Effects of environmental heterogeneity on the distribution of sexes within and among populations in a gynodioecious species, Geranium maculatum

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Summary

- Populations containing both females and hermaphrodites (dimorphic) are generally found in drier sites than those with only hermaphrodites (monomorphic). The sex-differential plasticity hypothesis (SDP) suggests that this is caused by hermaphrodites reducing allocation to seeds in harsh environments, allowing female establishment. We proposed that a similar process could explain sex distribution within populations.
- We compared light availability and soil moisture between sites of three monomorphic and three dimorphic populations of Geranium maculatum and between microsites occupied by females and hermaphrodites within populations. We also correlated seed production in dimorphic populations with environmental measures.
- We found that dimorphic and monomorphic populations occurred in sites with similar soil moisture but within two dimorphic populations females occurred in drier microsites than hermaphrodites, as predicted by the SDP hypothesis. Contrary to the predictions, hermaphrodites' seed production was not influenced by the environment. Rather, females' seed production was correlated with environmental conditions in two populations, although the direction of the correlation differed between populations.
- Our results suggest that in this species, the SDP hypothesis does not explain sex distribution among or within populations. However, microsite environments may influence the distribution of sexes within a population and potentially aid in maintaining gynodioecy.

Key words: environmental differences, Geranium maculatum, gynodioecy, microsite differences, sex-differential plasticity (SDP) hypothesis.

Introduction

Gynodioecy, the co-occurrence of female and hermaphroditic individuals within a population, has interested biologists since Darwin first noted that females often produced more seeds than hermaphrodites (Darwin, 1877). Theory has shown that this seed fitness increase is necessary to allow females to invade hermaphroditic populations and coexist with their hermaphroditic counterparts (Charlesworth & Ganders, 1979; Charlesworth, 1981). Although the requirements and mechanisms of increased seed fitness in females have been frequently studied in populations containing both sexes, fitness differences in these populations speak little to the absence of females in other populations. One proposed explanation for the distribution of sexes among populations is that the frequent establishment and loss of populations does not allow populations to reach equilibrium, leading to the maintenance of females on a metapopulation level even if females have less of a fitness increase than typically required (Couvet et al., 1986; Gouyon & Couvet, 1987; Belhassen et al., 1989; Pannell, 1997). Metapopulation dynamics have had limited support, but are more likely to apply to a select group of species with high turnover rates in natural populations (Belhassen et al., 1989; Olson et al., 2005).

An alternative explanation for the presence or absence of females through an ecological mechanism has been proposed to explain the commonly observed pattern that females tend to be present in populations located in harsher environments (Delph, 1990b, 2003). This hypothesis proposes that environmental conditions are important in determining the relative seed fitness of the sexes and the relationship between fitness and the environment can explain why we see dimorphic populations in some areas and monomorphic populations in others. More specifically, it suggests that because hermaphrodites can gain fitness through both pollen and seeds, they may have evolved a plastic reproductive strategy that decreases the
emphasis placed upon seed production under harsh conditions. By contrast, because females can only gain fitness through seeds, they may have evolved to keep seed production relatively consistent regardless of the environmental conditions. Under this hypothesis (hereafter the sex-differential plasticity (SDP) hypothesis), one expects to see that hermaphrodites have higher seed fitness in benign environments and lower seed fitness in harsh environments, leading to females being able to invade and become established in populations in harsh environments because they can better compete with the lower hermaphrodite seed production.

Several lines of evidence suggest that the scenario proposed by the SDP hypothesis might be important in determining the distribution of females among populations for some gynodioecious species. First, in a study comparing 38 environmental factors of 65 populations of *Wurmbea biglandulosa* across a large geographical scale, Vaughn & Ramsey (2005) concluded that the best predictor for the presence of females (i.e. dimorphic populations) was higher temperatures, higher radiation (light intensity) and less rainfall (i.e. conditions assumed to be harsher). Similar results have also been found in *Lobelia siphilitica* (Caruso & Case, 2007). In addition, studies that either vary the environmental conditions directly (Barr, 2004; Dorken & Mitchard, 2008) or evaluate the environmental quality indirectly by using surrogate measurements, such as plant size (Delph, 1990a,b; Ashman, 1999; Sarkissian et al., 2001), have shown that hermaphrodites are more affected by environmental conditions than females, a pattern consistent with the SDP hypothesis. Combined, these studies support the hypothesis that the sex-specific relationship between the environment and seed production could be key to understanding the mechanisms behind the distribution of dimorphic and monomorphic populations in gynodioecious species.

