each population, paying no attention to whether plants were flowering. For every branch longer than one inter-node, we recorded the flowers or fruits present using a 5-point scale: 0; 1–10; 11–30; 31–60; and > 60. Flower production was estimated as the sum of flower scars and developing fruits. We then transformed these indices to the middle value of the corresponding interval (80 for the > 60 category) and summed the values across all branches on each plant. Developing fruits per flower were then calculated for each plant.

The size of each plant (branches per plant) was estimated as the number of primary branches originating directly from the rootstock plus one half the number of secondary branches which originated from axillary meristems on primary branches, which were given reduced weight because of their smaller size. The ratio of secondary to primary branches did not differ between monomorphic (mean ± SE = 0.52 ± 0.05) and trimorphic (0.42 ± 0.06) populations (t = 1.3, d.f. = 26, P = 0.20). Clonal reproduction (clonal progeny per branch) was estimated for each plant as the number of branches that were rooting at their tip divided by the weighted number of branches. We also scaled the flower production (flowers per branch) of each plant by plant size.

Pollen deposition and pollen tube growth
We sampled single flowers collected about 48 hours after anthesis from up to 100 plants throughout each population during peak flowering in August 1996, except for two monomorphic populations (ME-M5 and ME-M14) which produced very few flowers. Although D. verticillatus can be pollinated up to 2 days after anthesis, virtually all stigmatic pollen capture occurs during the first 12 hours (C.G. Eckert, G.P. Grabas and C. Münch, unpublished data) and pollen tube growth is complete by 48 hours after pollination (Eckert & Allen 1997). Pollen deposition was quantified by removing the stigmas from a subsample of 20 flowers per population, mounting them individually in fuschin jelly, and counting the number of D. verticillatus and other pollen grains under 50x (Kearns & Inouye 1993). We calculated the number of conspecific and heterospecific grains per stigma and the proportion of conspecific pollen. The number of pollen tubes in each style and the number of ovules per ovary penetrated by a tube were counted in a separate random sample of 10 pistils from each trimorphic population and 20 pistils from each monomorphic population following Eckert & Allen (1997). Pollen tube counts were performed blind to the identity of the population from which samples originated.

Fruit and seed production
We revisited all 28 populations in October 1996 to estimate mature fruit and seed production. We counted the number of fruits on every branch on each of 30 haphazardly sampled plants per population, and the number of seeds in a sample of up to five fruits per plant.
Average seeds per fruit was multiplied by the number of fruits to estimate the number of seeds produced per plant. The average number of mature fruits and seeds per flower were calculated for each population using the September estimate of flowers per plant.

Statistical analysis

We used nested analysis of variance (ANOVA) to evaluate differences between monomorphic and trimorphic populations for flowers per plant, flowers per branch, conspecific pollen per stigma, heterospecific pollen per stigma, proportion conspecific pollen, pollen tubes per style, penetrated ovules per ovary, developing fruits per flower, seeds per fruit, seeds per plant, branches per plant, clonal progeny per plant and clonal progeny per branch. Population type (monomorphic vs. trimorphic) was a fixed effect and population, nested within population type, was a random effect. Because we sampled a different number of monomorphic and trimorphic populations, we used the Satterthwaite method to perform approximate tests of significance (Sokal & Rohlf 1995).

Residuals were rarely normally distributed and usually correlated with predicted values, and variables could not be transformed to meet assumptions of ANOVA because of the substantial number of zeros in the data, particularly in monomorphic populations. However, values for monomorphic and trimorphic populations were sufficiently different that changing the scale of the data had no effect on the outcome of any particular F-test. In addition, all significant contrasts between monomorphic and trimorphic populations remained significant when population means were compared using non-parametric Wilcoxon tests, so the main results presented below are probably robust to the violations of ANOVA assumptions. All statistical analyses were performed using JMP (version 3.2.1, SAS Institute 1997).

GENOTYPIC DIVERSITY IN MONOMICROPHIC VS. TRIMORPHIC POPULATIONS

In June 1997, 9–13 cuttings (mean = 12) were collected from throughout each of the 18 monomorphic populations and seven of the 10 trimorphic populations (Dorken 1998). Each plant was then assayed following Eckert & Barrett (1993b) for its genotype at three polymorphic allozyme loci, all of which can be reliably scored and obey strict Mendelian segregation (Eckert & Barrett 1994b). We calculated the number of distinct three-locus genotypes detected per sample (G) as well as a measure of genotypic richness, R = (G–1)/(n – 1), which varies from 0 when all n plants in a sample possess the same genotype, to 1.0, when all plants possess a different genotype. Style morph was not recorded, although its inclusion as a fourth genetic marker would have increased the estimates of G for trimorphic but not for monomorphic populations (see Eckert & Barrett 1993b), such that the reported difference in genotypic diversity is probably an underestimate.

One monomorphic population (CT-M1) included a few plants where the flowers consisted of petals only. Although all 12 ramets sampled exhibited the same three-locus genotype and possessed normal long-styled flowers, the additional floral variation is likely to have a genetic basis (Coen & Carpenter 1993), and it is therefore possible that this population included more than one genotype.

