



Spatial sex segregation in the dioecious grass *Poa ligularis* in northern Patagonia: the role of environmental patchiness

MONICA B. BERTILLER^{1,*}, CLAUDIA L. SAIN¹, ALEJANDRO J. BISIGATO¹,
FERNANDO R. CORONATO¹, JORGE O. ARES^{1,2} and PAMELA GRAFF²

¹Centro Nacional Patagónico (CONICET), Boulevard Brown s/n, 9120 Puerto Madryn, Chubut;
²Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur San Juan 670,
8000 Bahía Blanca, Argentina; *Author for correspondence (e-mail: bertil@cenpat.edu.ar;
fax: +54-2965-451543)

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Abstract. We examined the effect of environmental patchiness on the spatial segregation of the sexes in the dioecious anemophilous grass *Poa ligularis*. Because the species is sensitive to grazing, a better understanding of environmental factors that control its spatial distribution and abundance could improve conservation efforts. We hypothesized that (i) males and females are spatially segregated in the microenvironments created by plant patches as the result of sexual specialization in habitat and/or resources use, (ii) sexual specialization is related to different tolerance to competition and reproductive costs of males and females, and (iii) changes in patch structure affect the microenvironment and the intensity of spatial segregation of the sexes. We analyzed the spatial distribution of sexes at three sites with different plant and micro-environmental patchiness and performed a controlled competition experiment with different substitution of males and females. Our results showed that large plant patches created larger sheltered soil fertility islands than small patches. As patch size and their area of influence increased, the density and the spatial segregation of the sexes of *P. ligularis* also increased, resulting in biased habitat-specific sex ratios. In accordance with their higher reproductive costs, females were more frequent in sheltered (low air evaporative demand) and nitrogen-rich areas inside patch perimeters than males. Females were also better able to tolerate inter-sexual competition than males. In contrast, males tolerated low nitrogen concentration in soil and low sheltering, probably gaining advantage in pollen dispersal. Inter- and intra-sexual competition, however, affected the reproductive output of both sexes. From the point of view of conservation, environmental patchiness is important to the status of *P. ligularis* populations. The reduction of patch size limits the available microsites, biases the sex ratio towards males inside patches, increases inter- and intra-sexual competition, and it might be expected to decrease overall seed and pollen production and consequently potential recruitment.

Key words: dioecy, niche segregation, sheltering, soil nitrogen, soil water

Abbreviations: BC – Bahía Cracker; ESL – Estancia San Luis; LB – Laguna Blanca; SSS – Spatial segregation of the sexes

Introduction

Dioecy is common in water- and nutrient-limited environments and is probably a major key for the success of some plant species in patchy habitats (Freeman et al.

1980). Discontinuities in plant canopies of arid ecosystems induce microenvironments with varying conditions of fertility and shelter from wind (Klemmedson and Barth 1975; Rostagno and del Valle 1988; Garner and Steinberg 1989; Rostagno et al. 1991; Aguiar et al. 1992; Aguiar and Sala 1994; Vinton and Burke 1995). These contrasting microhabitats would increase the opportunities to successfully sustain spatially separated sex functions (Freeman et al. 1976; Lloyd and Bawa 1984; Freeman et al. 1993). Spatial segregation of the sexes might optimize the use of resources in space and time, increasing the fitness of males and females in comparison with cosexuals (Freeman et al. 1976, 1984; Lloyd 1982; Lovett Doust and Lovett Doust 1988; Pannell and Barrett 1998; Charlesworth 1999).

The avoidance of inbreeding or selective advantage of sexual specialization have been indicated as possible reasons for the evolutionary success of dioecy in plants. The former has probably been a major force in the evolution of dioecy in animal-pollinated plants, while sexual specialization probably underlies the prevalence of separated sexes in plants with abiotic pollination (Freeman et al. 1997). Sex-related division of labor may eventually be associated with morphological (Midgley and Bond 1989) and functional differences (Dawson and Bliss 1989; Dawson and Ehleringer 1993) resulting in habitat segregation between sexes (Harper 1977; Lloyd and Webb 1977; Cox 1981; Lovett Doust et al. 1987; Lloyd and Bawa 1984; Gehring and Linhart 1993). Slatkin (1980, 1984) suggested that competition for limiting resources would be the main cause underlying the evolution of sexual dimorphism in resource use and habitat occupancy, but competition alone would not necessary lead to a consistent pattern of male dominance in resource-poor habitats as observed in most situations (Geber 1999). Other researchers centered their attention on disruptive selection led by higher reproductive costs of females as compared to males (Freeman et al. 1976; Cox 1981; Bierzychudek and Eckard 1988; Dawson and Bliss 1989; Dawson and Ehleringer 1993; Freeman et al. 1997; Geber 1999).

Poa ligularis Nees. ap. Steudel is a widespread wind-pollinated dioecious bunchgrass (Correa 1978) in arid ecosystems of Patagonia and the Patagonian-Monte and is one of the most preferred species by herbivores (Boelcke 1957; Correa 1978). Long-term observations in a wide range of field and greenhouse environmental conditions did not indicate sex lability in this species (Bertiller et al. 2000; Bertiller unpublished). *Poa ligularis* colonizes many ecosystems with patchy vegetation, where soil nitrogen and microclimate variations are strongly related to discontinuities of the plant canopy (Aguiar et al. 1992; Aguiar and Sala 1994; Soriano et al. 1994; Mazzarino et al. 1996; Bisigato and Bertiller 1997; Mazzarino et al. 1998). The species abundance and cover has been reduced in areas with moderate to high grazing impacts (Soriano 1959; Soriano et al. 1995), where also the structure of the shrub canopy has been disturbed by grazing (Bisigato and Bertiller 1997; Bertiller and Bisigato 1998). Previous evidences of partial SSS in *P. ligularis* in relation to the occurrence of plant patches (Bertiller et al. 2000), led us to analyze the role of environmental patchiness on SSS and the likely consequences for reproductive assurance in *P. ligularis*. This

knowledge would contribute to the information set required for developing active policies aimed to the conservation of this species.