In addition to variation in the presence or absence of females among populations, many gynodioecious species have variation in the distribution of females within populations. The sexes are often spatially aggregated within populations (Graff, 1999; Olson et al., 2006; this study), with patches of females interspersed with hermaphrodites. A simple explanation may lie in the genetic control of sex. In most gynodioecious species, femaleness is determined by a mitochondrial gene while male function can be restored by a nuclear gene (Budar & Pellietier, 2001). Because mitochondria are primarily inherited from the maternal individual, limited seed dispersal coupled with founder effects may lead to spatial structuring of sex (Olson & McCauley, 2002; Klaas & Olson, 2006; Olson et al., 2006). Less common is a pure nuclear control of sex, which under limited pollen and seed dispersal could also lead to spatial structuring. Alternatively, the mechanism proposed by the SDP hypothesis could operate within populations, so that females persist in harsh microsites within populations where hermaphrodites produce fewer seeds. It is well documented that environmental conditions, such as soil moisture and light availability, are often quite heterogeneous among microsites within a population (Hutchison & Matt, 1977; Robertson et al., 1993; Palmer, 2003). This is particularly true for forest understory habitats, where gaps created by tree fall or the forest edge effect can dramatically change the water and light availability for a particular microsite (Hutchison & Matt, 1977; Chen et al., 1993, 1995; Xu et al., 1997). It has also been shown that microsites of similar quality tend to aggregate (Fortin et al., 1989; Rossi et al., 1992; Legendre, 1993; Clark et al., 1996; Nicotra et al., 1999) in natural populations. Based on the SDP hypothesis, the aggregation of sexes may reflect the underlying aggregation of microsites, with females more prevalent in harsher microsites and hermaphrodites more prevalent in less harsh microsites. Thus, the SDP hypothesis can potentially explain both among and within population variation in sex distribution. To our knowledge, this hypothesis has not been tested at the local scale within populations.

In this study, we tested several predictions derived from the sex-differential plasticity hypothesis at two spatially hierarchical scales: among populations and among microsites within populations of *Geranium maculatum*. Specifically, we asked four main questions. Do sites occupied by monomorphic and dimorphic populations differ in their light availability or soil moisture? Within a population, are the sexes aggregated? Within a population do females and hermaphrodites live different light availability or soil moisture environments? Within a population does seed production vary with the environmental conditions? We predicted that if SDP contributes significantly to the distribution of females among populations of this species populations in wetter sites would lack females because it would be more difficult for females to gain a seed fitness advantage over hermaphrodites in those populations. Similarly, we predicted that mechanisms functioning within a population would lead to the aggregation of females in drier microsites, where they would have a seed production advantage over hermaphrodites.

**Materials and Methods**

*Geranium maculatum* L. is a gynodioecious, rhizomatous perennial ranging from the South Eastern USA to Canada and west to the Great Plains (Radford et al., 1968). Populations used in this study were located in Georgia, USA, and were in the forest understory near streams. *Geranium maculatum* leaves emerge before canopy tree leaves and remain until the autumn (in the OT population (see Populations section) used in this study, *G. maculatum* leaves emerged before 17 March 2008, while the canopy leaves did not fully emerge until approx. 29 April 2008). Flowering begins in early spring (approx. 24 March 2008 for the OT population), with individuals producing, on average, six flowers per inflorescence (Chang, 2006). Flowers are visited by generalist pollinators including bees, flies and butterflies. Hermaphrodites are self-compatible and selfing rates range from 0 to 17% depending on the population (M. L. Van Eten et al., unpublished). Inbreeding depression is high and variable between populations (cumulative postdispersal
inbreeding depression ranges from 0.38 to 0.84, Chang, 2007). Seeds are dispersed by the elastic dehiscence of the schizocarp, to an average of 3 m from the maternal plant (Stamp & Lucas, 1983). Based on field and glasshouse observations, sex appears to be genetically determined. Preliminary data rule out the possibility that sex is controlled entirely by cytoplasmic genes, but the exact genetic control has not yet been determined (M. L. Van Etten & S.-M. Chang, unpublished). Females have small aborted anthers and smaller petals when compared with hermaphrodites (Ågren & Willson, 1991; Chang, 2006). Flower number is approximately the same between sexes in natural populations (Ågren & Willson, 1991; Chang, 2006), although in one glasshouse study females produced slightly more flowers than hermaphrodites (Van Etten et al., 2008). In natural populations, females produce more seeds than hermaphrodites (20–50% more; Ågren & Willson, 1991; Chang, 2006) and seeds that have a higher germination rate (Chang, 2006). Higher seed production is probably caused by higher seed set (more seeds produced per flower; Ågren & Willson, 1991; Chang, 2006), although the ovule number is the same between sexes. Local populations around Athens, GA, USA, range in female frequency from 0 to 50% (Chang, 2006).

