Reproduction and genetics in fragmented plant populations
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Variable herkogamy and autofertility in marginal populations of Gentianella germanica in The Netherlands

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Abstract: In The Netherlands, Gentianella germanica (Willd.) Börner is a rare plant species, occurring at the margin of its distribution area. It is found only in isolated patches of chalk grassland surrounded by intensively used agricultural land. In 1997 and 1998, we investigated the spatial and temporal separation of anthers and stigma in relation to autofertility. We performed our experiments in one large and two small populations to investigate possible selection against herkogamy and for increased autofertility in marginal populations under pollination limitation. Dichogamy appeared to be very weak and varied within individuals. Homogamous and protandrous flowers were most frequent. Dichogamy is apparently no effective barrier against self-fertilization in this species. Herkogamy varied significantly between individuals and between populations, and plant and flower size were not correlated with anther-stigma separation. Both observations suggest a genetic basis to herkogamy. Autofertility was generally high in all three populations. Only in one small population a correlation between autofertility and herkogamy was found. Here, most plants had the stigma positioned above the anthers (mean herkogamy +0.8 mm), while in the other two populations the stigma was positioned mainly in between or below the anthers (mean herkogamy -0.55 mm). In comparison with earlier data from 1991 and 1992, plants in the large population had become less herkogamous and more self-fertile in 1998. This population has been mown one month earlier than usually since 1993, leading to dramatic reductions (bottlenecks) in population size. In addition, the early mowing time may have caused a further reduction of the already scarce visitation by pollinating insects. We hypothesize that both adverse conditions may have caused a selection for plants with smaller anther-stigma separation and higher autofertility. Through this mechanism, human influence can have profound effects on the reproductive success and evolution of rare plant species, even if it occurs in the context of nature conservation.

INTRODUCTION
The most widespread feature of floral morphology promoting outcrossing in plants is the separation of male and female functions (Baker & Hurd 1968, Fægri & van der Pijl 1979). Herkogamy is the spatial separation of anthers and stigmas (Webb & Lloyd 1986) whereas in dichogamous flowers, pollen presentation and stigma receptivity are separated in time (Lloyd & Webb 1986). In animal-pollinated flowers, protandry is more common than protogyynz (Bertin & Newman 1993) although the latter has been said to give a better protection against self-fertilization. Herkogamy and dichogamy often occur simultaneously in many plant species and are interpreted as mechanisms to reduce self-fertilization.

The separation between anthers and stigma is reflected in many different flower types. Therefore, Webb and Lloyd (1986) described three main classes of herkogamy: interfloral, in which flowers present only pollen or stigmas (monoeicism), reciprocal, with two or three types of hermaphroditic flowers that show reciprocal differences in the position of anthers
and stigma, (heterostyly) and homomorphic, in which all flowers are of one form and hermaphroditic, and pollinator contact is unordered or ordered. In ordered herkogamy, pollen and stigma presentation is related to the foraging path of the pollinator. The nectar-bearing flowers are gullet-, bell- or tube-shaped. A sophisticated pollinator behaviour in these flowers is essential for an effective transfer of pollen to the stigmas. Approach herkogamy, in which the stigma is placed before the anthers, is found for example in the families Liliaceae, Boraginaceae, Ericaceae and Gentianaceae (Webb & Lloyd 1986).

In many angiosperms, herkogamy appears to be a continuously distributed trait (Barrett & Eckert 1990, Carr & Fenster 1994, Lennartsson 1997). In self-compatible plant species with a mixed mating system, an increase in self-pollination will occur in less-herkogamous and homogamous plants when pollinators are scarce (Lloyd 1992). Habitat fragmentation, small population size and isolation may increase pollen limitation because of low visitation rates (Kwak 1988, Kwak et al. 1991). Here, selection for highly autogamous individuals may be expected, but only when inbreeding depression is low (Charlesworth & Charlesworth 1987, Lande & Schemske 1985). However, the high production of seeds that is associated with autofertility may allow for substantial selection on the offspring without serious demographic consequences, even when there is considerable inbreeding depression.