CONTRIBUTION OF GENETIC VS. ECOLOGICAL FACTORS IN LIMITING SEX

The fertility of the 18 monomorphic and seven trimorphic populations surveyed for sexual reproduction and genotypic diversity has been compared in a common glasshouse environment (Eckert et al. 1999). The cuttings sampled from each plant (one each from self- and cross-pollinations) were grown to flowering in a randomized array under standard glasshouse conditions. For each plant in the experiment that flowered during the summer of 1997, one sample of flowers was self-pollinated and another was cross-pollinated with pollen from a plant from the same population. Treatments were subsequently pooled because there were no differences in components of fertility between self- and cross-pollinations (Eckert et al. 1999).

We used a subsample of about eight pollinated flowers per plant (average = 31 flowers per population) to estimate pollen tubes per style and penetrated ovules per ovary (as above). The remaining pollinated flowers (average = 107 flowers per population) were allowed to produce fruits and seeds, which were counted to estimate the number of seeds per fruit for each plant.

Pollen tubes per style, penetrated ovules per ovary and seeds per fruit (which were determined similarly in both studies) were compared between environments. Higher reproductive performance in the glasshouse (subset of data from Eckert et al. 1999) than the field (this study) would indicate that environmental factors limit sexual reproduction in natural populations. If the field environment is partly responsible for the large difference in sexual fertility between monomorphic and trimorphic populations, we would expect the former to experience the larger increase in fertility when pollinated under glasshouse conditions, providing that all populations exhibit near-maximal fertility under glasshouse conditions. Variation in the fertility change across environments was evaluated by testing the interaction between the effects of population type (monomorphic vs. trimorphic: a between-subject factor) and environment (field vs. glasshouse: a within-subject factor) in a repeated-measures ANOVA (Neter et al. 1990). A significant interaction indicates that monomorphic and trimorphic populations responded differently to the change in environment. We used the Box-Cox method (Neter et al. 1990) to transform the raw data to meet assumptions of ANOVA, with Y′ = (Y + 1 – 0.2)/0.0211 for pollen tubes per style, Y′ = 4.0 ln(Y + 1) for penetrated ovules per ovary and Y′ = ([Y + 1]^{1/2} – 1)/0.0465 for seeds per fruit.
Decodon in Northern Reduced sexuality


Variable Mono Tri Mono Tri Mono Tri
Flowers per plant 74.0 ± 13.9 92.1 ± 17.2 51.5 79.3 0.0–173.7 33.5–169.3
Flowers per branch 3.0 ± 0.7 10.5 ± 1.7 1.6 11.2 0.0–11.1 3.5–20.1
Conspicuous pollen per stigma 99.3 ± 16.2 167.4 ± 14.8 96.3 171.5 17.6–214 73.3–292.7
Heterospecific pollen per stigma 24.2 ± 7.9 11.8 ± 3.7 29.1 5.3 1.3–122.7 3.0–36.9
Proportion conspecific pollen 0.824 ± 0.037 0.933 ± 0.017 0.885 0.957 0.564–0.989 0.835–0.985
Pollen tubes per style 14.1 ± 4.3 62.6 ± 6.8 7.3 54.4 1.4–73.0 33.5–106.2
Penetrated ovules per ovary 0.63 ± 0.13 11.88 ± 1.88 0.55 11.60 0.00–1.60 2.60–22.30
Developing fruits per flower 0.30 ± 0.05 0.67 ± 0.04 0.29 0.68 0.00–0.72 0.44–0.95
Seeds per fruit 0.80 ± 0.31 21.74 ± 2.89 0.21 21.83 0.00–4.01 2.18–37.17
Seeds per plant 14.7 ± 7.0 1139.0 ± 222.0 2.8 1338.8 0.0–114.0 48.6–1855.3

Table 1 Differences in components of sexual reproduction between 18 monomorphic (Mono) and 10 trimorphic (Tri) populations of Decodon verticillatus in New England, USA. All the summary statistics presented are based on population means

<table>
<thead>
<tr>
<th>Variable</th>
<th>Population type</th>
<th>Population</th>
<th>F</th>
<th>P</th>
<th>P</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowers per plant</td>
<td>0.6</td>
<td>0.43</td>
<td>5.9</td>
<td>&lt;0.0001</td>
<td>5.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Flowers per branch</td>
<td>22.5</td>
<td>&lt;0.0001</td>
<td>7.9</td>
<td>&lt;0.0001</td>
<td>4.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Conspicuous pollen per stigma</td>
<td>8.2</td>
<td>0.0084</td>
<td>9.7</td>
<td>&lt;0.0001</td>
<td>15.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Heterospecific pollen per stigma</td>
<td>4.8</td>
<td>0.038</td>
<td>12.8</td>
<td>&lt;0.0001</td>
<td>12.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pollen tubes per style</td>
<td>41.4</td>
<td>&lt;0.0001</td>
<td>7.3</td>
<td>&lt;0.0001</td>
<td>4.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Penetrated ovules per ovary</td>
<td>86.8</td>
<td>&lt;0.0001</td>
<td>4.3</td>
<td>&lt;0.0001</td>
<td>6.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Developing fruits per flower</td>
<td>27.7</td>
<td>&lt;0.0001</td>
<td>17.3</td>
<td>&lt;0.0001</td>
<td>6.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Seeds per fruit</td>
<td>91.4</td>
<td>&lt;0.0001</td>
<td>17.3</td>
<td>&lt;0.0001</td>
<td>6.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Seeds per plant</td>
<td>48.3</td>
<td>&lt;0.0001</td>
<td>6.1</td>
<td>&lt;0.0001</td>
<td>16.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Branches per plant</td>
<td>5.5</td>
<td>0.027</td>
<td>4.5</td>
<td>&lt;0.0001</td>
<td>4.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Clonal progeny per plant</td>
<td>0.004</td>
<td>0.048</td>
<td>5.1</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Clonal progeny per branch</td>
<td>4.3</td>
<td>0.048</td>
<td>5.1</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Results