Populations

Six populations were used in this study: three monomorphic (CA, 33°54′0.84″ N, 83°23′9.24″ W; WT, 33°54′30.29″ N, 83°23′53.11″ W; HP, 33°45′48.56″ N, 83°26′37.96″ W) and three dimorphic (OT, 33°54′4.92″ N, 83°22′47.34″ W; OTG, 33°54′4.90″ N, 83°22′44.93″ W; RL, 33°45′44.34″ N, 83°15′53.58″ W). The CA, WT, OT and OTG populations were located in Athens, GA, while the HP and RL populations were in Oconee County, GA. The dimorphic populations varied in the percentage of females: the OT and OTG populations had approx. 50% female, and the RL population had approx. 25% female. All populations were located in the forest understorey within 20 m of a creek or river with continuous water flow. All populations used appear to be well established and persistent; the areas in which populations have been found have been protected from major disturbance for several decades and four of the populations used in this study have been monitored for other experiments for 6.7 yr. A demographic study also suggested that the mortality of established plants was very low over a 3-yr period (M. L. Van Etten, unpublished data). The populations chosen were within a larger group of populations that have been surveyed for their sex ratio. The populations’ sex ratio does not appear to have a spatial component; dimorphic populations are interspersed with monomorphic populations. For example, the OT and OTG population (dimorphic) are within 0.48 km of the CA populations (monomorphic), while the HP and CA populations (both monomorphic) are over 18 km apart.

To make sampling and mapping of plants easier in the dimorphic populations, permanent sampling points were placed throughout the populations. Parallel transects were laid down every 3 m and on each transect a sampling point was placed every 3 m, forming a grid of sampling points. This led to 49–53 sampling points per population, which were used in plant selection, mapping and measuring of the environmental conditions. All flowering plants in the dimorphic populations were mapped by stretching a meter tape down the length of a row of sampling points and then measuring the distance from the plant to the meter tape with another meter tape. Locations were then converted to x and y coordinates. We also recorded the sex of every plant mapped.

Environmental conditions

Light availability and soil moisture were measured in all populations. Light and moisture were chosen as environmental variables of interest because many studies have found that soil moisture affects the sex ratio (see the Introduction). In addition, light availability is often the cause of soil moisture differences; areas with higher light exposure have greater evapotranspiration and therefore lower soil moisture (Rosenberg et al., 1983). Light availability measures were taken using hemispherical photos (Nikon CoolPix885 digital camera with Nikon FC-E8 fisheye converter lens). Pictures were taken approx. 25 cm off the ground, with the top of the camera pointing north and the camera level. Pictures were taken either on an overcast day or just after sunrise or just before sunset. In dimorphic populations, pictures were taken every third sampling point in the spring (20–22 March 2008, before canopy leaf emergence) and at each sampling point in the autumn (7 October 2008–4 November 2008, before significant leaf loss). In monomorphic populations, two 15-m long transects were laid down to span the population and photos were taken every 3 m. For these populations, photos were only taken in the autumn (16 October 2008–4 November 2008). Pictures were analysed for per cent canopy openness using Gap Light Analyzer (Frazer et al., 1999).

Soil moisture was measured as per cent volumetric water content (VWC) using a hand-held moisture sensor (Hysens-10 by Spectrum Technologies Inc., 12 cm probes). Leaf litter was removed and readings were taken by completely inserting the probes perpendicular to the soil surface. In the dimorphic populations, three soil moisture readings were taken and their average recorded at each of the sampling points. Readings for the dimorphic populations were taken twice – once in the spring of 2008 (10 May) and once in the autumn of 2008 (14–15 October). For the monomorphic populations, at each of the places where light photos were taken, three soil moisture readings were taken and their average recorded. Readings for the monomorphic populations were taken in the autumn of 2008 (16–17 October). In order to obtain data that were comparable among populations, each set of measurements (autumn or spring) were taken within 4 d of each other, during which time there was no significant rainfall.
Kriging for the environmental conditions

To determine if hermaphrodites and females in dimorphic populations were located in different environments, we used ordinary kriging to estimate soil moisture and light availability for each plant mapped in a population. Kriging is a method that uses the measurement of a variable taken from several sampling sites to estimate the value of that variable at unmeasured sites based on a model that takes into account the pattern of spatial correlation of that variable (Rossi et al., 1992; Wackernagel, 2003; Schabenberger & Gotway, 2005). This method was developed primarily for mining purposes, aiming to predict the location of desirable ores, and has been adopted by ecologists to predict variation in natural populations (Rossi et al., 1992).