A previous study (Luijten et al. 1998) on the reproduction biology of Gentianella germanica (Willd.) Börner in The Netherlands showed that this rare species is largely self-compatible. However, it was concluded that pollinators are essential for a high seed set. Although mean seed production per flower was reduced significantly in caged, untouched plants (spontaneous seed set <30% as compared to 86% for manually selfed plants), the ability of auto-deposition clearly varied between individuals (seed set ranged from 0 to 90%). We hypothesized that differences in auto-deposition of pollen on stigmas were caused by variation in spatial separation between the anthers and stigma among individuals. This has already been observed by floral biologists from the late 19th century observed that the stigma in G. germanica was always higher than the anthers (Ricca 1870) or that anther-stigma distance varied (Kerner von Marilau 1891, Müller 1881, Schultz 1888). They assigned this variation in herkogamy to differences in style length.

![Figure 1](image_url)

**Figure 1.** A part of the main distribution area of *G. germanica* and the location of our study populations.
Our research questions were as follows: (1) is auto-deposition of pollen on the stigma in *G. germanica* related to the separation of anthers and stigma, and (2) has selection occurred against herkogamous and for more autogamous individuals in small populations? In order to answer these questions, we investigated not only the spatial but also the temporal separation between anthers and stigma and their development during anthesis. We measured various flower parts, and tested plants for their ability to self-fertilize spontaneously. Experiments were performed in one large and two small populations. The results obtained on anther-stigma distance and autofertility in 1998 were compared with data from previous experiments in 1991 (Luijten et al. 1998), 1992 and 1997.

**MATERIALS AND METHODS**

**Study species**

*Gentianella germanica* occurs mainly in central Europe, with some outposts in England and Slovakia (Fig. 1, Meusel et al. 1978). The Dutch populations are situated at the northern margin of the range (Fig. 1).

In The Netherlands, *G. germanica* is a strict biennial (Verkaar & Schenkeveld 1984). Germination occurs in early spring, during the first year a rosette is formed and the plants flower in late summer (August-September) of the second year. Plants vary in size from rather short in stature (<10 cm) bearing few (1-5) flowers to very tall (>40 cm) with many flowers (>100). The purple to pinkish, nectar-producing flowers have a relatively narrow corolla tube with a characteristic rim of fringes in the throat. Flower visitors on *G. germanica* in our study populations were rather scarce. Some small bumblebees and sirphid flies have been seen visiting the flowers. In some years, migrating noctuid moths like *Autographa gamma* may be frequent flower visitors of *G. germanica*. Whether this species is pollinating the flowers is not known, although pollen of *Gentianella* has been found on its body (T. Petanidou, pers. comm.).

**Study populations**

Populations of *G. germanica* are found in small patches of chalk grassland, isolated from each other by large areas of intensively used agricultural land. Most of these chalk grasslands are nature reserves. Our experiments were performed in one large population and two small populations.

The large population WRAKELBERG (abbreviated WRAK) consists of approximately 100,000 individuals and has been present for at least 45 years. The two smaller populations (±100 individuals) have been recently founded. Population EXPERIMENTAL SLOPE (abbr. EXP), located in the valley Gerendal, was founded in 1991 in a rather species-rich grassland vegetation. This formerly intensively used grassland has been restored by an intensive mowing and hay-making regime. A nearby larger population was probably the seed source. The third study population EYS dates from approximately 15 years ago. How and when this population was founded is unknown.

**Development of the different flower parts during anthesis**

In population EXP, the spatial development of various flower parts during anthesis was investigated in early September 1997. On 35 randomly selected plants, flowerbuds were marked with acrylic paint on the calyx. During one week, plants were visited daily. The
spatial arrangement of the flower parts was investigated by measuring the following floral characters (Fig. 2): corolla tube length, pistil length, stamen length, connate part of the filament, free part of the filament and the distance between the top of the anthers and the lowest part of the stigma with papillae (the ‘anther-stigma separation’). Since, these floral parts were measured with a dissecting microscope, the floral development during anthesis could not be determined within the same flower. Per plant different flowers were picked representing different stages (ages) of anthesis. Flowers were picked on the first, second, third and fourth (last) day of anthesis.