SEXUAL REPRODUCTION IN MONOMORPHIC VS. TRIMORPHIC POPULATIONS

All components of sexual reproduction were much lower in populations that were monomorphic for style length than those that were trimorphic (Tables 1 & 2). Individual branches in monomorphic populations produced 71% fewer flowers and stigmas, carried 41% fewer conspecific pollen grains. Although heterospecific pollination was frequent in some monomorphic populations, the substantial variation among populations within types meant that the effect of type was not significant. The conspecific fraction of the pollen load was 12% lower in monomorphic populations. The number of pollen tubes per style and penetrated ovules per ovary were reduced by 77% and 95%, respectively, with only one monomorphic population (ME-M12) in the range of trimorphic populations for pollen tubes per style and no overlap for penetrated ovules per ovary. Developing fruits per flower were 55% lower in monomorphic populations (Table 1), mature fruits per flower were reduced by 91% (mean ± SE: 0.042 ± 0.012 vs. 0.474 ± 0.094, t = 5.8, df = 25, P < 0.0001), seeds per fruit by 96%, seeds per flower by 99% (0.089 ± 0.043 vs. 10.4 ± 1.8, t = 7.3, df = 25, P < 0.0001), and seeds per plant by 99%. On average, monomorphic populations produced only 15 seeds per plant compared to 1139 in trimorphic populations, and, while all 10 trimorphic populations produced seed, seven of 18 monomorphic populations produced no seed at all (Fisher’s exact test P = 0.027). Among the 11 monomorphic populations that did produce seed, the number of seeds per plant was very low (mean ± SE = 24.1 ± 10.8, median = 7.9, range = 0.08–114). For each of the four measures of fruit and seed production, only one trimorphic population (MA-T6) fell within the range of monomorphic populations.

A more detailed inspection of the data (not shown) revealed little heterogeneity in reproductive performance among plants within populations. Individual plants within monomorphic populations exhibited uniformly low values for most components of sexual reproduction, well below the range of the means for trimorphic populations.
We detected 13 distinct three-locus genotypes among 18 monomorphic (Mono) and 10 trimorphic (Tri) populations of *Decodon verticillatus* in New England, USA. All the summary statistics presented are based on population means.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Means ± SE</th>
<th>Medians</th>
<th>Ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branches per plant</td>
<td>33.4 ± 5.3</td>
<td>16.1 ± 2.6</td>
<td>26.1 ± 1.8</td>
</tr>
<tr>
<td>Clonal progeny per plant</td>
<td>1.72 ± 0.26</td>
<td>1.75 ± 0.29</td>
<td>1.78 ± 0.18</td>
</tr>
<tr>
<td>Clonal progeny per branch</td>
<td>0.077 ± 0.016</td>
<td>0.134 ± 0.024</td>
<td>0.067 ± 0.130</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.001–0.201</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.028–0.288</td>
</tr>
</tbody>
</table>

Table 3 Differences in components of vegetative growth and clonal regeneration between 18 monomorphic (Mono) and 10 trimorphic (Tri) populations of *Decodon verticillatus* in New England, USA. All the summary statistics presented are based on population means.