Environmental conditions, like ore deposits, are not randomly located in an environment, but are aggregated in space. This method takes advantage of such aggregation and uses it to predict, for example, ore concentrations or light availability, by creating mathematical models relating the variable of interest with a given location. To parameterize these models, several methods are typically used, including maximum likelihood, restricted maximum likelihood (REML), least-square methods and Bayesian approaches. We used these methods to obtain parameters for models describing each environmental variable separately (light availability and soil moisture) and compared the models with nonspatial models using the Akaike Information Criterion (AIC; Akaike, 1974) values to determine if the environmental variables were spatially structured. The nonspatial model outperformed the spatial model in two cases and thus kriging was not used for those measures: for the spring light measures, there was insufficient data to construct an appropriate model; in the OTG population for spring soil moisture, the nonspatial model was better. For the other variables, parameters estimated using REML yielded the best-fit models and were used to estimate the light availability and soil moisture for each of the individuals mapped in the dimorphic populations. Models and estimations were calculated using the geoR package in R (Ribeiro & Diggle, 2001).

Demographic traits

To determine if seed production was correlated with either of the environmental conditions measured, approx. 50 plants per sex per population were selected and data recorded in 2006–08 for the OT population and in 2007–08 for the OTG and RL populations. In the OT and OTG populations, at each of the sampling points, the closest female and hermaphrodite were selected. In the RL population, because females were at a lower density, female plants were chosen throughout the population as well as the nearest hermaphrodite within 1 m.

For each year observed, the number of flowers, fruits and seeds were recorded for each of the plants sampled. All seeds and fruits were collected by covering the inflorescence with a bridal veil bag until maturation to prevent seed dispersal. From these measures, we calculated the cumulative seed production as the total number of seeds produced over the 2- to 3-yr study period.

Statistical analysis

Environmental differences between monomorphic and dimorphic populations were tested using the average measured environmental data for each population as the response variable and the population type as the predictor variable in an ANOVA.

To determine if the sexes were spatially aggregated, we used a spatial autocorrelation method often used to determine if there is fine-scale genetic structure. In the context of genetic structure, this analysis measures the genetic similarity between pairs of individuals separated by a particular distance (Sokal & Oden, 1978; Ennos, 2000) and calculates relatedness using an autocorrelation coefficient, $r$, described in Smouse & Peakall (1999). Though the coefficient $r$ is normally used when analysing genotypes from multiple genetic marker loci, it can be adapted to other types of variables. In our case, we use sex (female or hermaphrodite) as the ‘genotypes’ to calculate the $r$ coefficient. This coefficient is bounded by 1 and –1, with positive values indicating that individuals are more similar than expected by chance (in our case the same sex) and negative values indicating individuals are less similar than expected by chance (in our case the opposite sex). The significance test for $r$-values was carried out against the null hypothesis that the sexes were randomly distributed throughout the population, corresponding to an $r$-value of zero. For each distance class, a mean similarity between pairs of individuals at that distance class is calculated, and tested to see if it is significantly different from zero by bootstrapping of the ‘genotypes’. Significant positive $r$-values would indicate that plants of the same sex tend to aggregate with each other while significant negative $r$-values would indicate that opposite sexes tend to aggregate together. In the case where the $r$-values are significantly different from zero at small distances but not at large distances, the distance at which the $r$-value changes from significantly to not significantly different from zero approximates the size of sex patches. This analysis was performed using GENALEX V6.2b (Peakall & Smouse, 2006).

To determine if females and hermaphrodites were located in significantly different environmental conditions, the estimated soil moisture and light availability values from kriging for each mapped individual were used in an ANOVA. To validate the results from the kriging estimates, we carried out two other analyses. First, each mapped individual was assigned the measured value (light availability or soil moisture) from the nearest sampling point, which was never $>1.5$ m away. These values were then used in an ANOVA with the environmental measure as the response variable and sex as the predictor variable to determine if the sexes differed in their environment. Second, the sex ratio within a 3-m radius circle of each measuring point...
was determined and was regressed against light availability and soil moisture using Proc Reg in SAS (SAS Institute Inc., Cary, NC, USA). In this analysis, the spring light availability measures were used in addition to those used in the kriging analysis. We expected to see a significant influence of environmental factors on the sex ratios in this analysis if females and hermaphrodites differ significantly in their preference for light availability or soil moisture.