We hypothesized that (i) males and females of *P. ligularis* are spatially segregated in the range of microenvironments created by plant patches as a result of sexual specialization in habitat and/or resources use, (ii) sexual specialization is related to different tolerance to competition and reproductive costs of males and females, and (iii) changes in plant patch structure affect the microenvironment and the intensity of SSS. Based on probable higher resource costs of reproduction of females and better opportunities for pollen dispersal of males, we predicted that SSS occurs along gradients of water/N-fertility and air evaporative demand. This would result in females occupying N/water-rich and sheltered areas inside plant patches while males would be frequent in exposed and non-fertile areas outside plant patches. We also predicted that large patches would create larger sheltered areas and induce higher SSS than small patches.

Materials and methods

Study area

We selected three study sites of about 2 ha each in northeastern Patagonia at places where grazing by large herbivores had been excluded for at least 5 years (Estancia San Luis: ESL, 42°39' S, 65°23' W, 115 m a.s.l.; Laguna Blanca: LB, 42°48' S, 65°08' W, 120 m a.s.l.; and Bahía Cracker: BC, 42°59' S, 64°34' W, 90 m a.s.l.). Mean annual precipitation ranges from 160 mm in the western site (ESL) to 200 mm in the eastern site (BC) (Barros and Rivero 1982). In ESL and LB, soils are a complex of Typic Petrocalcids-Typic Haplocalcids (Del Valle 1998; Soil Survey Staff 1998). In the upper soil, the clay content ranges from 4–6% beneath vegetation patches to 7–8% in the bare soil (Mazzarino et al. 1996). The vegetation is characteristic of the southern portion of the Monte Phytogeographic Province (Soriano 1950; León et al. 1998). The plant canopy is dominated by tall shrubs (*Larrea divaricata* Cav., *Chuquiraga hystrix* Don., *Lycium chilense* Miers ex Bert, *Schinus johnstonii* Barkley), medium shrubs (*Chuquiraga avellanadae* Lorentz, *Atriplex lampa* Gill. ex Moq), and dwarf shrubs (*Nassauvia fuegiana* (Speg.) Cabrera, *Acantholippia seriphioides* (A. Gray) Mold., *Junellia seriphioides* (Gillies and Hook) Mold.). Shrubs cover more than 25% of the soil in a patchy pattern. The grass stratum is dominated by *Stipa tenuis* Philippi, *P. ligularis* and *Stipa speciosa* Trinius et Ruprecht. In LB, the total cover is ca. 40–60% and *Chuquiraga avellanadae* is the dominant medium shrub species while in ESL the cover is about 30–35% and *Atriplex lampa* dominates among medium shrubs (Bertiller et al. 1991; Mazzarino et al. 1996; Bisigato and Bertiller 1997). In BC, soils are Xeric Haplargids with some Xeric Calcargids and Typic Torriorthents (Del Valle 1998; Soil Survey Staff 1998). The vegetation is characteristic

of the Patagonian Phytogeographic Province with some components of the Monte Phytogeographic Province (Soriano 1950; Soriano 1956; León et al. 1998). The dominant species are medium shrubs (*Ch. avellanadae*) and small shrubs (*N. fuegiana*) which cover about 30–40% of the soil, in a patchy pattern. The grass stratum is dominated by *S. tenuis*, *P. ligularis*, *Piptochaetium napostaense* (Spegazzini) Hackel ap. Stuckert and *S. speciosa* (Beeskow et al. 1995).

Sampling plant patches and micro-environmental characteristics

a. Vegetation

We evaluated the spatial distribution of perennial vegetation patches and the distribution of *P. ligularis* with respect to them along linear transects (four 40 m, six 50 m, and eight 50 m in LB, BC, and ESL, respectively) during the flowering season of *P. ligularis* (October–November). The number of transects at each site was varied in proportion to the local density of *P. ligularis* plants intercepted by transects. This was done in order to obtain similar total numbers of plants sampled at each site. For the purpose of this study, we define a ‘patch’ as a group of perennial plant species, including at least one shrub life form, such that the projection of its canopy over the soil was separated from neighbor patches by at least 15 cm of bare ground. In each transect, the number, maximum height and length intercepted by each patch were measured and the internal cover of each patch was visually estimated. The plants of *P. ligularis* on each transect were categorized according to their position with respect to patches (center, edge, and outside). The position ‘center’ corresponded to those plants located at distances to patch center smaller than half of the radius (r) and ‘edge’ to those ranging from $1/2r$ to r .

b. Soil nitrogen

In order to evaluate different aspects of the spatial distribution of nitrogen in soil, we obtained two series of samples at each study site. One was aimed at identifying differences in soil nitrogen concentration among positions with respect to plant patches (center, edge, outside) at BC, LB, and ESL. Five vegetation patches of about 50–70 cm radius (in LB and ESL) and 30–40 cm radius in BC were selected in March–April 1998. Soil samples were taken with a metallic corer (depth: 10 cm, diameter: 5 cm) at the center, at the edge (N, S, E, W), and outside patches (N, S, E, W, at 30–50 cm from patch border). A second series of samples was used to evaluate differences in soil nitrogen concentration under males or females of *P. ligularis* plants growing inside (center + edge) or outside patches. In October 1998, twenty cylindrical soil cores (depth: 10 cm, diameter: 10 cm) beneath plants of each sex of *P. ligularis* growing inside and outside patches were taken at each site. The soil obtained in all cases was air-dried and sieved (0.5 mm mesh) and total nitrogen concentration (mg N per g soil) was determined by the Kjeldahl technique (Bremner and Mulvaney 1982).

c. Microclimate

The evaporative demand was measured at 10 cm aboveground during day-light hours (9–17 h) with calibrated Piche evaporimeters (Stoutjesdijk and Barkman 1992) at 30–90 days intervals, from March 1997 to February 1999. Measurements were made at the edge (N, S, E, W), and outside (N, S, E, W, at 30–50 cm from patch border) of one vegetation patch (radius: 50–70 cm in LB and ESL, radius: 30–40 cm in BC) at each site. The soil water content (g of water per 100 g of soil dried at 105 °C) was determined at three vegetation patches at each site by extracting soil cores (depth: 30 cm, diameter: 5 cm) at the same positions where the evaporative demand was measured.