**Flower measurements and autofertility experiments**

In 1998, the same flower parts as in 1997 were measured, but this time we only used flowers that were in their first day of anthesis. In addition, we performed experiments in three populations. We were able to study 32 plants in population WRAK, 20 plants in population EYS and 21 plants in population EXP. Of these selected plants, the height was measured and the total number of flowers per plant counted. Then, plants were placed in wire cages covered with fine-meshed gauze to prevent insects from visiting. In order to relate anther-stigma separation to the ability to autofertilize, we needed to measure both variables on the same plant. For each variable, 5 to 10 flower-buds in the same stage of development were marked with acrylic paint. One group of buds was left untouched to test their seed production after spontaneous selfing, while another group was collected to measure the various flower parts. Of each collected flower, the developmental stage of anthers and stigma was also recorded to determine the degree of dichogamy. Anther development was classified in the following categories: (a) closed, (b) just open, (c) open and filled with pollen, (d) empty. Categories of stigma development were: (a) closed, (b) stigma lobes slightly open, (c) lobes fully open (d) lobes curled. Using combinations of these classes within flowers, they could be classified as protandrous, homogamous or protogynous.

Four weeks after the experiment, the mature fruits were collected and filled and empty seeds were counted to determine the reproductive success. Filled and empty seeds could be readily distinguished under a dissecting microscope. Seed set was calculated as the ratio of

![Figure 2. Schematic drawing of the relative lengths and positions of anthers and stigma in flowers of Gentianella germanica, showing the various flower parts that have been measured. \(t\)=corolla tube, \(c\)=connate part of filament, \(f\)=free part of filament, \(s\)=entire stamen, \(p\)=pistil, and \(ASS\)=anther-stigma separation (\(p\)-\(s\)).](image-url)
filled seeds to the initial ovule number. A small number of the fruits was infested by insect larvae or fungi and had to be discarded from further analysis. Data on herkogamy and autofertility in 1992 were obtained in a similar manner.

Data analysis
For the statistical analysis we used Systat 5.2.1 for Apple Macintosh (Wilkinson 1989). All data were tested for normality and heteroscedasticity. When data did not fulfill the assumptions of parametrical statistics, and transformation did not improve this, we had to resort to non-parametrical tests. Differences in size of the measured flower parts during four days of anthesis were tested using an ANOVA (length of the pistil and free part of the filament) with linear contrasts between days or by using a Kruskal-Wallis test (length of the corolla tube, stamen, connate part of the filament and anther-stigma separation). Variation in anther-stigma separation was tested using a nested ANOVA, with plants nested within populations, and the measured flowers nested within plants. For differences among populations in flower size, anther-stigma distance, number of ovules, seed set and correlations between the various flower parts we used the individual measurements per flower. For any correlation that involved seed set, we used the mean values per plant, as there was no direct correspondence between the (destructively sampled) measured flowers and the flowers that could set seed.

RESULTS

Flower development during anthesis
The lifespan of a flower was approximately four days. On the fourth day, the stigma lobes were curled backwards and the flower remained closed. During these four days a significant increase was found for corolla tube length, stamen length and pistil length (Fig. 3). This increase in length was most pronounced between the second and third day. Despite the increase in length of the measured floral parts, the spatial separation between anthers and stigma did not change significantly during anthesis.

Variation in pollen and stigma presentation
Flowers within individual plants varied considerably with respect to the temporal separation of sexual functions on their first day of anthesis. Hence, it was not possible to classify a plant as entirely protandrous, homogamous or protogynous. Of all 74 plants examined, only 7% could be classified as fully protandrous. However, differences in the proportion of protandrous and protogynous, but not homogamous, flowers per plant were found between

![Figure 3](image-url)
Table 1. The median proportion per plant of flowers (and range) in each dichogamy category in the three study populations of *G. germanica*. Values that have no letter in common (in horizontal comparisons, between populations) are significantly different (MW U-tests, \( p \leq 0.05 \)).

