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Changes in sex ratios of a dioecious grass with grazing intensity: the interplay between gender traits, neighbour interactions and spatial patterns

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Summary

1. Dioecious plants frequently face sex-related resource allocation trade-offs. Differential traits of male and female plants can have important consequences that influence their direct and indirect interactions with neighbours and herbivores.

2. Gender differences in traits have been considered to be inherently linked to sex-biased ratios along environmental stress gradients. However, the mechanisms behind the variation in sex ratios with increasing biotic stress remain elusive.

3. We linked the results of process-based field experiments with fine-scale spatial patterns of naturally established plants (both sexes) at different grazing intensities. We demonstrated that slow-growing females were better defended against insects and small mammals but were more susceptible than males to competition from neighbours.

4. In large-herbivore exclosures, we found that sex-biased spatial patterns at the microsite scale can be predicted from gender-specific traits, with females more segregated from competitors (unpalatable grasses) than males. Traits related to growth/defence trade-off in females, coupled with their higher competition costs than males, suggest that gender dimorphism in traits can directly influence species interactions and spatial organization.

5. In the presence of large herbivores, female individuals were preferentially browsed despite their larger investment in antiherbivore compounds. The greater distance of females from unpalatable competitors was the main determinant of female-biased consumption by domestic herbivores. Females growing close to neighbours successfully eluded browsing but also faced competition. Scaling up to a population level, population sex ratios drift from female- to male-bias with increasing domestic grazing intensity.

6. *Synthesis*: The sexual dimorphism in traits of a dioecious bunchgrass results in differential herbivory by natural enemies, differential competitive ability and differential spatial distribution of sexes. Domestic grazers disrupt this balance by shifting species interactions and their spatial organization. Changes in dioecious plant populations towards male-biased sex ratios with grazing intensity could not be predicted solely by gender-specific traits.

Key-words: arid ecosystems, dioecy, life-history traits, O-ring statistics, plant–herbivore interactions, plant–plant interactions, sex ratio, sexual dimorphism, spatial pattern, trade-offs

Introduction

Unlike animals, plants with separate sexes are rare (*c.* 6% of species) (Renner & Ricklefs 1995). However, as in animals, differences in morphological, physiological and life-history traits occur between genders of dioecious plant species (i.e. sexual dimorphism) (Geber, Dawson & Delph 1999). Among dioecious plants, reproductive investment is often higher for females than for males (Putwain & Harper 1972; Lloyd & Webb 1977; Dannel *et al.* 1985; Obeso 2002) and is linked with lower growth rates (Nicotra 1999; Obeso 2002), smaller plant size (Hoffmann & Alliende 1984), greater physiological stress and higher mortality rates (Lloyd & Webb 1977; Allen & Antos 1988; Obeso 2002). These trade-offs are likely to be stronger when resources are scarce (Allen & Antos 1988;

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Delph 1990, 1999), and sexual dimorphism in traits is expected to be more marked under resource limitation (Gehring & Linhart 1993; Sakai, Sasa & Sakai 2006). As a result of this, male-biased sex ratios within populations seem to arise with increasing environmental stress, revealing the consequences of trade-offs in resource allocation (Delph 1999; Obeso 2002).