In order to highlight contrasts between the micrometeorological conditions prevailing at the patch edge and outside patches, irrespective of seasonal variation, we computed the average relative evaporative demand and the average relative soil water content, at dates i from average (N, S, E, W) absolute values as:

$$\lambda r_{e,i} = \lambda a_{e,i} / (1/2(\lambda a_{e,i} + \lambda a_{o,i}))$$

$$\lambda r_{o,i} = \lambda a_{o,i} / (1/2(\lambda a_{e,i} + \lambda a_{o,i}))$$

$$\theta r_{e,i} = \theta a_{e,i} / (1/2(\theta a_{e,i} + \theta a_{o,i}))$$

$$\theta r_{o,i} = \theta a_{o,i} / (1/2(\theta a_{e,i} + \theta a_{o,i}))$$

where λr – λa refer to the relative and absolute air evaporative demand and θr – θa to the relative and absolute soil water content at edge (e) or outside (o) positions, respectively.

Plant competition experiment

In October 1996, 44 plants (22 females and 22 males) were obtained from Laguna Blanca site, transplanted to pots with 2 kg soil and maintained in the greenhouse until September 1997. At this time, three individual tillers with two expanded green leaves were separated from plants and pooled into groups by sex. Individual tillers in different sex combinations and densities were placed in rectangular prismatic boxes (21 × 9 × 2 cm) filled with 360 g of air-dried soil. The soil was obtained from the upper layer (0–10 cm) underneath vegetation patches at Laguna Blanca site. Six competition treatments with seven replicates were established as follows: 2 males (MM), 2 females (FF), 4 males (MMMM), 4 females (FFFF), 1 male + 1 female (MF), and 2 males + 2 females (MMFF). Each box was provided with an irrigation porcelain capsule connected to a water reservoir to allow a nearly constant and homogeneous soil water content (10% dry weight basis). The boxes were maintained during approximately 14 months in a ventilated greenhouse where temperature was similar to that in the

field. At the flowering time in October 1998, the plants of each box were removed, washed and the above- and below-ground biomass was dried at 45 °C and weighed.

Statistical analysis

We used SPSS package (Norusis 1993) to perform all statistic tests. Prior to statistical analysis, we tested all variables for normality and homogeneity of variances (Sokal and Rohlf 1981). We used a one way and three way Anova to determine the significance of differences in patch characteristics among sites and nitrogen concentration in soil among and within sites, respectively. Differences in relative air evaporative demand (λr) and in the non-normally distributed relative soil water content (θr) between positions with respect to patches at each site were tested by one way Anova and the non-parametric Mann–Whitney’s test, respectively. We used a two-way Anova to analyze differences in plant density between sexes and sites. To analyze the spatial distribution of the sexes within and among sites, we used χ^2 -tests. In the plant competition experiment, we used a three-way Anova to evaluate the significance of differences in plant performance between sexes and among treatments. Stepwise regression analysis was used to analyze the relationship between patch and environmental variables, total plant densities (independent variables) and the relative frequency of males and females (dependent variables).

Results

Plant patches and micro-environmental characteristics

The vegetation in ESL and BC consisted of a significantly higher number of patches per unit transect length than in LB. The radius and height of the patches did not differ significantly between LB and ESL, but were higher than in BC. The internal patch cover was significantly higher in LB and BC than in ESL (Table 1). The range of soil nitrogen depended both on patch size (radius and height) and patch cover (ESL > LB > BC). In large patches in LB and ESL, the highest soil nitrogen concentration was found beneath patch centers while in small patches of BC there was no significant spatial pattern of soil nitrogen variability. The lowest soil nitrogen concentration was found outside patches in ESL (Tables 2a,b). Relative evaporative demand increased towards outside the patches at all sites (Table 3). The relative soil water content differed among sites in accordance with their patch structure. In LB, where patches were large and dense, the soil at the edge of the patches was significantly drier than the soil outside them. In BC, (small patches with high internal cover), the soil water content was comparatively higher at the edge than outside the patches. In ESL no differences in relative soil water content were found between inside and outside the patches (Table 3).

Table 1. Characterization of the vegetation structure at Laguna Blanca, Estancia San Luis, and Bahía Cracker sites.

| Site | No. of patches per 10 m transect | Mean patch radius (cm) | Mean height of patches (cm) | Mean internal patch cover (%) |
|-------------------|----------------------------------|------------------------|-----------------------------|-------------------------------|
| Laguna Blanca | 3.4 ^a | 49.9 ^a | 120.6 ^a | 66.1 ^a |
| Estancia San Luis | 5.7 ^b | 43.8 ^a | 112.0 ^a | 54.1 ^b |
| Bahía Cracker | 5.7 ^b | 34.2 ^b | 49.8 ^b | 75.2 ^a |

Different lower case letters indicate significant ($P \leq 0.05$) differences between sites (One way ANOVA test, Norusis 1993).

Table 2a. Nitrogen concentration of soil at different sites and positions with respect to patches: center, edge, and outside.

| Site | Mean nitrogen concentration at the center ($\text{mg} \cdot \text{g}^{-1}$) | Mean nitrogen concentration at the edge ($\text{mg} \cdot \text{g}^{-1}$) | Mean nitrogen concentration outside ($\text{mg} \cdot \text{g}^{-1}$) |
|-------------------|---|---|---|
| Laguna Blanca | 0.906 ^a | 0.621 ^b | 0.582 ^b |
| Estancia San Luis | 0.844 ^a | 0.553 ^b | 0.471 ^c |
| Bahía Cracker | 0.772 ^a | 0.787 ^a | 0.765 ^a |

Different lowercase letters indicate significant ($P \leq 0.05$) differences in N concentration of soil among different positions with respect to patches and sites (Tukey's test for multiple comparisons, Norusis 1993).