To determine the relationship between seed production and the microsite environment, the estimated light availability and soil moisture around plants for which seed production data were collected were regressed with cumulative seed number. In addition, an ANCOVA including cumulative seed number as the response variable and sex, environmental measures and their interaction as the predicting variables was used to test if the slope of the regression was significantly different between females and hermaphrodites (indicated by a significant sex × environment interaction). Levene’s test for equality of variance was used to test if variation in seed fitness differed between females and hermaphrodites. To determine which factor(s) were most important for seed production, we compared models containing one or more of several predicting variables, sex (dummy coded as a one or a zero), light availability, soil moisture, the density of pollen donors within a 3-m radius circle, the density of flowering plants within a 3-m radius circle and the sex ratio (% females) within a 3-m radius circle using the AIC option in Proc Reg in SAS to determine the best fit model. Statistical tests were performed in SAS 9.2.

Results

Does the environment differ between monomorphic and dimorphic populations?

Average light availability but not soil moisture differed significantly between sites occupied by monomorphic and dimorphic populations. Monomorphic populations were located in significantly brighter sites than dimorphic populations (monomorphic mean = 13.08% canopy openness, dimorphic mean = 10.51%; P = 0.004, df = 1,4, Fig. 1a). The brightest site was CA with 13.7% average canopy openness, probably because it is parallel to a trail, while the shadiest was OT with 10.2% average canopy openness. Although the average soil moisture did not differ significantly between sites occupied by monomorphic and dimorphic populations (P = 0.16, df = 1,4, Fig. 1b), there was variation among populations, with RL being particularly wet. The RL site had an average of 22.18% VWC in the autumn compared with 16.8% and 18.2% in the other dimorphic population sites (Fig. 1b). The within-population range of microsite soil moisture was also highest in RL, ranging from 14 to 42% VWC in the spring and 14 to 40% in the autumn, whereas the ranges in the other populations were between 12% and 30% in the spring and 11% and 23% in the autumn.

Are the sexes aggregated within a population?

All populations showed significant spatial structure of sex (Figs 2, 3). However, the populations differed in the strength and spatial range of the structure. The OTG population had the highest initial r-value (r at 1 m = 0.744, Fig. 2b), indicating that, compared with the OT and RL populations, individuals in this population are surrounded by individuals of the same sex more often. The OT and RL populations had lower initial r-values (r = 0.387, Fig. 2a, and r = 0.459, Fig. 2c, respectively) indicating that there is slightly more mixing of the sexes at small distances than in the OTG population, although individuals are usually still surrounded by the same sex. In the OT population, the sex structure was significant for a farther...
distance, until c. 11 m, than the other two populations. The other populations had shorter distances before \( r \) reached zero (OTG, 7 m; RL, 4 m), indicating that sex patches were smaller. These results can also be seen in the population maps (Fig. 3): in the OT population there are large areas with primarily one of the sexes. In addition, in both OT and OTG populations, \( r \) becomes significantly negative at large distances indicating that at large distances individuals are of the opposite sex (Fig. 2a,b). This, too, is evident in the population maps where there are distinct female patches and hermaphrodite patches (Fig. 3). In the RL population, however, the sex patches are more intermixed leading to a nonnegative \( r \)-value at long distances.

Are hermaphrodites and females located in different environments within a population?

Hermaphrodites and females were located in significantly different environments within a population. In all three populations, females were in significantly brighter microsites than hermaphrodites (Figs 3, 4a, OT \( P < 0.0001, \text{df} = 1,1282; \) OTG \( P < 0.0001, \text{df} = 1,1666; \) RL \( P = 0.038, \text{df} = 1,1966 \)). In two of the populations (OT and OTG), females were also in drier environments although in RL they were in significantly wetter environments (Figs 3, 4b, OT \( P < 0.0001, \text{df} = 1,1282; \) OTG \( P < 0.0001, \text{df} = 1,1666; \) RL \( P < 0.0001, \text{df} = 1,1966 \)).

Results were qualitatively similar in the analysis using the values from the nearest measuring point, with the exception that some results were not significant (OT: \( \text{df} = 1,1282, \text{light } P < 0.0001, \text{soil spring } P = 0.0004, \text{soil autumn } P < 0.0001; \) OTG: \( \text{df} = 1,1666, \text{light } P = 0.02, \text{soil spring } P = 0.23, \text{soil autumn } P < 0.0001; \) RL: \( \text{df} = 1,1966, \text{light } P = 0.10, \text{soil spring } P < 0.0001, \text{soil autumn } P < 0.0001 \)). Similar results were also found when using the sex ratio within a 3-m radius circle around each measuring point (Table 1). In the RL population, wetter environments tended to be associated with a higher percentage of females (spring \( P = 0.034, \text{autumn } P = 0.0022 \)), as was found in the kriging analysis. In contrast to the RL population, in the OT and OTG populations drier environments tended to be associated with a higher percentage of females (OT, spring \( P = 0.077, \text{autumn } P = 0.052; \) OTG, spring \( P = 0.667, \text{autumn } P = 0.082 \)) and in the OTG