Sexual dimorphism in traits is likely to be a major cause of the success of dioecious plant species in patchy habitats (Freeman, Klikoff & Harper 1976; Freeman, Harper & Ostler 1980; Bertiller *et al.* 2002a). Females are more common in favourable microsites whereas males predominate in low resource microsites, resulting in a sex ratio bias at a local scale (Dawson & Bliss 1989; Dawson & Ehleringer 1993; Eppley, Stanton & Grosberg 1998; Bertiller *et al.* 2000, 2002a; Eppley 2005). This pattern is referred to as 'spatial segregation of the sexes' (Bierzychudek & Eckhart 1988) and has been observed in the majority of the dioecious plant populations. Gender segregation seems to reflect adaptations to different costs associated with reproduction of individuals that do not express sex expression lability (i.e. plastic alteration of sex expression in relation to its environment) (Cox 1981; Bierzychudek & Eckhart 1988; Eppley, Stanton & Grosberg 1998). Spatial segregation of the sexes can also be the result of interactions with other species such as competition for limited resources (Freeman, Klikoff & Harper 1976; Cox 1981; Eppley 2006; Mercer & Eppley 2010). Inter and intrasexual competition has been proposed as one of the probable mechanisms behind local spatial segregation of the sexes (Bertiller, Sain & Carrera 2002b; Eppley 2006; Mercer & Eppley 2010). Nevertheless, there has been a conspicuous lack of investigation into sex-related effects on the competitive interaction with other plant species (Sánchez-Vilas, Turner & Pannell 2010). Differences in competitive ability between the sexes are expected as a result of allocation trade-offs associated with gender-specific reproductive costs. Thus, the negative effects of competition should be stronger on females, since reproductive costs in females are higher than in males (Lloyd & Webb 1977). As a result, females are expected to be found further away from other plant competitors than males (Schurr *et al.* 2004). Nonetheless, this process–pattern relationship remains largely untested.

Interactions with herbivores have also been proposed as an explanation for female-biased sex ratios in a wide range of habitats and geographic regions (Dannel *et al.* 1985; Elmqvist *et al.* 1988; Boecklen, Price & Mopper 1990; Hjältén 1992). According to the resource availability hypothesis, the higher cost of reproduction, which slows growth, confers a selective advantage to females with traits that increase defence against herbivores (Coley, Bryant & Chapin 1985; Jing & Coley 1990; Herms & Mattson 1992; Cepeda-Cornejo & Dirzo 2010). Consequently, dioecious populations frequently experience male-biased herbivory (as reviewed by Ågren *et al.* 1999; Cornelissen & Stiling 2005). However, most consumers strongly interact with plant spatial patterns (Adler, Raff & Lauenroth 2001). For example, mammal herbivory could be more related to the distance from less palatable neighbours than to investments in antiherbivore defences (Atsatt & O'Dowd 1976; Milchunas & Noy-Meir 2002; Graff & Aguiar 2011). Empirical evidence for sex-biased herbivory has been historically based on isolated plants growing in controlled situations or in correlative field surveys of naturally established co-occurring plants, which are partially inconclusive because of multicausality (Cornelissen & Stiling 2005).

Consumers strongly alter the outcome of species interactions through direct and indirect effects. Studies in terrestrial as well as in aquatic ecosystems confirmed that consumer-incurred biotic stress (i.e. 'disturbance' *sensu* Grime 2001) has a substantial impact on the interactions between sessile species (Bertness & Callaway 1994; Smit, Rietkerk & Wassen 2009). Competing neighbours (negative interaction) can be transformed into refuges (positive interaction) for palatable plants depending on the intensity of selective herbivory or browsing (Graff, Aguiar & Chaneton 2007; Graff & Aguiar 2011; Le Bagousse-Pinguet, Gross & Straile 2011). Herbivory by domestic ungulates is also a major driver of fine-scale plant distribution, increasing spatial aggregation among plant species with different palatability (Bisigato *et al.* 2005; Graff & Aguiar 2011 and references therein). If genders interact differently with neighbours, and those interactions determine fine-scale spatial organization of sexes, it is expected that variation of consumer-incurred biotic stress will affect the population sex ratio. As far as we are aware, no studies have disentangled the impact of the biotic components leading to changes in sex ratios along biotic-stress gradients or explored the mechanisms behind those patterns.

Since spatial segregation of the sexes may be adaptive (Freeman, Klikoff & Harper 1976; Bierzychudek & Eckhart 1988; Geber, Dawson & Delph 1999), herbivory by domestic animals and the subsequent changes in resource availability or neighbour interactions may change sex ratios and reproductive success of many dioecious species populations. Furthermore, since only half of the population (i.e. the females) sets seeds, the number of local patches reached by seeds is potentially reduced in dioecious populations (Heilbuth, Ilves & Otto 2009). Therefore, increases in male-biased sex ratios could compromise population viability.