Table 2b. Two-way analysis of variance of nitrogen concentration of soil by site and position with respect to patches.

| Source of variation | Sum of squares | d.f. | F |
|-----------------------------|----------------|------|---------|
| <i>Main effects</i> | 1.77 | 4 | 40.95** |
| Site | 1.11 | 2 | 51.18** |
| Position | 0.66 | 2 | 30.72** |
| <i>Two-way-interactions</i> | | | |
| Site \times Position | 0.32 | 4 | 7.50** |

Significance: ** $P \leq 0.01$.

Table 3. Mean ($n = 18$) relative evaporative demand (λr) and soil water content (θr) inside patches (edge) and outside patches in LB, ESL, and BC.

| Site | λr | | θr | |
|-------------------|-------------------|-------------------|-------------------|-------------------|
| | Edge | Outside | Edge | Outside |
| Laguna Blanca | 0.90 ^a | 1.10 ^b | 0.98 ^a | 1.02 ^b |
| Estancia San Luis | 0.86 ^a | 1.14 ^b | 1.00 ^a | 1.00 ^a |
| Bahía Cracker | 0.90 ^a | 1.10 ^b | 1.09 ^a | 0.91 ^b |

Different lowercase letters indicate significant ($P \leq 0.05$) differences between different positions with respect to patches (edge, outside) at each site (One way ANOVA test and Mann-Whitney's test, respectively, Norusis 1993).

Poa ligularis density and sexual spatial segregation (SSS)

The total density and the density of males and females of *P. ligularis* were significantly higher in LB than in the other two sites (Tables 4a,b). Females and males were found at the same density in BC and ESL, while in LB the density of females was greater than that of males. The relative position of sexes of *P. ligularis* with respect to the patch (center, edge or outside) was significantly different within and among sites (Figure 1). At LB, plants were more uniformly distributed inside and outside the patches than at the other two sites where almost all the plants were concentrated inside patches. At LB, significant differences ($\chi^2 = 14.74$, $P < 0.01$) in the spatial distribution of the sexes were observed. The frequency of males increased towards outside patches. Females were more abundant at the edge of the patches. Female-biased sex ratios occurred inside patches (center and edge) and male-biased sex ratios occurred outside patches. At ESL and BC, males were significantly more frequent at the edge and females at the center of the patches ($\chi^2 = 10.81$, $P < 0.01$, $\chi^2 = 8.35$, $P < 0.05$). In both sites, female-biased sex ratios occurred in the center and male-biased ratios at the edge of the patches. Outside patches, male-biased sex ratios were observed in ESL while in BC males and females occurred in equal proportions.

Table 4a. Mean density (total, male and female) of *P. ligularis* plants in the study sites.

| Site | Total plants per 10 m transect | Males per 10 m transect | Females per 10 m transect |
|-------------------|--------------------------------|-------------------------|---------------------------|
| Laguna Blanca | 10.9 ^A | 4.5 ^b | 6.4 ^a |
| Estancia San Luis | 3.8 ^B | 1.9 ^c | 1.9 ^c |
| Bahía Cracker | 5.1 ^B | 2.5 ^c | 2.6 ^c |

Different uppercase letters indicate significant ($P \leq 0.05$) differences in the total number of plants between sites (One way Anova). Lowercase letters indicate significant ($P \leq 0.05$) differences in the density of plants between sites and sexes (Tukey's test for multiple comparisons).

Table 4b. Two-way analysis of variance of density of *P. ligularis* plants by sex and sites.

| Source of variation | Sum of squares | d.f. | F |
|-----------------------------|----------------|------|---------|
| <i>Main effects</i> | 73.21 | 3 | 30.54** |
| Sex | 1.80 | 1 | 2.25NS |
| Site | 71.41 | 2 | 44.63** |
| <i>Two-way-interactions</i> | | | |
| Sex \times Site | 5.73 | 2 | 3.58* |

Significance: ** $P \leq 0.01$, * $P \leq 0.05$, NS: Not significantly different.