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>G</th>
<th>R</th>
<th>AA</th>
<th>AB</th>
<th>BB</th>
<th>AA</th>
<th>AB</th>
<th>BB</th>
<th>AC</th>
<th>BB</th>
<th>BC</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH-T2</td>
<td>13</td>
<td>6</td>
<td>0.67</td>
<td>0.46</td>
<td>0.46</td>
<td>0.08</td>
<td>0.00</td>
<td>0.54</td>
<td>0.46</td>
<td>0.08</td>
<td>0.15</td>
<td>0.00</td>
<td>0.15</td>
</tr>
<tr>
<td>MA-T7</td>
<td>13</td>
<td>6</td>
<td>0.42</td>
<td>0.00</td>
<td>0.38</td>
<td>0.62</td>
<td>0.08</td>
<td>0.62</td>
<td>0.31</td>
<td>0.00</td>
<td>0.46</td>
<td>0.00</td>
<td>0.38</td>
</tr>
<tr>
<td>MA-T2</td>
<td>12</td>
<td>6</td>
<td>0.82</td>
<td>0.17</td>
<td>0.75</td>
<td>0.08</td>
<td>0.00</td>
<td>0.25</td>
<td>0.75</td>
<td>0.00</td>
<td>0.08</td>
<td>0.08</td>
<td>0.67</td>
</tr>
<tr>
<td>MA-T1</td>
<td>10</td>
<td>3</td>
<td>0.22</td>
<td>0.00</td>
<td>1.00</td>
<td>0.00</td>
<td>0.20</td>
<td>0.70</td>
<td>0.10</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>MA-T10</td>
<td>14</td>
<td>4</td>
<td>0.27</td>
<td>0.00</td>
<td>0.83</td>
<td>0.17</td>
<td>0.00</td>
<td>0.00</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.50</td>
<td>0.00</td>
</tr>
<tr>
<td>MA-T9</td>
<td>9</td>
<td>5</td>
<td>0.50</td>
<td>0.00</td>
<td>0.44</td>
<td>0.56</td>
<td>0.00</td>
<td>0.12</td>
<td>0.88</td>
<td>0.00</td>
<td>0.00</td>
<td>0.22</td>
<td>0.67</td>
</tr>
<tr>
<td>CT-T1</td>
<td>12</td>
<td>7</td>
<td>0.54</td>
<td>0.08</td>
<td>0.67</td>
<td>0.25</td>
<td>0.00</td>
<td>0.00</td>
<td>1.00</td>
<td>0.08</td>
<td>0.08</td>
<td>0.17</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Although plants in monomorphic populations consisted of twice as many branches, they produced the same number of clonal progeny as those in trimorphic populations. Clonal progeny per branch was therefore the same number of clonal progeny as those in trimorphic populations, no more than three genotypes were detected in each of the monomorphic populations, no more than three genotypes were detected in each of the monomorphic populations.

**Table 4** Genotype frequencies for three polymorphic allozyme loci in seven trimorphic and 18 monomorphic populations of *Decodon verticillatus*. The electrophoretic migration distances are denoted in alphabetical order with A as the fastest migrating allele. The number of distinct genotypes detected (G) and a measure of genotypic richness (R) are also presented. In each population, the number of ramets sampled (n) was the same for all loci. The style morph fixed in each of the monomorphic populations is indicated in brackets.

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>G</th>
<th>R</th>
<th>MDH</th>
<th>IDH</th>
<th>ACP</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH-M1 (L)</td>
<td>12</td>
<td>2</td>
<td>0.09</td>
<td>0.25</td>
<td>2.70</td>
<td>0.28</td>
</tr>
<tr>
<td>ME-M14 (L)</td>
<td>12</td>
<td>2</td>
<td>0.09</td>
<td>0.25</td>
<td>2.70</td>
<td>0.28</td>
</tr>
<tr>
<td>ME-M16 (L)</td>
<td>12</td>
<td>2</td>
<td>0.09</td>
<td>0.25</td>
<td>2.70</td>
<td>0.28</td>
</tr>
<tr>
<td>ME-M17 (L)</td>
<td>12</td>
<td>2</td>
<td>0.09</td>
<td>0.25</td>
<td>2.70</td>
<td>0.28</td>
</tr>
<tr>
<td>ME-M18 (L)</td>
<td>12</td>
<td>2</td>
<td>0.09</td>
<td>0.25</td>
<td>2.70</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Although plants in monomorphic populations consisted of twice as many branches, they produced the same number of clonal progeny as those in trimorphic populations. Clonal progeny per branch was therefore the same number of clonal progeny as those in trimorphic populations. Clonal progeny per branch was therefore the same number of clonal progeny as those in trimorphic populations. Clonal progeny per branch was therefore the same number of clonal progeny as those in trimorphic populations. Clonal progeny per branch was therefore about 50% lower (Tables 2 and 3). Distributions of all three variables overlapped between population types.

**Genotypic diversity in monomorphic vs. trimorphic populations**

We detected 13 distinct three-locus genotypes among 18 florally monomorphic populations (n = 214 ramets) and 23 genotypes among seven trimorphic populations (n = 81 ramets). The number of distinct genotypes per sample (G) was much lower for monomorphic than trimorphic populations (Table 4; t = 8.2, d.f. = 23, P < 0.0001). We found only single genotypes, of which only one was homozygous for all loci, in 15 of the 18 florally monomorphic populations. In the other three monomorphic populations, no more than three genotypes were detected, and one heterozygous genotype predominated in each. The average genotypic richness (R) was less than 0.02 (se = 0.012). In contrast, samples from trimorphic populations included 3–10 distinct genotypes with an average G of 0.49 ± 0.08 (t = 9.1, d.f. = 23, P < 0.0001).
Reduced sexuality in Northern Decodon

Different monomorphic populations were rarely fixed for the same multilocus genotype. Only three pairs of single-genotype populations shared the same combination of style morph and allozyme genotype (Table 4), and only one of these (ME-M3 and ME-M4), located 3 km apart, were in the same watershed.