Here, we worked with a dioecious native perennial grass of the Patagonian steppe (*Poa ligularis*), which is also a valuable forage species that has declined in the last decades (Perelman, Leon & Bussacca 1997) compromising the sustainability of sheep production in this region (Texeira & Paruelo 2006). We carried out a set of manipulative field and greenhouse experiments in combination with field-based descriptive studies. We hypothesized that *P. ligularis* shows sexually dimorphic traits that are directly related to their interactions with neighbouring grass species as well as with natural enemies. We also hypothesize that those differences in traits determine, indirectly, their interaction with domestic herbivores. The predictions were the following:

1 Females will have lower vegetative growth than males (Lloyd & Webb 1977; Obeso 2002), will also be better defended than males, and hence less preferred by natural herbivores (Coley, Bryant & Chapin 1985; Jing & Coley 1990; Herms & Mattson 1992; Cepeda-Cornejo & Dirzo 2010).

2. Female biomass will be more negatively affected by the competition with other grass species than males because of slower female growth and higher reproductive requirements (Lloyd & Webb 1977).
3. In the absence of domestic herbivores, females will be more spatially segregated from other unpalatable grasses than males because of competitive effects. Assuming that grasses with the same rooting depth show low horizontal overlap (Schenk & Robert 2002), the distance at which plants will segregate from each other should match root distribution patterns (Schurr *et al.* 2004; Graff & Aguiar 2011).
4. Neighbouring unpalatable species would reduce the probability of herbivore attack, and plants may gain protection by shared defence or associational resistance (Atsatt & O'Dowd 1976; Milchunas & Noy-Meir 2002; Baraza, Zamora & Hódar 2006; Smit *et al.* 2007). Therefore, distance to less palatable grasses, and not the innate palatability of sexes, will control browsing rate between males and females in domestic grazing situations.
5. As a consequence of females distributing more distantly from unpalatable species, we expect that overall females will be more consumed than males in grazing situations, and male-biased sex ratio will intensify with grazing pressure.

In addition,

6. We expect that the protective role of the benefactor unpalatable species on their *protégés* will peak at moderate grazing situations and decline with increasing grazing pressure (Smit, Rietkerk & Wassen 2009; Graff & Aguiar 2011). Therefore,
7. Within grazed conditions, we expect stronger spatial aggregations (at the herbivore's foraging scale) of both male-unpalatable and female-unpalatable plant pairs with decreasing grazing intensity (Graff & Aguiar 2011).

Materials and methods

SPECIES DESCRIPTION

We worked with *Poa ligularis* Nees. ap. Steudel (hereafter, *Poa*), a dominant species in nondegraded Patagonian shrub grass steppes. *Poa* is a wind-pollinated and strictly dioecious bunchgrass (sect Dioicopoa) native to South American 500 000 km^2 rangelands (Correa 1978). *Poa* is a highly preferred species by domestic and wild herbivores (Correa 1978; Pazos, Bisigato & Bertiller 2007) and is the main foraging resource in these Patagonian steppes. *Poa* populations have experienced significant reductions in over-stocked fields (Perelman, Leon & Bussacca 1997), which have compromised the sustainability of sheep production in this region (Texeira & Paruelo 2006).

This C$_3$, dioecious bunchgrass is a perennial species approximately 30 cm tall (Soriano & Sala 1983). It is active most of the year, but vegetative growth is limited in winter by low temperatures and in late spring and summer by soil water availability (Soriano & Sala 1983). Green leaves rapidly expand during spring and flowering occurs in early summer. Seeds are dispersed in late summer. Reproduction is predominantly sexual: stolons and rhizomes are absent (Aguiar & Sala 1998).

Populations from the southern distributional range exhibit abundant seed production every year but germination events are rare (< 10% germination in 78% of years) (Rotundo 2005). Seedling survival is also low (*c.* 7%), and recruitment chances are exclusively restricted to infrequent good years (Rotundo 2005).