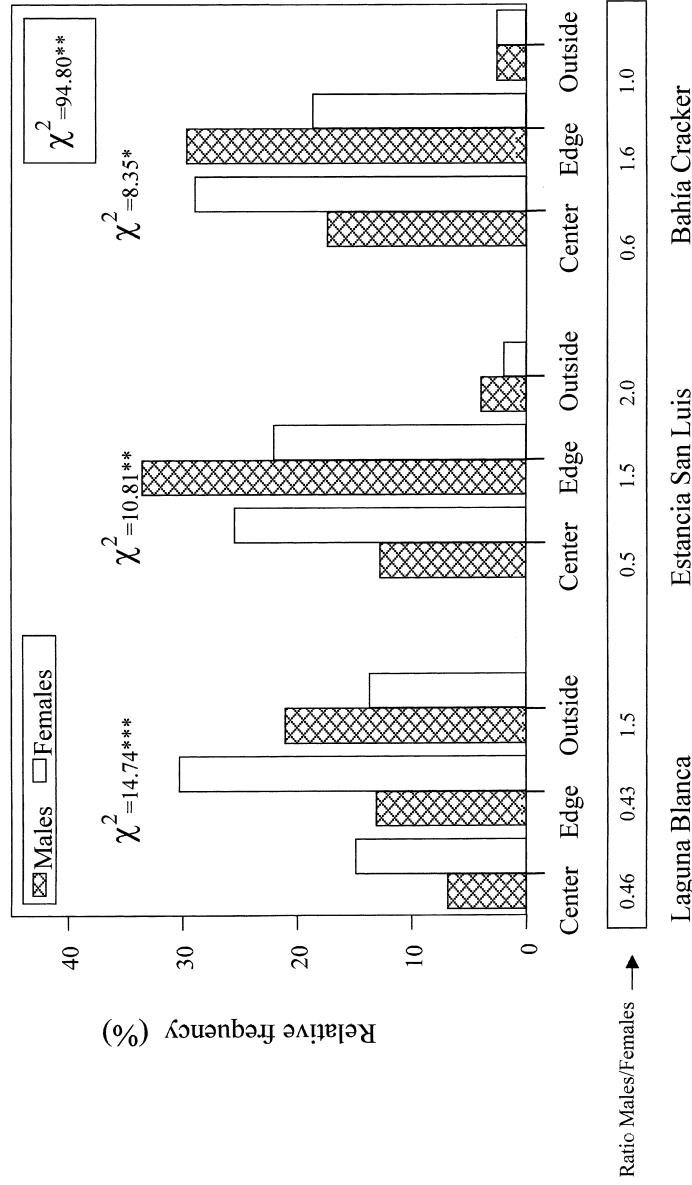


Figure 1. Distribution of relative frequencies of sexes in relation to the positions of *P. ligularis* plants with respect to patches (center, edge, or outside) in the study sites (Laguna Blanca, Estancia San Luis or Bahía Cracker). Significant sex × patch position interactions at each site are indicated by the χ^2 values included in each graph. Differences in the distributions among sites (sex × position × site) are indicated by the χ^2 value in the upper right corner. Significance: ** $P \leq 0.01$, * $P \leq 0.05$. Below ratios Male/Female.

Table 5a. Nitrogen concentration ($\text{mg} \cdot \text{g}^{-1}$) in the soil beneath plants of *Poa ligularis* growing inside or outside patches.

| | Inside | | Outside | |
|-------------------|--------------------|--------------------|-------------------|-------------------|
| | Males | Females | Males | Females |
| Laguna Blanca | 0.65 ^{cd} | 0.66 ^{cd} | 0.45 ^a | 0.49 ^a |
| Estancia San Luis | 0.56 ^c | 0.67 ^d | 0.43 ^a | 0.51 ^b |
| Bahía Cracker | 0.90 ^e | 0.83 ^e | 0.67 ^d | 0.76 ^d |

Significant ($P \leq 0.05$) differences between sexes and position with respect to patches (inside or outside) are indicated with different lowercase letters (Tukey's test for multiple comparisons, Norusis 1993).

Table 5b. Three-way analysis of variance of nitrogen concentration in soil by sex, position, and site.

| Source of variation | Sum of squares | d.f. | F |
|---------------------|----------------|------|---------|
| Main effects | 2.39 | 4 | 38.34** |
| Sex | 0.06 | 1 | 3.96* |
| Position | 0.77 | 1 | 49.55** |
| Site | 1.55 | 2 | 49.93** |

Significance: ** $P \leq 0.01$, * $P \leq 0.05$. All two and three way interactions are not significantly different.

N-concentration in microsites occupied by each sex

Females of *P. ligularis* occupied N-richer microsites (inside or outside patches) than males. These differences, however, were more marked in ESL, the N-poorest site (Tables 5a,b).

Inter- and intra-sexual competition

Plant density was the main factor affecting the total biomass of both sexes of *P. ligularis* (Figure 2, Table 6a). Males and females growing in unisexual or bisexual groups at high densities (MMMM, MMFF, FFFF) had the lowest aerial and root biomass. At low densities, males growing in pure sex groups (MM) had significantly higher total biomass than those growing in bisexual mixtures. In this latter, total biomass did not differ from that at high densities. Females, however, were better able to tolerate inter-sex competition since the total biomass did not differ between plants growing in unisexual and bisexual groups at low or high densities. Plants of both sexes growing in pure groups (MM, MMMM, FF, or FFFF) allocated more biomass to panicles than those growing in mixtures, independently from the plant density. In all treatments, panicle biomass was higher in females than in males and in both sexes was affected by inter-sex competition (Figure 2, Table 6b).

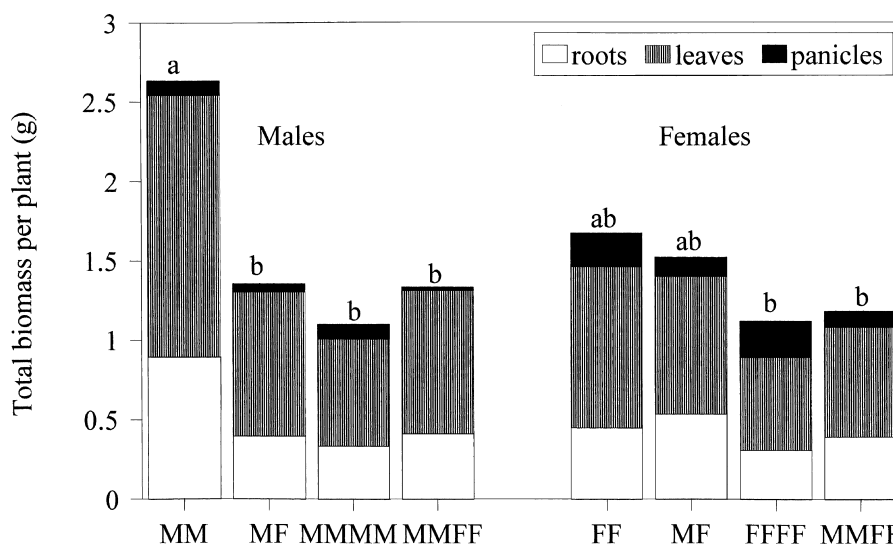


Figure 2. Results of a controlled competition experiment with different substitution of male and female plants of *P. ligularis* growing under homogeneous and constant water supply (10%). Different lowercase letters indicate significant ($P \leq 0.05$) differences in total biomass.