<table>
<thead>
<tr>
<th></th>
<th><em>WRAK</em> ((n=32))</th>
<th><em>EYS</em> ((n=20))</th>
<th><em>EXP</em> ((n=22))</th>
</tr>
</thead>
<tbody>
<tr>
<td>protandrous</td>
<td>0.50 (0.13-1.00)*b</td>
<td>0.21 (0.00-0.75)*a</td>
<td>0.35 (0.00-1.00)*b</td>
</tr>
<tr>
<td>homogamous</td>
<td>0.40 (0.00-0.75)*a</td>
<td>0.50 (0.00-0.88)*a</td>
<td>0.40 (0.00-1.00)*a</td>
</tr>
<tr>
<td>protogynous</td>
<td>0.00 (0.00-0.43)*a</td>
<td>0.23 (0.00-0.56)*b</td>
<td>0.18 (0.00-0.75)*b</td>
</tr>
</tbody>
</table>

populations (Table 1). Plants in population *WRAK* and *EXP* were predominantly protandrous or homogamous, whereas they were mainly homogamous in population *EYS*. The proportion of protogyny was significantly lower in *WRAK*, and protandry was less common in *EYS* (Table 1).

The time that passed between pollen presentation and stigma receptivity (opening) was always less than one day.

**Differences in herkogamy between populations in 1998**

The measurements of anther-stigma separation per plant per population are shown in Fig. 4. The proportion of variance in herkogamy among flowers within plants was small (13.7%) and not significant (Table 2), but the variance among plants within a population (38.9%) and

![Figure 4](image-url)  
**Figure 4.** The anther-stigma distribution per plant in the three study populations. Differences among plants were tested with ANOVA (statistics are presented in the graphs).
Table 2. Nested analysis of variance of the spatial separation between anthers and stigma in flowers of G. germanica. Significance levels of the F-ratios were determined using the Satterthwaite approximation (Sokal & Rohlff 1981). The variance components (in %) are indicated between brackets.

<table>
<thead>
<tr>
<th>Source of variation (%)</th>
<th>SS</th>
<th>df</th>
<th>F&lt;sub&gt;s&lt;/sub&gt;</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among populations (47.4%)</td>
<td>163.86</td>
<td>2</td>
<td>26.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Among plants within populations (38.9%)</td>
<td>210.01</td>
<td>70</td>
<td>20.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Among flowers within plants (13.7%)</td>
<td>63.02</td>
<td>438</td>
<td>0.17</td>
<td>ns</td>
</tr>
<tr>
<td>Total</td>
<td>436.89</td>
<td>510</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Means and standard deviation (between brackets) of plant height and the measured flower parts per population. Values in a row that have no letter in common are significantly different (ANOVA, p<0.001). The number of flowers per plant was tested with a Kruskal-Wallis ANOVA (p<0.001), the median instead of mean value is given for this variable, and the range is given between the brackets.

<table>
<thead>
<tr>
<th>Variable</th>
<th>WRAK</th>
<th>EYS</th>
<th>EXP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height (cm)</td>
<td>25.9</td>
<td>26.7</td>
<td>34.2</td>
</tr>
<tr>
<td>Number of flowers per plant</td>
<td>36.5</td>
<td>19.0</td>
<td>26.0</td>
</tr>
<tr>
<td>Corolla length (mm)</td>
<td>14.5</td>
<td>12.9</td>
<td>16.8</td>
</tr>
<tr>
<td>Pistil length (mm)</td>
<td>15.3</td>
<td>13.0</td>
<td>18.4</td>
</tr>
<tr>
<td>Filament length (commate part, mm)</td>
<td>6.4</td>
<td>5.5</td>
<td>7.4</td>
</tr>
<tr>
<td>Filament length (free part, mm)</td>
<td>8.6</td>
<td>7.5</td>
<td>9.4</td>
</tr>
<tr>
<td>Total stamen length (mm)</td>
<td>15.8</td>
<td>13.5</td>
<td>17.6</td>
</tr>
<tr>
<td>Anther-stigma separation (mm)</td>
<td>-0.5</td>
<td>-0.6</td>
<td>+0.8</td>
</tr>
</tbody>
</table>

among populations (47.4%) was highly significant (Table 2). In populations WRAK and EYS, the stigma was positioned below the top of the anthers in most of the plants, while the stigma in flowers of population EXP mainly protruded above the tops of the anthers.