Reproductive structures during flowering and fruiting phases allow gender identification. Some panicles produced the previous year are useful for sex determination, since pistillate florets are markedly more hairy than staminate florets (Giussani 2000). Identifying the sex in prereproductive plants is not possible. Male and female flowering dates can overlap (Graff personal observation). However, females complete reproductive growth (production of seeds) about 30–60 days later than males during the growing season and during the driest part of the year, long after pollen production has ceased (Bertiller, Beeskow & Coronato 1991). Therefore, gender related differences in fitness arise from different reproductive costs (Bertiller *et al.* 2002a).

Sex determination mechanisms in this species are unknown. Nonetheless, long-term observations in a wide range of field and greenhouse environmental conditions indicate that sex expression in *Poa* is constant through time and does not show sex expression lability (Bertiller *et al.* 2000, 2002a; Graff personal observation).

Reports of spatially segregated sexes of *Poa* have been related to the physical microenvironment (soil moisture, nitrogen availability, wind intensity, etc.) (Bertiller *et al.* 2000, 2002a).

STUDY SITE

Field studies were conducted in the Patagonian steppe in south-western Chubut, Argentina (lat 45°41_S, long 70°16_W, 500 m a.s.l.). The study sites were located within a 150-km^2 area at the Río Mayo Experimental Field Station (Instituto Nacional de Tecnología Agropecuaria) and on nearby private properties. Río Mayo is located in the southern edge of the distribution range of *Poa* and corresponds to a grass-shrub steppe of the Occidental District of Patagonia (Leon *et al.* 1998). Tussock grass patches account for 67% of above-ground net primary productivity (ANPP), while shrubs account for the remaining 33% (Fernández, Sala & Golluscio 1991). The other dominant perennial grass species of the steppe are from the genus *Pappostipa* (formerly *Stipa*). *Pappostipa* grasses are fairly unpalatable to wild and domestic herbivores relative to *Poa* (Pazos, Bisigato & Bertiller 2007).

The climate is semi-arid with a mean annual precipitation of 170 mm (mostly falling during fall and winter). The scarce precipitation and its distribution during winter lead to a high summer water deficit (Paruelo, Aguiar & Golluscio 1988; Paruelo *et al.* 1998). Average monthly temperatures range from 2 °C in winter to 14 °C in summer (Paruelo *et al.* 1998). Soils are sands with gravel and stones derived from fluvial deposits (Paruelo, Aguiar & Golluscio 1988).

Grazing intensity levels

We worked at three levels of sheep grazing pressure: ungrazed (UN), moderate grazing (MG) and intense grazing (IG). We selected 12 sampling areas (4 per grazing intensity level) at different grazing management units (grazing sites) to avoid pseudoreplication. 'Ungrazed' sites were paddocks (> 5 ha) that had been fenced to exclude large herbivores for 14–54 years. 'MG' and 'IG' sites were different paddocks (> 200 ha) that had been grazed at moderate (*c.* 0.2 sheep ha^{-1}), and very high stocking rates (*c.* 0.4 sheep ha^{-1}), respectively, for the last 40 years. These sites were used in different combinations for the different experiments. All sites shared the same precipitation regime and had similar slope, aspect and topography. Thus, the main component that strictly shifts along this gradient is sheep herbivory pressure. Studies examining grazing effects on vegetation in the region have shown an overall loss of plant cover, but rarely with substantial shifts in the dominant life-form (Bisigato *et al.* 2005; Cipriotti & Aguiar 2005). In most cases, grazing decreased the abundance of palatable grass species (*Poa ligularis* and *Bromus pictus*), increased the relative cover of unpalatable grass species (*Pappostipa speciosa* and *Pappostipa humilis*) (Perelman, Leon & Bussacca 1997; Pazos, Bisigato & Bertiller 2007) and magnifies the patchiness of the vegetation (Bisigato *et al.* 2005; Cipriotti & Aguiar 2005).