Table 6. Three-way analysis of variance of (a) total biomass, (b) panicle biomass by sex (male or female), diversity (1 sex or 2 sexes per pot), and density (2 plants or 4 plants per pot).

| Source of variation | Sum of squares | d.f. | <i>F</i> |
|---------------------|----------------|------|----------|
| (a) | | | |
| Main effects | 17.36 | 3 | 3.86* |
| Sex | 1.53 | 1 | 1.02NS |
| Diversity | 0.37 | 1 | 0.25NS |
| Density | 15.46 | 1 | 10.32** |
| (b) | | | |
| Main effects | 0.106 | 3 | 7.24** |
| Sex | 0.064 | 1 | 13.27** |
| Diversity | 0.041 | 1 | 8.42* |
| Density | 0.001 | 1 | 0.03NS |

Significance: ** $P \leq 0.01$, * $P \leq 0.05$, NS: Not significantly different. All two and three way interactions are not significantly different.

Relative sex frequency, plant and environmental variables

Relative evaporative demand was the single most important environmental variable explaining female frequencies in stepwise regression including plant and environmental variables (Table 7a). The increase in the relative frequency of females was related to the decrease of the relative evaporative demand, which explained 46% of the total variance. The increase in the relative frequency of males was significantly related to

Table 7. Stepwise regression between the relative frequency of each sex (dependent variable) at the different sites and microsities and independent variables: patch (height, radius, cover), microenvironment (relative evaporative demand, relative soil moisture, and nitrogen concentration in the soil), and total density of *P. ligularis*.

| Variables entered | <i>R</i> | <i>R</i> ² | Sum of squares | <i>F</i> | Regression coefficients | <i>t</i> |
|--|----------|-----------------------|----------------|----------|-----------------------------|-------------------|
| a. Dependent variable: relative frequency of females | | | | | | |
| 1. Evaporative demand (constant) | 0.68 | 0.46 | 0.43 | 43.58** | -0.79 (0.12) 0.94 (0.12) | -6.60** 8.05** |
| b. Dependent variable: relative frequency of males | | | | | | |
| 1. Evaporative demand | 0.47 | 0.22 | 0.24 | 14.31** | -0.99 (0.17) | -5.77** |
| 2. Soil nitrogen | 0.60 | 0.34 | 0.40 | 14.17** | -0.60 (0.15) | -3.99** |
| 3. Patch cover (constant) | 0.64 | 0.42 | 0.46 | 11.41** | 0.004 (0.002) 1.27(0.21) | 2.03* 6.02** |

Significance: ** $P \leq 0.01$, * $P \leq 0.05$.

the decrease of the evaporative demand, the decrease of N concentration in soil and the increase of patch cover (22, 12, and 8% of the total variance, respectively).

Discussion

The sexes of *P. ligularis* were spatially segregated in the microenvironments created by plant patches. The SSS varied according to the characteristics of environmental patchiness (Figure 1). As in other arid ecosystems (Klemmedson and Barth 1975; Charley and West 1977; Garner and Steinberg 1989; Soriano et al. 1994; Callaway 1995; Vinton and Burke 1995; Breshears et al. 1998), vegetation patches at our study sites were associated with fertility and shelter islands (Tables 1, 2, and 3). Our results showed that as the patch size and their area of influence increased, the intensity of SSS and the density of *P. ligularis* also increased ($BC \leq ESL < LB$, Figure 1, Table 4). These patterns are consistent with an increase of fertile and sheltered microsities inside patches (center + edge) or immediately nearby outside, resulting in enhanced opportunities for establishment. In LB, where patches were large and dense, the total number of *P. ligularis* was higher and the SSS more intense than in ESL and BC. Females were concentrated in sheltered (low evaporative demand) and N-rich areas inside plant patches while the frequency of males increased with distance from the center of patches. These results are in agreement with other studies (Freeman et al. 1976; Harper 1977; Lloyd and Webb 1977; Cox 1981; Lovett Doust et al. 1987; Lloyd and Bawa 1984; Gehring and Linhart 1993; Dawson and Geber 1999; Ågren et al. 1999) showing a consistent pattern of female-biased sex ratios in favorable habitats and male-biased ratios in stressful habitats.

Sex specialization and SSS are important adaptive plant traits, which may in turn increase reproductive efficiency (Freeman et al. 1976; Lloyd 1982) or permit access to resources that each sex may require for successful growth and reproduction (Dawson

and Ehleringer 1993). Biased sex ratios of *P. ligularis* in specific habitats and microhabitats may be analyzed in the framework of the current models of the evolution of sexual dimorphism (Geber 1999). According to these, competition for limiting resources between sexes would lead to sexual dimorphism in resource and habitat use in such a way that females would use portions of the resource spectrum different from those used by males (Slatkin 1980, 1984). This mechanism is likely to be operative in *P. ligularis* as shown by the higher competitive ability of females over males (Figure 2). However, as previously argued by Geber (1999) and also found in our study, competition alone would not necessarily lead to a consistent pattern of male dominance in resource-poor habitats. SSS has also been related to the effect of disruptive selection acting on male-female populations with different reproductive costs (Freeman et al. 1976; Lovett Doust et al. 1987; Lyons et al. 1994; Bierzychudek and Eckard 1988; Geber 1999). Our data are consistent with a mechanism of SSS based on sex-specific disruptive selection, which does not depend on interactions with the other sex. In this context, the preferential distribution of females at fertile and sheltered microsites, where the evaporative demand is low (Tables 2, 3 and 7), may result from their higher reproductive costs as compared to males (Figure 2). On the contrary, males with lower reproductive costs than females may eventually be at a reproductive advantage in N-poor and exposed microsites because of enhanced pollen dispersal (Freeman et al. 1976; Geber 1999). Even at a micro-scale, we also found spatial sex segregation inside and outside patches (Tables 5a,b). Sex-related phenological traits of *P. ligularis* give further support to a mechanism of SSS based on sex-specific disruptive selection. Males flower earlier during the growing season than females and stop their vegetative and reproductive growth when pollen is dispersed (Bertiller et al. 1991). In northern Patagonia, the annual precipitation is concentrated in autumn-winter and pollen dispersion occurs in spring when water and inorganic-N are still available in the soil profile explored by grasses (Mazzarino et al. 1998). Thus, male reproduction occurs at a time when resources are not strongly limiting, even at exposed areas far from a patch. Females complete reproductive growth about 30–60 days later than males during the growing season. For females growing in exposed areas, reproductive growth would occur under limiting conditions during the driest part of the year, long after pollen production had ceased (Bertiller et al. 1991; Coronato and Bertiller 1997; Mazzarino et al. 1998; Bertiller, unpublished). Accordingly, we interpret that SSS and sexual specialization in *P. ligularis* have resulted from differential fitness of each sex derived from their reproductive costs as well as the outcome of competition for limiting resources and better opportunities for pollen dispersal of males.