CONTRIBUTION OF GENETIC VS. ECOLOGICAL FACTORS IN LIMITING SEX

When grown in a common glasshouse environment, plants from monomorphic populations again exhibited lower values for all components of sexual reproduction, including pollen tubes per style, penetrated ovules per ovary and seeds per fruit (Eckert et al. 1999). However, monomorphic populations were generally much more fertile in the glasshouse than in the field (Fig. 2). The number of pollen tubes per style increased by more than 30% between environments, although the difference is not significant (paired \( t = 0.7, P = 0.47 \)). Much larger and statistically significant increases were seen for both penetrated ovules per ovary (\( > +500\%, t = 3.9, P = 0.0015 \)) and seeds per fruit (\( > +1500\%, t = 5.2, P = 0.0002 \)). In contrast, the average fertility of trimorphic populations did not differ between environments for pollen tubes per style (\( -45\%, t = 1.7, P = 0.15 \)), penetrated ovules per ovary (\( -16\%, t = 0.6, P = 0.59 \)), or seeds per fruit (\( +17\%, t = 0.7, P = 0.53 \)). The differential response of monomorphic vs. trimorphic populations to the change from field to glasshouse is more formally reflected in a significant interaction between the effects of population type and environment in a repeated-measures ANOVA for all three reproductive parameters (Table 5), though the ANOVA model for pollen tubes per style is not quite significant (\( P = 0.066 \)).

We can estimate the contribution of environmental factors to the suppression of sex in northern populations by comparing the difference in fertility between monomorphic and trimorphic populations measured in the field with that observed in the glasshouse. The mean difference (± SE) was reduced by 66% for pollen tubes per style (55.0 ± 9.3 vs. 18.5 ± 9.7), 40% for penetrated ovules per ovary (13.5 ± 1.4 vs. 8.1 ± 1.9) and 20% for seeds per fruit (24.0 ± 2.2 vs. 19.3 ± 4.8).

Table 5 Analysis of variance in fertility between monomorphic vs. trimorphic populations of Decodon verticillatus in field vs. glasshouse growth environments. A significant interaction between the effects of population type and environment indicates that monomorphic and trimorphic populations are responding differently to the change in growth environment. Because the effect of population was far from significant in all analyses (all \( P > 0.6 \)), it is not reported below. Population means are in Fig. 2.

<table>
<thead>
<tr>
<th>Source of variation (d.f.)</th>
<th>Tubes per style</th>
<th>Penetr. ovules</th>
<th>Seeds per fruit*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole model (22/19*)</td>
<td>2.0 (0.066)</td>
<td>24.3 (0.0003)</td>
<td>7.0 (0.0001)</td>
</tr>
<tr>
<td>Population type (1)</td>
<td>26.1 (0.0001)</td>
<td>110.9 (0.0001)</td>
<td>200.6 (0.0001)</td>
</tr>
<tr>
<td>Environment (1)</td>
<td>0.8 (0.39)</td>
<td>3.7 (0.068)</td>
<td>16.1 (0.0010)</td>
</tr>
<tr>
<td>Type × Env. (1)</td>
<td>5.1 (0.037)</td>
<td>8.9 (0.0075)</td>
<td>9.8 (0.0064)</td>
</tr>
<tr>
<td>( r^2 )</td>
<td>0.69</td>
<td>0.46</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Fig. 2 Comparison of components of sexual fertility between field and glasshouse growth environments for New England populations of Decodon verticillatus. Means for monomorphic and trimorphic populations are open circles and filled triangles, respectively. The broken diagonal line shows equal performance in both environments; points above the line indicate higher fertility in the glasshouse than the field. Analyses of pollen tubes per style and penetrated ovules per ovary involve 15 monomorphic and six trimorphic populations. The analysis of seeds per fruit involves 13 monomorphic and five trimorphic populations. Log_{10} scales are used for display purposes only. Analysis of these data is in Table 5.

LATITUDINAL VARIATION IN SEXUAL REPRODUCTION

As expected from the latitudinal trend in floral polymorphism (Fig. 1), all components of sexual reproduction
Table 6 Correlations between latitude (°N) and components of sexual reproduction, vegetative growth and clonal reproduction among 28 populations of Decodon verticillatus in New England. Correlation coefficients are given for analyses involving all populations (All), monomorphic populations only (Mono; \( n = 18 \)) and trimorphic populations only (Tri; \( n = 10 \)). For components of sexual reproduction, correlations were evaluated using a 1-tailed test of significance with the expectation that the correlation would be negative. For components of vegetative growth and clonal reproduction, 2-tailed tests of significance were used.