Studies

SEX-RELATED TRAITS

To test the hypothesis that *Poa* individuals show sex-related trait differences and that females grow slower and are less palatable to herbivores (Prediction 1), we conducted an experiment under controlled conditions to evaluate vegetative growth and insect attacks on female and male plants (Growth traits and insect herbivory under controlled conditions). We also collected plants from the field to explore if females produce more phenolic compounds than males (Leaf chemistry under field conditions). We estimated plant area under field conditions to test whether differences in vegetative growth were also reflected in plant size (Plant size at field conditions).

Growth traits and insect herbivory under controlled conditions

In May 2006, we collected male and female plants from field exclosures. Plants were cultivated in pots filled with sand in a common garden at the Facultad de Agronomía, Universidad de Buenos Aires, Argentina (34° 35.4′ S; 58° 28.8′ W). After the acclimation period (30 days), plants were fragmented to obtain homogeneous individual transplants (5-tiller, 8-cm-tall plants, $n = 100$ males and $n = 100$ females), and moved to a semicontrolled greenhouse for the following 2 months. Plants were weekly relocated within the greenhouse to avoid microlocation effects and watered with 100 mL of Hoagland solution per pot once a week. We monitored root growth following the first 15 days and recorded the appearance and the number and length of new roots from 50 female and 50 male plants. After growing in the semicontrolled greenhouse for 4 months, final vegetative and root biomass were measured ($n = 50$ male and 50 female plants). Roots were separated from sand by flotation and sieving (0.2 mm mesh sieve). At harvest, specific leaf area (g cm^{-2}) was calculated. We selected two fully expanded green leaves per plant, leaf area was measured and weighed and then averaged into a unique value of leaf area and leaf weight per plant. The area was estimated by scanning the leaves and counting pixels. The pixels were transformed to area by the relationship between the number of pixels and the area of a known shape. We performed t-tests to compare sexes (response variables: number of new roots, root length and biomass, shoot biomass, shoot/root ratio and specific leaf area, $n = 50$) (SAS 1996).

During the greenhouse experiment in 2006, *Poa* plants experienced a natural infestation of *Sipha maydis* (Passerini) aphids, mostly on male plants. However, insects were removed soon after counting to avoid impact on plant growth. So, in May 2007, we collected female and male plants ($n = 100$) from the experimental field site in Patagonia and cultivated them in pots filled with sand in a common garden in the Facultad de Agronomía campus. After an acclimation period of 30 days, plants were fragmented to obtain homogeneous plants (60 female and 60 male plants) and moved to a semicontrolled greenhouse for the following 3 months. Plants were spatially arranged creating a checkerboard, alternating male and female plants in order to separate spatial effects (i.e. sex aggregation, distance to edge and other neighbourhood effect) from the aphids' sex preference. Number of aphids was counted in this second spontaneous infestation during the course of the experiment (3 months). We performed nonparametric Wilcoxon–Mann–Whitney to assess differences in aphid number between female and male plants (SAS 1996).

Leaf chemistry under field conditions

Greenhouse roofs could reduce phenolic production by reducing UV radiation (Mazza *et al.* 2000). Therefore, tissue samples for total soluble phenolic determination were collected in December 2012 from plants (20 female and 20 male randomly selected individuals) growing in one of the Patagonian field exclosures. Leaf samples (5 mg) were placed in 1.4 mL of a methanol : HCl solution (99 : 1 v/v) and allowed to extract for 48 h at −20 °C (following Mazza *et al.* 2000). Absorbance of extracts was read in a spectrophotometer 305 nm (A_{305}) and 320 nm (A_{320}), (UV-1700 series; Shimadzu, Kyoto, Japan). We performed *t*-tests to compare absorbance values between sexes (SAS 1996).

Plant size at field conditions

Using the maps drawn in the field (Spatial patterns of sex distribution), we calculated the area (cm^2) of each naturally established male and female plant present in the nine grazing sites (paddocks). We compared the mean value of sizes between sexes under the three grazing intensity levels ($n = 3$) using a split-plot ANOVA (SAS 1996).