From the point of view of conservation, environmental patchiness is an important indicator of the status of *P. ligularis* populations, related to the SSS and the reproductive success of this species. The reduction of the patch size and/or internal cover modifies the proportion of favorable microsites for each sex. This in turn leads to the decrease of SSS, to the reduction of plant density, and to a bias in the sex ratio

towards males inside patches. As previously reported for dioecious species (Freeman et al. 1976, 1984; Lovett Doust and Lovett Doust 1988; Pannell and Barrett 1998), decreased SSS would affect the reproductive assurance of *P. ligularis* through the increase of inter-sexual competition for limiting resources, the reduction of pollen dispersal, and the decrease of overall seed production (Figure 2, Table 6b). The assessment of biological consequences of intra- and inter-sexual interactions under scenarios of different environmental patchiness, a subject scarcely explored in most ecosystems (Ågren et al. 1999) is the theme of ongoing research at our sites and will contribute to the information set required for *P. ligularis* conservation in Patagonia.

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References

- Aguiar M, Soriano A and Sala OE (1992) Competition and facilitation in the recruitment of seedlings in a Patagonian steppe. *Functional Ecology* 6: 66–70
- Aguiar M and Sala OE (1994) Competition, facilitation, seed distribution, and the origin of patches in a Patagonian steppe. *Oikos* 70: 26–34
- Ågren J, Danell K, Elmqvist T, Ericson L and Hjältén J (1999) Sexual dimorphism and Biotic interactions. In: Geber MA, Dawson TE and Delph LF (eds) *Gender and Sexual Dimorphism in Flowering Plants*, pp 217–246. Springer-Verlag, Heidelberg
- Barros V and Rivero M (1982) Mapas de probabilidad de precipitación de la Provincia del Chubut. Monografía Nro. 54, pp 1–12. Centro Nacional Patagónico, Puerto Madryn, Chubut, Argentina
- Beeskow AM, Elissalde NO and Rostagno CM (1995) Ecosystem changes associated with grazing intensity on the Punta Ninfas rangelands of Patagonia, Argentina. *Journal of Range Management* 48: 517–522
- Bertiller MB, Ares JO, Graff BP and Baldi R (2000) Sex-related spatial patterns of *Poa ligularis* in relation to patch occurrence in northern Patagonia. *Journal of Vegetation Science* 11: 9–14
- Bertiller MB, Beeskow AM and Coronato FR (1991) Seasonal environmental variation and plant phenology in arid Patagonia (Argentina). *Journal of Arid Environments* 21: 1–11
- Bertiller MB and Bisigato AJ (1998) Vegetation dynamics under grazing disturbance. The state-and-transition model for the Patagonian steppes. *Ecología Austral* 8: 191–199
- Bierzchudek P and Eckard V (1988) Spatial segregation of the sexes in dioecious plants. *American Naturalist* 132: 34–43
- Bisigato A and Bertiller MB (1997) Grazing effects on the spatial patterns of vegetation in northern Patagonia. *Journal of Arid Environments* 36: 639–653
- Bremner JM and Mulvaney CS (1982) Nitrogen Total. In: Page AL, Miller RH and Keeney DR (eds) *Methods of Soil Analysis. Part 2 Chemical and Microbiological Properties*, pp 595–698. American Society of Agronomy, Soil Science Society of America, Madison, Wisconsin