Floral morphology and autofertility

Plants in population EXP were significantly taller than in WRAK and EYS, but the mean number of flowers per plant was highest in population WRAK. Flower size differed significantly between the three study populations (Table 3). Flowers were the largest in population EXP, the smallest in population EYS, whereas flowers in population WRAK were of intermediate size. Although the lengths of the corolla tube, pistil and stamens were highly correlated with each other within each population, anther-stigma separation varied independently from these variables in all populations (Table 4). In all populations, herkogamy was also not

Figure 5. The relation between autofertility and herkogamy in the three study populations. Pearson's product-moment correlation coefficients are given in each graph.
Table 4. Pearson's product-moment correlation coefficients among measured flower and plant traits in three populations of *G. germanica*.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Anther-stigma separation</th>
<th>Corolla length</th>
<th>Pistil length</th>
<th>Connate part filament</th>
<th>Free part filament</th>
<th>Stamen length</th>
<th>Plant height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corolla length</td>
<td>-.196</td>
<td>-.109</td>
<td>-.034</td>
<td>.845</td>
<td>.927</td>
<td>.814</td>
<td>.847</td>
</tr>
<tr>
<td>Pistil length</td>
<td>.214</td>
<td>-.033</td>
<td>.462</td>
<td>.845</td>
<td>.927</td>
<td>.814</td>
<td>.847</td>
</tr>
<tr>
<td>Connate part filament</td>
<td>-.388</td>
<td>-.263</td>
<td>-.277</td>
<td>.847</td>
<td>.893</td>
<td>.773</td>
<td>.613</td>
</tr>
<tr>
<td>Free part filament</td>
<td>-.090</td>
<td>-.170</td>
<td>.220</td>
<td>.706</td>
<td>.613</td>
<td>.691</td>
<td>.822</td>
</tr>
<tr>
<td>Stamen length</td>
<td>-.305</td>
<td>-.278</td>
<td>-.025</td>
<td>.923</td>
<td>.928</td>
<td>.935</td>
<td>.865</td>
</tr>
<tr>
<td>Plant height</td>
<td>-.069</td>
<td>-.046</td>
<td>-.246</td>
<td>.176</td>
<td>.133</td>
<td>.045</td>
<td>.100</td>
</tr>
<tr>
<td>Flowers/plant</td>
<td>-.183</td>
<td>-.087</td>
<td>-.277</td>
<td>-.026</td>
<td>-.170</td>
<td>-.075</td>
<td>-.115</td>
</tr>
</tbody>
</table>

Significance levels: italics = $p \leq 0.025$, underlined = $p \leq 0.001$
Figure 6. Frequency distribution diagrams of anther-stigma separation between different study years given for the three populations. Note that the comparison is between 1997 and 1998 for population EXP and between 1992 and 1998 for populations WRAK and EYS.
correlated with plant height and the number of flowers per plant (Table 4). Plants in population EXP had a significantly higher anther-stigma (+0.80 mm) than plants in both population WRAK (−0.49 mm) and population EYS (−0.56 mm). The latter two populations did not differ. Autofertility (seed set on untouched flowers on caged plants) was 76.4%, 54.4% and 67.4% in populations WRAK, EYS and EXP respectively, and varied significantly among populations (Anova, $F_{2,70}=13.01, p<0.001$). In populations WRAK and EYS no significant relationship was found between herkogamy and autofertility (Figs. 5a and b). In population EXP, herkogamy was negatively correlated with the ability to self spontaneously (Fig. 5c).