FIELD EXPERIMENT: SEX-RELATED INTERACTIONS WITH NEIGHBOURS AND HERBIVORES

To test for differences in gender interactions with neighbours and herbivores (both natural and domestic herbivores), we began a manipulative field experiment to disentangle competitive effects from protective effects of unpalatable grasses (*Pappostipa speciosa*) on the growth of male and female plants of *Poa* at three grazing intensity conditions (Predictions 2 and 4).

Pappostipa speciosa (formerly *Stipa speciosa*) is a perennial tussock grass native to the Patagonian steppe which is less palatable than *Poa* and therefore acts as a facilitator of palatable species in the presence of herbivores (Graff, Aguiar & Chaneton 2007; Graff & Aguiar 2011). Within each of the UN, MG and IG sites, we worked in a 1 ha area (in MG and IG that area was located 1 km away from water points and fences) where we selected 48 naturally established *P. speciosa* individuals (hereafter, *Stipa*) of similar height (14 cm), basal diameter (14–16 cm) and < 40% standing dead material. Selected tussocks were far from other grasses and shrubs (closest grass neighbour > 0.8–1 m, closest shrub neighbour > 3 m) to control for neighbour interactions that could obscure our treatments. *Stipa* plants were paired with *Poa* female and male transplants (24 for each gender). Male and female transplants were obtained by fractioning *Poa* plants into 5-tiller individual transplants. In the previous spring–summer (November–January), *Poa* plants growing within the exclosures were sexed and marked in the field to enable the collection in May 2002 (when the experiment began). Since *Poa* plants are perennial, we selected stem bases with green tillers coming from the tussock periphery to form the 5-tiller individuals. We transplanted male and female *Poa* individuals at two distances from *Stipa* ('close' = touching canopies and 'far' = 30 cm away from *Stipa*) and at two below-ground competition level treatments (control and reduced competition). Before transplanting, we dug a hole 0.20 m in diameter by 0.30 m deep, and the extracted soil was sieved to remove any roots and stones. Reduced competition treatment was achieved with a fine mesh cloth that was fitted before replacing the sieved soil in the pits, which functioned as a barrier to growth of roots of neighbour plants under *Poa* target plants (Aguiar, Soriano & Sala 1992; Graff, Aguiar & Chaneton 2007). Transplants with competition had no mesh around the soil core. All transplants were located east of *Stipa* plants. Strong winds blow mainly from the west throughout the year, and evaporation rates are 46% lower near *Stipa* tussocks than far from them (Graff, Aguiar & Chaneton 2007). Instead of marking plant pairs which could alter the foraging behaviour of herbivores, we used global positioning system (Garmin II Plus, Olathe, KS, USA) to relocate transplants.

We harvested transplants in January 2004, after 2 growing seasons, by digging pits 0.20 m in diameter by 0.3 m deep where the transplants were located. Vegetative (shoot and root) and reproductive (panicles) material was separated, oven-dried at 70 °C for 48 h, and weighed. Roots were separated from sandy soils by flotation and sieving (0.2-mm-mesh sieve). Species were easily distinguished by their root morphology (Leva, Aguiar & Oesterheld 2009).

We recorded herbivory in November 2002, January 2003, May 2003, November 2003 and January 2004. In the UN site, insect or small mammal leaf attacks were recorded. In MG and IG sites, we only accounted for sheep herbivory because sheep bites could mask any sign of insect herbivory. Plants were considered 'attacked' or 'consumed' when they showed clear insect or mammal bite marks and decreased in height since the previous census.

We performed ANOVAS in SAS (1996). Response variable was total vegetative biomass (roots and shoots). Panicle biomass was not included since some seeds from female plants were dispersed before we harvest the plants. We had three crossed factors: sex (S) (males and females), distance to neighbour (D) (close and far) and barrier to root competition (B) (with or without) within each grazing site. We performed statistical inference only within each grazing site because the grazing intensity level was unreplicated in this study. Transformed data of total vegetative biomass (ln) were used to meet homoscedasticity ANOVA assumption. We further explored differences among treatments via two *a priori* orthogonal contrasts. We tested whether means differed between (i) females growing in the close vicinity of unpalatable plants with vs. without the barrier treatment and (ii) males growing in the close vicinity of unpalatable plants with vs. without the barrier treatment. These comparisons allow a direct evaluation of whether *Stipa* neighbours exert relatively greater competition impact on females than on males (Prediction 2).