<table>
<thead>
<tr>
<th>Component</th>
<th>All</th>
<th>Mono</th>
<th>Tri</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowers per branch</td>
<td>-0.58***</td>
<td>-0.08</td>
<td>-0.004</td>
</tr>
<tr>
<td>Conspicous pollen per stigma</td>
<td>-0.44*</td>
<td>-0.10</td>
<td>+0.03</td>
</tr>
<tr>
<td>Penetrated ovules per ovary</td>
<td>-0.64***</td>
<td>-0.15</td>
<td>+0.31</td>
</tr>
<tr>
<td>Mature fruits per flower</td>
<td>-0.74***</td>
<td>-0.17</td>
<td>-0.37</td>
</tr>
<tr>
<td>Seeds per fruit</td>
<td>-0.54**</td>
<td>-0.47*</td>
<td>+0.64</td>
</tr>
<tr>
<td>Seeds per plant</td>
<td>-0.70**</td>
<td>-0.70***</td>
<td>-0.07</td>
</tr>
<tr>
<td>Branches per plant</td>
<td>-0.70***</td>
<td>-0.31</td>
<td>-0.07</td>
</tr>
<tr>
<td>Clonal progeny per branch</td>
<td>+0.38</td>
<td>+0.15</td>
<td>-0.51</td>
</tr>
</tbody>
</table>

Statistical significance is denoted as: *** \( P < 0.001 \), ** \( P < 0.01 \), * \( P < 0.05 \).

Fig. 3 Correlations between latitude and components of sexual reproduction among 28 populations of *Decodon verticillatus* in New England. Means for monomorphic and trimorphic populations are open circles and filled triangles, respectively. Correlation coefficients for these and other variables are in Table 6.

There was a strong association between low seed production and reduced genotypic diversity that suggests, over the long-term, seed production limits sexual recruitment of *D. verticillatus* at its northern range margin.

**Discussion**

*Decodon verticillatus* exhibits extremely wide variation in sexual reproduction among natural populations over a modest geographical scale. Populations at the northern periphery of the range in New England produced little, if any, seed while most populations about 300 km further south set abundant seed. Low seed production has been noted in peripheral populations of several species of plants (e.g. Sculthorpe 1967; Hutchinson 1975) but comparisons with central populations (e.g. Barrett 1980a; McKee & Richards 1996; García et al. 2000) or investigations of the ecological and genetic factors causing such variation (e.g. Barrett 1980b) are rare. The glasshouse experiment revealed a strong genetic basis for the reduced sexual fertility of monomorphic populations, but our comparison of fertility measurements in the glasshouse and field indicated that environmental factors also limit fertility in natural populations (Fig. 2). The strong association between low seed production and reduced genotypic diversity suggests that, over the long-term, seed production limits sexual recruitment of *D. verticillatus* at its northern range margin.

**LOW GENOTYPIC DIVERSITY AND LONG-TERM SEXUAL RECRUITMENT IN NORTHERN POPULATIONS**

Broad, interspecific surveys of allozyme variation in plants suggest that clonal reproduction is not generally associated with greatly reduced genotypic diversity (Ellstrand & Roose 1987; Hamrick & Godt 1990), but intraspecific variation across ecological gradients has rarely been quantified (Les 1991; Eckert & Barrett 1993b; Piquot et al. 1996; Kudoh et al. 1999). In *D. verticillatus*, however, most northerly populations appeared to consist of single heterozygous clonal genotypes whereas all southerly populations were genotypically diverse (Table 3). These results are in accord with a smaller-scale survey of allozyme variation comparing four northern populations from Ontario and Michigan and 12 central and southern populations from Ontario, Michigan and Georgia (Eckert & Barrett 1993b).

Although the number of plants (\( n = 12 \) per population) and loci (\( n = 3 \)) sampled are small, the data do suggest that populations are almost exclusively clonal. Because of dominance at the two loci governing the inheritance of style length, sexual reproduction will usually produce more than one style morph (Eckert & Barrett 1993a), and only populations consisting only of the long-styled morph (eight of the 18 monomorphic populations in our sample) will remain monomorphic in the face of sexual recruitment. The confirmation, by extensive sampling, of floral monomorphism strongly supports the inference of clonal monomorphism based on allozymes. Furthermore, all but one of the monomorphic populations in which we detected single genotypes were heterozygous for at least one of the three marker loci. Genotypic monomorphism combined with...
Reduced sexuality in Decodon

Reduced sexuality strongly indicates that sex rarely, if ever, occurs in these monomorphic populations (see also Solis et al. 1988). Calculations of the probability of detecting only a single heterozygous genotype in a finite sample of ramets in a population experiencing a mixture of sexual and clonal reproduction confirms this interpretation (Appendix 1, in the Journal of Ecology archive on the World Wide Web; see the cover of a recent issue of the journal for the WWW address).

There are four possible explanations for the clonal monomorphism of northern populations: (1) A single individual genotype arrived at each population and then became established through exclusive clonal reproduction. (2) More than one individual genotype arrived at each population, but only one was able to cope with the biotic and/or abiotic environment in the north. (3) Several genotypes established but demographic stochasticity eventually eliminated all but one from each population. (4) A single genotype survived elimination by intraspecific competition among those initially present. It is also possible that different processes may be responsible for clonal monomorphism in different populations.

Factors Causing Reduced Sexual Reproduction in Northern Populations

Pollination, fertilization and seed maturation

Successful sexual reproduction in plants involves a series of events beginning with flower development and ending with the recruitment of progeny into the adult population, and is impaired here at several stages between flower development and seed maturation (Table 1). The lower flower production in monomorphic populations combined with their smaller population size may attract fewer pollinators than in trimorphic populations. Lower flower density may also result in low pollen loads on individual pollinators, further reducing the deposition of conspecific pollen. Data on pollinator abundance and visitation in monomorphic vs. trimorphic populations are needed to evaluate these possibilities (e.g. Sih & Baltus 1987; Jennensten 1988).