**Differences between years in herkogamy and autofertility**

Figure 6a-c shows the anther-stigma separation in two different study years. In populations WRAK and EYS, data of the year 1998 could be compared with data from a similar experiment in 1992, whereas in population EXP data from 1998 and 1997 could be compared. In population WRAK, a marginally significant shift was found towards a reduced anther-stigma separation. In this population, the mean separation between anthers and stigma had shifted from +0.15 mm in 1992 to −0.45 mm in 1998 (One-way ANOVA on log-transformed data: $F_{1,76}=3.39, p=0.069$). This suggests that the stigma was on average positioned higher than the anthers in 1992 in most flowers, and mostly under the level of the anthers in 1998 (Figure 6a). In the small population EYS, no significant shift was observed ($F_{1,39}=0.94, p=0.339$). Here, the mean separation between anthers and stigma was −0.28 mm in 1992 and -0.49 mm in 1998, which indicates that the stigma was mostly positioned under the level of the anthers in both study years (Figure 6b). Striking is, however, that in this population we observed no plants with the stigma above the anthers in 1998, while these were present in 1992. In the other small population EXP (Fig. 6c), the stigma was mostly positioned above the level of the anthers in both study years, +0.94 mm in 1997 and +0.8 mm in 1998 (difference not significant).

In population WRAK, we had data on autofertility for three years, and in population EYS for two years (Fig. 7). In population EYS, autofertility increased from 44% in 1992 to 54% in 1998 (Mann-Whitney U test, $p=0.051$). In population WRAK, mean autofertility increased from 27% in 1991 to 48% in 1992 and 76% in 1998 (Kruskal-Wallis, $p<0.001$).

**Figure 7.** Frequency distribution of seed set per fruit after spontaneous selfing in population WRAK in three different years.
VARIABLE HERKOGAMY AND AUTOFERTILITY IN MARGINAL POPULATIONS

DISCUSSION

Floral morphology and display

Late in the 19th century, floral biologists were already interested in dichogamy. At that time, different degrees of dichogamy were observed in *G. germanica* in various parts of its main distribution area and at different altitudes. The species was either considered clearly protandrous (Schultz 1888) or homogamous (Ricca 1870) or both (Kirchner 1888, Schultz 1890). Our results are in agreement with this apparently variable and weakly developed dichogamy. Moreover, dichogamy varied not only between, but also considerably within plants. Also, the temporal difference between male and female display was relatively short (measured in hours rather than in days) and most flowers can therefore effectively be considered homogamous. Hence, dichogamy may not be considered a very effective barrier to self-fertilization and heritability of this trait is probably very low. In this respect, *G. germanica* behaves similarly as its close relative *G. campestris*, studied in Sweden (Lennartsson 1997).

In *G. germanica*, most flowers were either ‘always herkogamous’ (*sensu* Robertson & Lloyd 1991), i.e., the stigma was positioned lower or higher than the anthers during the entire life span of the flower, or ‘never herkogamous’, i.e., the stigma and anthers were positioned at the same (or lower) level at all times. We found no potential for delayed self-pollination by corolla tube or filament stretching at a faster rate than the pistil during anthesis, as was found in *G. campestris* (Lennartsson 1997) and *Myosotis* (Robertson & Lloyd 1991). The increase in flower size from the second to the third day of anthesis is probably linked to the moment of (self-)pollination.

In *G. germanica*, the style is nearly absent (pers. observation) and has no role in the anther-stigma separation. This contradicts former reports, where herkogamy was assigned to differences in style length (Müller 1881, Kerner von Marilaun 1891). We found that herkogamy was caused by either pistil length, in population EXP, or stamen length as in populations WRAK and EYS. Most plants in population EXP had their stigma protruding above the anthers, while in populations WRAK and EYS, the majority of plants had their stigma below the level of the anthers.

Herkogamy varied independently from the size of different flower parts, plant height and the number of flowers per plant. Moreover, there was significant variation among plants and low within-plant variability. These observations strongly suggest that herkogamy is genetically controlled. Unfortunately, we were unable to determine the heritability of anther-stigma separation in *G. germanica* because seeds of selfed mothers failed to germinate. Our assumption is supported by Lennartsson (1997), who found a high heritability of herkogamy (0.85) in the close relative *G. campestris*. The degree of anther-stigma separation has a strong genetic component in other species (Ennos 1981, Carr & Fenster 1994, Robertson et al. 1994).