To analyse herbivory on male and female plants, we counted the number of plants that had been attacked at least once during the 2-year experiment. We used the GENMOD procedure in SAS (SAS Institute 1996). The model assumed a binomial distribution and used a logit link function because the response variable was binary (consumed or not consumed). The total number of plants per treatment was used as the binomial denominator.

SPATIAL PATTERNS OF SEX DISTRIBUTION

To test process-pattern relationships of female and male plants under field conditions (Predictions 3 and 7), we complemented the manipulative field experiment with a detailed vegetation mapping and spatial analysis.

Twenty-four maps per grazing intensity level were recorded in November–January 2006 (8 fine-scale maps × 3 grazing sites (paddocks) × 3 grazing levels = 72 maps). Maps (1 m^2 in area) were randomly located along transects within a 50 ha area and 1 km away from water points and fences. Because density of *Poa* in MG and IG sites is very low, some random points within transects did not have *Poa* individuals, so the next randomly assigned point was used instead. At the assigned location, a 1 m^2 square was placed in areas without shrubs in order to record spatial patterns primarily resulting from interactions among grasses. Maps had a resolution of 2 × 2 cm, related to the grain required to account for a plant-centred perspective (*sensu* Purves & Law 2002) and to explore changes in spatial distributions due to grass species interactions (Schurr *et al.* 2004). Therefore, we mapped all plants larger than 2 × 2 cm. Signs of recent herbivory (defoliated plants) were also indicated as a category (grazed or nongrazed). Maps were digitized with image software (IDRISI32 environment, 1987–2000) using three categories: *Poa* females, *Poa* males and *Stipa*. We were able to sex > 97% of *Poa* plants, only missing a few of the smaller ones growing in intensely grazed fields. Data were transformed from vector into raster format with a resolution of 2 × 2 cm to perform the spatial analysis.

Spatial patterns of *Poa* genders relative to nonpalatable grasses were analysed using bivariated O-ring statistics (O_{12} (r), Wiegand & Moloney 2004) that measure the density of neighbouring *Poa* sexes as a function of distance from the centre of an average unpalatable *Stipa* tussock. Calculations were performed with PROGRAMITA version 2010 (http://www.thorsten-wiegand.de/towi_programita.html – Accessed 2012 Feb 14) (Wiegand & Moloney 2004) using the real shape option and size of all plants (Wiegand *et al.* 2006). To detect possible facilitation or competition between species, we contrasted our data with a null model (Wiegand & Moloney 2004) that randomizes the locations of the sexes (e.g. females and males) and keeps the locations of the *Stipa* grasses fixed. Ninety-nine percentage confidence envelopes per grid were calculated from the highest and lowest values of $O_{12}(r)$ (Wiegand & Moloney 2004). In these analyses, if at a given distance r, $O_{12}(r)$ is above the upper (or below the lower) limit of the confidence envelope, we can say that females or males are positively or negatively associated with *Stipa* at the distance r. In order to analyse the vegetation data, we combined data from all eight maps per grazing site into a single weighted O-ring statistic (http://www.thorsten-wiegand.de/Programita/ManualCombineReplicates2009.pdf – Accessed 2012 Feb 14), resulting in three patterns per each grazing intensity level.