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**Table 1**  Slope, intercept and significance of regressions between environmental variables and sex ratio (% females) within a 3-m radius of each measuring point in each population of *Geranium maculatum*

<table>
<thead>
<tr>
<th>Population</th>
<th>Light Slope</th>
<th>Light Intercept</th>
<th>Light n</th>
<th>Soil Slope</th>
<th>Soil Intercept</th>
<th>Soil n</th>
<th>Light Slope</th>
<th>Light Intercept</th>
<th>Light n</th>
<th>Soil Slope</th>
<th>Soil Intercept</th>
<th>Soil n</th>
</tr>
</thead>
<tbody>
<tr>
<td>OT</td>
<td>0.0010</td>
<td>0.165</td>
<td>14</td>
<td>0.032</td>
<td>0.175</td>
<td>48</td>
<td>-0.019 a</td>
<td>0.879</td>
<td>47</td>
<td>0.032</td>
<td>0.097</td>
<td>48</td>
</tr>
<tr>
<td>OTG</td>
<td>0.081</td>
<td>-2.241</td>
<td>17</td>
<td>0.201***</td>
<td>-1.683</td>
<td>54</td>
<td>0.006</td>
<td>0.307</td>
<td>53</td>
<td>0.006</td>
<td>0.076</td>
<td>54</td>
</tr>
<tr>
<td>RL</td>
<td>-0.040</td>
<td>1.406</td>
<td>16</td>
<td>0.032</td>
<td>0.001</td>
<td>51</td>
<td>-0.032 a</td>
<td>0.989</td>
<td>54</td>
<td>0.006</td>
<td>-0.056</td>
<td>51</td>
</tr>
</tbody>
</table>

Light, % canopy openness; soil, % volumetric water content; \( n \), number of observations; \( a, P < 0.09; *, P < 0.05; **, P < 0.01; ***\), \( P < 0.0001 \). See the Materials and Methods section for details of the populations OT, OTG and RL.
population in brighter environments (spring $P = 0.165$, autumn $P < 0.0001$). Although some of the results were only marginally significant (with $0.1 < P < 0.05$), they were nonetheless consistent with results from kriging estimates.

Is seed production correlated with the environmental conditions?

Using cumulative seed number as a measure of seed production, we found contrasting trends among populations. In the OT population, plants in brighter sites had higher seed production than ones in darker sites regardless of their sex ($P = 0.002$, Table 2). Conversely, in the RL population, plants in darker environments tended to have higher seed production ($P = 0.04$ and $P = 0.03$, respectively, Table 2) although sex and local sex ratio also influenced seed production. Further analysis shows that in this population, the sexes differed in the strength of their response to soil moisture ($P = 0.01$, df = 1.42, Fig. 5e,h). In the RL population, for both spring and autumn soil moisture, females had higher seed production.
in drier areas (spring $P = 0.03$; autumn $P = 0.01$; Fig. 5e,h), while the seed production of hermaphrodites did not vary with soil moisture (Fig. 5e,h). Higher female seed production in drier sites in this population was probably caused by females producing fewer fruits (female slope in spring = $-0.035$, $P = 0.03$; female slope in autumn = $-0.69$, $P = 0.02$) and seeds (female slope in spring = $-0.18$, $P = 0.05$) in wet environments compared with dry environments. In the OTG population, although the model was not significant ($P = 0.066$, df = 3,39), the environmental variables did not affect seed production but the local density of pollen donors, local density of flowering plants and the local sex ratio did affect seed production. The local sex ratio was in the best model for two of the populations (RL and OTG) and in both cases the higher the percentage of females the lower the seed production (Table 2).

Hermaphrodites did not have more variation in their seed production; neither were they more strongly influenced by the environment than females, hence providing no support for the SDP hypothesis. More specifically, the variance in cumulative seed production did not differ between genders in any population (RL, females $\sigma^2 = 174.72$, hermaphrodites $\sigma^2 = 106.74$, $F = 1.52$, $P = 0.22$, df = 1.45; OTG, females $\sigma^2 = 28.10$, hermaphrodites $\sigma^2 = 26.39$, $F = 0.01$, $P = 0.94$, df = 1.66; OT, females $\sigma^2 = 73.44$, hermaphrodites $\sigma^2 = 40.68$, $F = 1.68$, $P = 0.20$, df = 1.95). Seed production by females in the OT and RL populations was also significantly correlated with light and light and soil moisture, respectively, while there was no significant correlation for hermaphrodites (Fig. 5).