- Breshears D, Nyhan JW, Heil CE and Wilcox B (1998) Effects of woody plants on microclimate in a semiarid woodland: soil temperature and evaporation in canopy and intercanopy patches. *International Journal of Plant Science* 159: 1010–1017
- Boelcke O (1957) Comunidades herbáceas del norte de la Patagonia y sus relaciones con la ganadería. *Revista de Investigaciones Agrícolas* 11: 5–98
- Callaway RM (1995) Positive interactions among plants. *The Botanical Review* 61: 306–349
- Charlesworth D (1999) Theories of the evolution of dioecy. In: Geber MA, Dawson TE and Delph LF (eds) *Gender and Sexual Dimorphism in Flowering Plants*, pp 33–60. Springer-Verlag, Heidelberg
- Charley JL and West NE (1977) Micropatterns of N-mineralization activity in soils of some shrub-dominated semidesert ecosystems of Utah. *Soil. Biology and Biochemistry* 9: 357–365
- Coronato FR and Bertiller MB (1997) Climatic controls of soil moisture dynamics in an arid steppe of northern Patagonia, Argentina. *Arid Soil Research and Rehabilitation* 11: 277–288
- Correa MN (1978) Flora Patagónica, Parte III. Colección Científica Tomo VIII. Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina
- Cox PA (1981) Niche partitioning between sexes of dioecious plants. *The American Naturalist* 117: 295–307
- Dawson TE and Bliss LC (1989) Patterns of water use and the tissue water relations in the dioecious shrub *Salix arctica*: the physiological basis of habitat partitioning between the sexes. *Oecologia* 79: 332–343
- Dawson TE and Ehleringer JR (1993) Gender-specific physiology, carbon isotope discrimination and habitat distribution in boxelder, *Acer negundo*. *Ecology* 74: 798–815
- Dawson TE and Geber MB (1999) Sexual dimorphism in physiology and morphology. In: Geber MA, Dawson TE and Delph LF (eds) *Gender and Sexual Dimorphism in Flowering Plants*, pp 175–215. Springer-Verlag, Heidelberg
- Del Valle HF (1998) Patagonian soils: a regional synthesis. *Ecología Austral* 8: 103–123
- Freeman DC, Klikof LG and Harper KT (1976) Differential resource utilization by sexes of dioecious plants. *Science* 193: 597–599
- Freeman DC, Harper KT and Ostler WK (1980) Ecology of plant dioecy in the intermountain region of western North America and California. *Oecologia* 44: 410–417
- Freeman DC, McArthur ED and Harper KT (1984) The adaptive significance of sexual lability in plants using *Atriplex canescens* as a principal example. *Annals of the Missouri Botanical Garden* 71: 265–277
- Freeman DC, McArthur ED, Sanderson SC and Tiedemann AR (1993) The influence of topography on male and female fitness components of *Atriplex canescens*. *Oecologia* 93: 538–547
- Freeman DC, Lovett Doust J, El-Keblawy A, Miglia KJ and McArthur ED (1997) Sexual specialization and inbreeding avoidance in the evolution of dioecy. *The Botanical Review* 63: 65–92
- Garner W and Steinberger Y (1989) A proposed mechanism for the formation of 'fertile Islands' in desert ecosystem. *Journal of Arid Environments* 16: 257–262
- Geber M (1999) Theories of the evolution of sexual dimorphism. In: Geber MA, Dawson TE and Delph LF (eds) *Gender and Sexual Dimorphism in Flowering Plants*, pp 97–122. Springer-Verlag, Heidelberg
- Gehring JL and Linhart YB (1993) Sexual dimorphisms and response to low resources in the dioecious plant *Silene latifolia* (Caryophyllaceae). *International Journal of Plant Sciences* 154: 152–162
- Harper JL (1977) *Population Biology of Plants*. Academic Press, London
- Klemmedson JO and Barth RC (1975) Distribution and balance of biomass and nutrients in desert shrub ecosystems. US/IBP Desert Biome Research Memo. 75-5, Utah State University Press, Logan, Utah
- León JJC, Bran D, Collantes M, Paruelo JM and Soriano A (1998) Grandes unidades de la Patagonia extra andina. *Ecología Austral* 8: 125–144
- Lloyd DG (1982) Selection of combined versus separate sexes in seed plants. *The American Naturalist* 120: 571–585
- Lloyd DG and Bawa KS (1984) Modification of the gender of seed plants in varying conditions. *Evolutionary Ecology* 17: 255–338
- Lloyd DG and Webb CJ (1977) Secondary sex characters in plants. *Botanical Review* 43: 177–216
- Lovett Doust J and Lovett Doust L (1988) *Plant Reproductive Ecology: Patterns and Strategies*. Oxford University Press, New York
- Lovett Doust J, O'Brien G and Lovett Doust L (1987) Effect of density on secondary sex characteristics and sex ratio in *Silene alba* (Caryophyllaceae). *American Journal of Botany* 74: 40–46

- Lyons EE, Miller D and Meagher TR (1994) Evolutionary dynamics of sex ratio and gender dimorphism in *Silene latifolia*: I. Environmental effects. *Journal of Heredity* 85: 196–203
- Mazzarino MJ, Bertiller MB, Sain CL, Laos F and Coronato F (1996) Spatial patterns of nitrogen availability, mineralization and immobilization in northern Patagonia (Argentina). *Arid Soil Research and Rehabilitation* 10: 295–309
- Mazzarino MJ, Bertiller MB, Sain CL, Satti P and Coronato FR (1998) Intra and interannual nitrogen dynamics under different plant patches in northeastern Patagonia. *Plant and Soil* 202: 125–131
- Midgley JJ and Bond W (1989) Leaf size and inflorescence size may be allometrically related traits. *Oecologia* 78: 427–429
- Norusis MJ (1993) SPSS for Windows: Base System's User Guide. SPSS Inc., Chicago
- Pannell JR and Barrett SCH (1998) Baker's law revisited: reproductive assurance in a metapopulation. *Evolution* 52: 657–668
- Rostagno CM and del Valle HF (1988) Mound associated with shrubs in aridic soils of northeastern Patagonia. Characteristics and probable genesis. *Catena* 15: 347–359
- Rostagno CM, del Valle HF and Videla L (1991) The influence of shrubs on some chemical and physical properties of an aridic soil in north-eastern Patagonia, Argentina. *Journal of Arid Environments* 20: 179–188
- Slatkin M (1980) Ecological character displacement. *Ecology* 61: 163–177
- Slatkin M (1984) Ecological caused of sexual dimorphism. *Evolution* 38: 622–630
- Soil Survey Staff (1998) Key to Soil Taxonomy. USDA, Washington, DC
- Sokal RR and Rohlf FJ (1981) *Biometry*. Freeman, San Francisco
- Soriano A (1950) La vegetación del Chubut. *Revista Argentina de Agronomía* 17: 30–66
- Soriano A (1956) Los distritos florísticos de la Provincia Patagónica. *Revista de Investigaciones Agrícolas* 10: 323–348
- Soriano A (1959) Síntesis de los resultados obtenidos en las clausuras instaladas en la Patagonia en 1954 y 1955. *Revista de Agronomía del Noroeste Argentino* 3: 163–176
- Soriano A, Sala OE and Perelman S (1994) Patch structure and dynamics in a Patagonian arid steppe. *Vegetatio* 111: 127–135
- Soriano A, Nogués Loza M and Burkart S (1995) Plant biodiversity in the extra-andean Patagonia: comparisons with neighbouring and related vegetation units. In: Montes L and Oliva G (eds) *Actas del Taller Internacional sobre Recursos Fitogenéticos, Desertificación y uso sustentable*, pp 36–45. Río Gallegos, Santa Cruz, Argentina
- Stoutjesdijk PH and Barkman JJ (1992) The influence of vegetation on microclimate. In: Stoutjesdijk PH and Barkman JJ (eds) *Microclimate, Vegetation and Fauna*, pp 83–132. Opulus Press, Sweden
- Vinton MA and Burke IC (1995) Interactions between individual plant species and soil nutrient status in shortgrass steppe. *Ecology* 76: 1116–1133