Reduced pollen deposition in monomorphic populations was, in turn, associated with fewer pollen tubes in styles and ovules penetrated. Some of the difference in pollen tube numbers could be due to higher levels of self-pollination because natural pollinations will involve mostly self-pollen in single-genotype monomorphic populations, compared with a mixture of self- and cross-pollen in trimorphic populations. Because pollen tube growth in this species is faster for cross- than self-pollen, and this is manifested in greater numbers of cross-tubes and, in natural populations of *D. verticillatus*, almost all pollen deposition occurs within the first 12 hours after anthesis (Eckert, G.P. Grabas and C. Münch, unpublished data), so that the difference in tube numbers disappears by 36 hours after pollination (Eckert & Allen 1997). We collected flowers 48 hours after anthesis. Slower growth of self-tubes is therefore unlikely to explain much of the large difference in pollen tube numbers.

Ultimately, low pollination and pollen tube growth in monomorphic populations is associated with extremely scant seed production. In fact, plants sampled from seven of 18 monomorphic populations produced no seed at all. There is very little difference in seed production by *D. verticillatus* after self-compared with cross-pollination (Eckert & Barrett 1994a), suggesting that early acting inbreeding depression following self-pollination accounts for little of the striking reduction in seed production in monomorphic populations.

Sex is impaired at several stages

If sexual reproduction was being impaired at only one critical developmental stage, the average proportional difference between monomorphic and trimorphic populations should remain more or less constant throughout later stages. Our results, however, show a consistent increase from 41% for pollen deposition, to 77% for pollen tube number, 95% for ovules penetrated by a pollen tube, and 99% for seeds per flower. More detailed analysis (not shown) revealed that, although the relative reproductive performance of each monomorphic population tends to decrease incrementally at each stage, populations differ in terms of which stage is most severely impaired. For example, low seed production in populations ME-M5 and ME-M14 was due to the production of very few flowers whereas ME-M13 produced abundant flowers but few were pollinated, and as much pollen was deposited in ME-M3 and ME-M15 as in trimorphic populations but pollen tube growth and ovule penetration were severely impaired. Reduced sexual reproduction is probably due to the impairment of almost all stages, including pollination, fertilization and seed formation, to varying degrees in individual populations, rather than the failure of any single stage.

The association between seed fertility and genotypic diversity is seen among monomorphic populations as well as very clearly between monomorphic and trimorphic populations. All of the seven monomorphic populations that failed to produce any seed consisted of single genotypes. Of the 11 populations that produced some seed, three, including two with the highest seed production, contained more than one genotype. Low seed production therefore seems to limit the recruitment of sexually produced progeny in monomorphic populations. Too few seeds were produced to test whether sexual recruitment is further limited by germination and seedling establishment, but even if this does not occur the difference in seed fertility between monomorphic
and trimorphic populations (more than two orders of magnitude) is sufficient to cause very large differences in sexual recruitment.

Both genetic and ecological factors cause reduced sexual reproduction

Monomorphic populations exhibited much lower fertility than trimorphic populations when compared in a common, and probably more benign, glasshouse environment (Fig. 2), and this was also observed in the glasshouse conditions (Eckert et al. 1999). Moreover, reduced pollen tube growth and ovule penetration, which contributed substantially to reduced seed production under field conditions, were also observed in the glasshouse. It seems therefore that one or more intrinsic, probably genetic, factors were the primary cause of the reduced sexual fertility of monomorphic populations observed in the field (Eckert et al. 1999). Detailed genetic analysis involving an infertile population from Ontario suggests that infertility may be the result of specific nuclear sterility mutations (Eckert et al. 1999). Sexual infertility is unlikely to involve the accumulation of generally deleterious mutations in old clones, as recent glasshouse experiments have shown that there is no difference in vegetative growth between monomorphic and trimorphic populations (C.G. Eckert, M.E. Dorken and F. Thompson, unpublished data).

A substantial role for ecological factors in reducing the fertility of monomorphic populations is also indicated because most exhibited higher fertility in the glasshouse than in the field (Fig. 2). For example, monomorphic population ME-M8 produced almost as many seeds per pollination in the glasshouse as trimorphic populations, whereas seed production in the field was well below the range of trimorphic populations. Moreover, this population appears to be fixed for a genotype that is heterozygous at two loci (Table 3), implying that sexual reproduction is rare (see Appendix 1 in the Journal of Ecology archive on the World Wide Web). In contrast, fertility of trimorphic populations differed little between environments. Our comparisons suggest that environmental factors may be responsible for about 20% of the difference in seeds per fruit between monomorphic and trimorphic populations observed in the field, although because most monomorphic populations have extremely low fertility even under benign glasshouse conditions, this may be an underestimate. Reciprocal transplants of inherently fertile genotypes between the locations of trimorphic and monomorphic populations would help clarify this issue.