**Discussion**

This study investigated the relationship between the environment and the distribution of sexes both within and among populations. Among populations, previous studies in other species had found that dimorphic populations were in drier environments than monomorphic populations. However, we found that the soil moisture was similar for both types of populations but that the dimorphic populations were in darker environments than the monomorphic populations.

**Table 2** Parameter estimates ($t$-values) of the top model predicting cumulative seed number for each population of *Geranium maculatum*

<table>
<thead>
<tr>
<th>Population</th>
<th>Sex</th>
<th>Soil moisture</th>
<th>Light availability</th>
<th>Hermaphrodite density</th>
<th>Adult density</th>
<th>Sex ratio</th>
<th>df1, df2</th>
<th>Model F</th>
<th>Model P</th>
</tr>
</thead>
<tbody>
<tr>
<td>OT</td>
<td>Hermaphrodite</td>
<td>2.24 (3.10)**</td>
<td>-1.37 (-2.26)*</td>
<td>0.33 (1.53)</td>
<td>-17.22 (-2.60)*</td>
<td>1, 95</td>
<td>9.59</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>OTG</td>
<td>Hermaphrodite</td>
<td>7.93 (2.27)*</td>
<td>-6.75 (-2.85)**</td>
<td></td>
<td></td>
<td>3, 39</td>
<td>2.59</td>
<td>0.066</td>
<td></td>
</tr>
<tr>
<td>RL</td>
<td>Hermaphrodite</td>
<td>9.59 (2.27)**</td>
<td>-6.75 (-2.85)**</td>
<td></td>
<td></td>
<td>3, 43</td>
<td>5.55</td>
<td>0.0026</td>
<td></td>
</tr>
</tbody>
</table>

Soil moisture, % volumetric water content; light availability, % canopy openness; hermaphrodite density, number of hermaphrodites in 3-m radius circle; adult density, number of flowering plants in 3-m radius circle; sex ratio, % females in 3-m radius circle; df1 and df2 are the numerator and denominator degrees of freedom for the model, respectively. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$.

See the Materials and Methods section for details of the populations OT, OTG and RL.

![Fig. 4](image-url)

**Fig. 4** Mean and SE of light availability (a) and autumn soil moisture (b) for hermaphrodites (closed bars) and females (open bars) of *Geranium maculatum* populations. VWC, volumetric water content. See the Materials and Methods section for details of the populations.

*, $P < 0.05$; ***, $P < 0.0001$. 

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Contrary to this result, we found the expected pattern of females being located in drier microsites within two of the dimorphic populations. This expectation was based on the SDP hypothesis, which suggests that hermaphrodites’ seed fitness would be negatively affected in drier microsites, giving females an advantage under these conditions. We found no indication that this had occurred in the populations studied. We also did not find any evidence that seed production by hermaphrodites was more strongly influenced by the environment than seed production by females. Our results suggest that while the sexes may be associated with different microsites within populations, the SDP hypothesis does not provide a
satisfactory mechanism to explain this pattern. This hypothesis is therefore unable to explain the sex distribution patterns of females either within or among populations in this species.

Among population environmental differences

Previous studies have shown that soil moisture may play an important role in large-scale patterns of female distribution, with dimorphic populations found more often in drier areas (Vaughton & Ramsey, 2005) and a higher frequency of females in sites with drier soils (Wolfe & Shmida, 1997; Alonso & Herrera, 2001; Asikainen & Mutikainen, 2003; Case & Barrett, 2004; but see Alonso et al., 2007; Caruso & Case, 2007). One of our main goals in this study was to test whether the same pattern occurred at smaller scales. We compared populations with and without females located within approx. 19 km of each other. Our results showed quite a different picture. We found that dimorphic populations, on average, tended to be in darker environments but in similar soil moisture environments. Contrary to this pattern, at the within-population level, we found that females tended to be located in brighter microsites, consistent with what we expected (discussed further in next section). One explanation for the contrasting results could be that different selective and stochastic processes might be responsible for patterns at different geographical scales. Contrasting results depending on the spatial scale were also found in two previous studies that examined ecological correlations with female frequencies in natural populations of Daphne laureola at different geographical scales (Alonso & Herrera, 2001; Alonso et al., 2007). Comparisons among populations separated at most by 600 km showed that drier populations had a lower frequency of females (Alonso et al., 2007) while a subset of more local populations (within 60 km) had previously shown the opposite (Alonso & Herrera, 2001). Alonso and colleagues suggested that site-specific plant traits and mating system variation could potentially lead to the contrasting results at different spatial scales, though supporting evidence remained absent. It is possible that characteristics such as founder effect and mating systems are more important for the presence of females at this spatial scale than the ecological conditions such as soil moisture and light availability measured in this study. Future studies that increase the number of populations and the geographical spatial scale will allow a more direct comparison for our system and the D. laureola studies.