Herkogamy and its significance for autofertility

Several studies demonstrated that anther-stigma separation is positively correlated with outcrossing rate (Ennos 1981, Dole 1992, Holtsford & Ellstrand 1992, Belaoussoff & Shore 1995, Karron et al. 1997, Brunet & Eckert 1998). The opposite, the decrease of the degree of autogamous selfing when herkogamy increases, has been much less investigated. In some
Mimulus species, weak associations were found between autofertility and anther-stigma separation (Carr & Fenster 1994). In Aquilegia caerulea (Brunet & Eckert 1998) and Ipomoea purpurea (Ennos 1981) herkogamy was strongly negatively associated with autogamous selfing. In these species, herkogamy was much more pronounced than in G. germanica, with anther-stigma distances up to 8 mm. Nevertheless, we still found a negative correlation between herkogamy and autofertility, but only in population EXP, where the stigma was clearly protruding up to 3 mm above the anthers in some plants. In populations WRAK and EYS, most of the flowers had the stigma in between the anthers, so a significant relationship between herkogamy and autofertility was less likely.

In comparison with an earlier study of G. germanica (Luijten et al. 1998), autofertility by increased with more than 30%. This might be a result of our experimental method, because herkogamy and autofertility had to be determined on the same plant. Although we moved the cage up and down with extreme care to prevent disturbance of the plant, it still might have increased pollen transport from anthers to the stigma, and increased the overall seed set. Another explanation might be the numerous thrips we observed in the flowers of especially population EXP. Baker and Cruden (1991) demonstrated that thrips within flowers or plants could cause significant (self-)pollination in Ranunculus sceleratus and Potentilla rivalis.

It seems strange that populations EYS and WRAK, which have on average a similar range of anther-stigma separations, differ significantly in autofertility. Especially the lower autofertility in population EYS might be explained by other factors than anther-stigma separation, such as pollen production or viability, since the anthers looked slightly different and were even malformed in several flowers (which were not included in the experiment).

Selection for decreased herkogamy and increased autofertility

Whether the explanations discussed above were also responsible for the increased autofertility in population WRAK is questionable. Here, a high autofertility could be expected, since anther-stigma separation was less than in population EXP. However, it was interesting to find that on average herkogamy had changed from positive (+0.15 mm) in 1992 to negative (−0.45 mm) in 1998. Plants in this population also started to flower one month earlier in both 1997 and 1998, as compared with 1992. We can dismiss weather conditions as an explanation for these changes, since we did not observe a similar shift in the other two populations.

It seems more likely that self-fertile individuals were selected for under conditions of extreme pollen limitation in this population. During our field work, we have hardly observed any insect visitation to G. germanica. In addition, a shift in the mowing time from late October to early September in population WRAK has made the site very unattractive for pollinators during the flowering period. Moreover, the earlier mowing also drastically reduced the number of flowering gentians to tens rather than thousands of plants. Reductions in population size may have an effect on visitation rates and seed set (Jennersten 1988, Kwak et al. 1991, Oostermeijer et al. 1998). In a recent study, hardly any pollen of G. germanica was found on the bodies of various insect species (D. van Hest, unpublished data).

On the basis of our data, we hypothesize that selection for autofertility occurs especially in marginal populations with low insect visitation rates. This is supported by the high autofertility observed in G. germanica in the Austrian Alps by Wagner and Mitterhofer (1998). Here, the habitat is marginal because of its altitude of 1980 m a.s.l., where pollen limitation is frequently reported (Gugerli 1997, Totland 1997). Our populations are situated at
the margin of the species’ distribution area where the habitat is strongly fragmented. In the more centrally located Swiss Jura, the observed autofertility rate was much lower, which might be associated with a higher availability of pollinating insects (Fischer & Matthies 1997). It is remarkable that in that area, small and isolated populations had lower seed set than large ones (Fischer & Matthies 1997). Our theory predicts that in such populations selection will occur on less herkogamous and more autofertile individuals. The same may of course be expected in population EXP if the observed pollen limitation continues.

It can be concluded that pronounced herkogamy is effective in reducing self-fertilization in G. germanica, but that its degree is easily influenced by adverse situations. It seems that in the strongly man-made landscape of today, isolation by habitat fragmentation, pollinator limitation and badly timed mowing or grazing management may all have a strong effect on the reproduction of ecologically or distributionally marginal populations.

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