It is likely that sexual reproduction is limited by one or more biotic and/or abiotic factors that covary with latitude. Both the prevalence of monomorphic populations (Fig. 1 and Eckert & Barrett 1992) and sexual reproduction of our study populations (Table 6) covary strongly with latitude. Although southerly, trimorphic populations are inherently much more fertile than northerly, monomorphic populations, even under glasshouse conditions (Fig. 2), latitude also correlates negatively with fruit and seed production when the analysis is restricted to monomorphic populations (Table 6), and these correlations are not confounded by any relation between latitude and the inherent sexual fertility of monomorphic populations (all $P > 0.22$).

Vegetative growth and survival may also covary with latitude. Plants in monomorphic populations produced fewer clonal progeny per branch but consisted of more branches than those in trimorphic populations (Table 3): both correlated negatively with latitude, although only branches per plant correlated significantly (Table 6). The propensity for individual branches to produce clonal progeny is largely a function of apical extension, because branches must make contact with wet substrate for rooting to occur, suggesting lower vegetative growth in monomorphic populations. Despite this, plants in monomorphic populations tended (to be larger (Table 3) suggesting that the recruitment of any type of progeny (either sexual or clonal) is less frequent. Preliminary results from an ongoing experiment suggest that the difference in vegetative performance between monomorphic and trimorphic populations observed in the field disappears when they are grown in a common glasshouse environment (C.G. Eckert, M.E. Dorken and F. Thompson, unpublished data).

Although we cannot, as yet, tell which of several potential biotic and abiotic factors are critical, temperature may well be a major determinant of sexual reproduction in northern populations of *D. verticillatus*. First, temperature has been shown to affect many aspects of sexual reproduction in plants (e.g. Herrera 1995; McKee & Richards 1996; Mikesell 1997; Woodward 1997) and is widely thought to shape the geographical distributions of plant species (e.g. Woodward 1990; Beerling 1993; reviewed in Salisbury 1942). Secondly, temperature usually varies with latitude and may therefore explain the common transition from trimorphism to monomorphism observed in *D. verticillatus* across geologically and ecologically diverse regions such as Ontario, Michigan and New England (Eckert & Barrett 1992). Finally, because both monomorphic and trimorphic populations occur in a wide variety of habitats (lake margins, marshes, swamps, bogs), there is unlikely to be a consistent difference in any other ecological variable that could so drastically affect long-term sexual reproduction across the northern edge of the species range.

**Ecology and evolution of geographic range limits**

Although few studies have investigated intraspecific variation in sexual reproduction, it is likely to be common in plants where clonal reproduction can occur across a wider range of environments than sexual reproduction (Sculthorpe 1967; Philbrick & Les 1996). Moreover, large-scale variation in sexuality may commonly affect the demography and genetics of populations near geographical range limits.
Compared to sexually derived seed, clonal propagules are usually larger and more vulnerable to desiccation and therefore have limited dispersal potential (Silander 1985). Even independent, floating clonal propagules, such as those produced by *D. verticillatus*, are likely to move much shorter distances than seeds in the still water of the habitats where this species typically occurs. The observation that northern populations of *D. verticillatus* usually consist of single clones and that individual clones are usually restricted to single populations also suggests that gene flow is infrequent at the range periphery. The geographical range may also be limited because the extremely low genotypic diversity associated with exclusively clonal reproduction restricts the capacity of peripheral populations to survive and adapt to various abiotic and biotic challenges (Burdon & Marshall 1980; Hoffman & Blows 1994). In general, severe declines in ecological and evolutionary potential towards geographic range boundaries may be expected in species where environmental factors cause variation in the relative importance of sexual vs. clonal reproduction.

**References**


Jennersten, O. (1988) Pollination in
Kudoh, H., Shibaike, H., Takasu, H., Whigham, D.F. &
Klekowski, E.J. Jr (1988b) Mutation, Developmental Selection
and Plant Evolution. Columbia University Press. New York, NY
Krahulcová, A. & Jarolímová, V. (1993) Ecology of two cyto-
types of Batrunus umbellatus I. Karyology and breeding.
Folia Geobotanica et Phytotaxonomica, 28, 385–411
Kudoh, H., Shibaike, H., Takasu, H., Whigham, D.F. &
McKeen, J. & Richards, A. J. (1996) Variability in seed produc-
tion and germinability in common reed (Phragmites australis) in Britain and France with respect to climate. New Phytologist, 133, 233–243.
Olivieri, L., Michalakos, Y. & Gouyon, P.H. (1995) Meta-
population genetics and the evolution of dispersal. American Naturalist, 146, 202–228.
Piquot, Y., Samitou-Laprade, P., Petit, D., Vernet, P. &
Epplin, JT. (1996) Genotypic diversity revealed by allozy-
mes and oligonucleotide DNA fingerprinting in French populations of the aquatic macrophyte Sparganium erectum. Molecular Ecology, 5, 251–258.
olution of self-incompatibility and breeding system con-
Unwin, London.
Received 3 January 2000
revision accepted 23 October 2000