Locations of sexes within populations

Contrary to the among-population patterns, the results from the within-population analysis were consistent, in two populations, with our original prediction that females would be more prevalent in sites with lower soil moisture and higher light availability. Females tended to grow in drier and brighter areas than hermaphrodites with the exception of the RL population where females were located in wetter sites. The aggregation of the sexes into specific microsites could be influenced by several factors. First, founder effects could cause the aggregation of the sexes such that females were originally introduced into a particular area and have not dispersed throughout the population. Founder effects may be especially important if sex is under cytonuclear control. With this type of genetic control, male sterility is caused by a gene in the mitochondria, which is usually passed on by the seed parent (but see McCauley et al., 2005, 2007; Pearl et al., 2009). Combined with limited seed dispersal, these two factors could cause a patchy sex distribution that happens to coincide with different light and soil environments. Although our data do not allow us to completely rule out this possibility, it seems unlikely to be the sole reason for the observed spatial structure because genetic structure analyses on several populations have shown that genetic structure for neutral nuclear genes extends only to approx. 2–3 m in this species (M. L. Van Etten & S.-M. Chang, unpublished). Cytoplasmic genes would likely be even more spatially structured than nuclear markers because of their uniparental inheritance (Hu & Ennos, 1997). Thus, regardless of the type of genetic control of sex in this species, sex structure in all populations we studied extends past the genetic structure, indicating that the structuring of neutral genes does not fully explain the pattern we see in sex distribution.

Another possible cause for the observed sex distribution is that the sexes may have different seed fitness in different environments, such as suggested by the SDP hypothesis. If females produce relatively more seeds and/or produce seeds better able to establish in harsher environments, their frequency could increase in these environments. We found support for this pattern in terms of seed production in RL but not in the other two populations (see more discussion in next section). Beyond seed production, in other gynodioecious species, females have been found to produce larger and better provisioned seeds (reviewed in Shykoff et al., 2003), and thus may be better able to germinate or survive in harsher sites. In G. maculatum, female seeds are slightly heavier (Chang, 2006) and germinate at a higher rate in the glasshouse. However, whether germination or survival rates differ between the sexes in natural environments is unknown for this species.

The observed sex distribution could also result from the indirect effect that environmental conditions may have on seed fitness through its influence on mating systems. It has been suggested that the selfing rate may vary with the environment (Vaughton & Ramsey, 2005), which would differentially affect the sexes. In dry areas, the selfing rate of hermaphrodites may be higher than in wet areas, possibly because flower size in some species decreases in drier areas, leading to more selfing (Barrett & Eckert, 1990; Jonas & Geber, 1999; Herrera, 2005; Lambrechts & Dawson, 2007). If this occurs in G. maculatum, the strong inbreeding depression found in both pre- and post-seed dispersal traits (Chang, 2007) could lead to the reduction in the seed fitness of hermaphrodites to a level low enough for females to have an advantage and be maintained in those areas.
A mating system analysis taking into account the environment of the individuals would help determine if the selfing rate within a population depends upon the environment. Although we do not know the mechanism(s) behind the association of the sexes with different microsites, this association may aid in maintaining both sexes. Manipulative studies are needed to clarify the influence of light availability on the sexes in order to determine if heterogeneous environments may be aiding in maintaining both sexes.

Correlations between seed production and environmental gradients

The SDP hypothesis suggests that in harsh environments hermaphrodites reduce allocation to seed production, while the seed production of females remains comparatively constant, boosting the relative seed production of females in these areas. We predicted that if this were occurring in *G. maculatum*, seed production by hermaphrodites should be more dependent upon the environment than that of females, leading to a steeper decline for hermaphrodites than females in seed fitness as the environment became harsher. Our results do not show this trend – seed production by females generally was more affected by the environment than that of hermaphrodites. In addition, the sign of the slopes differed among populations (Fig. 5), suggesting that there is no consistent trend across populations in the type of environmental conditions that could lead to higher seed fitness. Instead, it appears that the relationships we found between the environmental factors and seed production might be specific to each population. Our results, thus, provide no support for the power of the SDP hypothesis as a general mechanism in explaining the sex distribution at the local level for this species. The influence of other mechanisms, such as pollen limitation, on seed production may simply outweigh the importance of seed production plasticity across soil moisture or light gradients.

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