SEX DENSITY AND SEX RATIO

To test for increases in male ratios with increasing grazing intensity as a consequence of female-biased sheep grazing (Prediction 5), in December 2009, we randomly established 50 m by 1 m linear transects at four UN sites (UN = 4), four MG sites (MG = 4) and four IG sites (IG = 4). These transects were located within a 50 ha area in each grazing site (paddock). To avoid areas of concentrated grazing pressure, we selected areas 1 km away from water points and fences. We counted the number of female and male *Poa* plants and calculated sex density and sex ratios in each transect. We also estimated the number of female and male plants that had signs of being consumed. We recorded insect or small mammal leaf attacks on plants growing in the UN sites. We only accounted for sheep herbivory at MG and IG sites since sheep bites could mask any sign of insect herbivory. We performed a complete randomized split-plot ANOVA with grazing intensity (G) as the main plot factor with three levels, and each level repeated four times for a total of 12 study plots (or grazing sites). Sex (S) was replicated at subplot level. We used Tukey tests for multiple *post hoc* comparisons (SAS 1996).

Results

SEX-RELATED TRAITS

Male plants grew faster than female plants (Table 1). Male roots were larger and more numerous (about 20% more new roots) ($P < 0.001$). At harvest, male plants exhibited 50% more root biomass ($P < 0.001$) and about 15% more green leaf biomass than female plants ($P = 0.0012$). Shoot/root ratio was higher for males than females ($P < 0.0001$). Neither specific leaf area nor leaf number varied between sexes. In the field, males were significantly larger than females (Sex, $P = 0.0178$) (Table 1), independent of grazing intensity level (Grazing × Sex, $P = 0.70$) (see Table S1 in Supporting Information for full statistics).

In our greenhouse experiment, aphid (*S. maydis*) densities were 6–10 times higher on male than female plants (Table 1). Under field conditions, female plants had higher levels of soluble phenolic compounds ($P < 0.001$) (Table 1).

Table 1. Sex-related differences in traits

Variables	Females	Males	*P*
Number of new roots	5.56	8.38	< 0.0001
Root length (cm)	35.74	42.76	0.0143
Root biomass (g)	0.27	0.56	< 0.0001
Specific leaf area ($cm^2\ g^{-1}$)	89.17	89.21	n.s.
Leaves number	9.69	9.50	n.s.
Shoot biomass (g)	0.52	0.70	0.0012
Shoot/root ratio	0.55	0.82	< 0.0001
Plant area (cm^2)	76.97	104.95	0.0178
Aphids/plant	0.47	6.08	< 0.0001
Aphids/leaf	0.03	0.38	< 0.0001
Phenolic content (A_{305})	0.82	0.41	< 0.0001
Phenolic content (A_{320})	0.80	0.40	< 0.0001

T- test for significance between mean values of female and male growth variables and leaf phenolic content. Split-plot ANOVA for plant area under field conditions. Wilcoxon (Mann–Whitney U) test for significance between female and male aphid attack.

FIELD EXPERIMENT: SEX-RELATED INTERACTIONS WITH NEIGHBOURS AND HERBIVORES

At the UN site, the presence of the root barrier (B) affected positively *Poa* biomass (B, $P = 0.0001$). The sex by root-barrier interaction (S × B) resulted in a *P*-value of 0.058, and female transplants growing close to neighbours grew twice as large with the barrier than without it (*a priori* contrast $F = 12.08$, $P = 0.001$, Fig. 1a), suggesting strong below-ground competition with *Stipa* neighbours. Males, on the contrary, did not show differences in total biomass between the barrier treatments when growing close to *Stipa* (*a priori* contrast $F = 0.63$, $P = 0.43$, Fig. 1b). Distance to the less palatable *Stipa* neighbours (D) did not affect the biomass of females or males (D, $P = 0.73$).

At the MG site, distance between *Stipa* and *Poa* affected *Poa* biomass (D, $P = 0.006$), but no gender differences were found (Fig. 1c,d). Plants, whether male or female, performed better near *Stipa* tussocks than far from them, demonstrating a positive effect of *Stipa* canopies on both genders. In addition, female transplants growing close to *Stipa* grew larger with the barrier than without it (*a priori* contrast $F = 5.05$, $P = 0.03$, Fig. 1c), suggesting below-ground competition in the close vicinity; whereas males did not show differences in total biomass (*a priori* contrast $F = 0.01$, $P = 0.93$, Fig. 1d).

At the IG site, no distance or barrier effect was detected for either male or female plants (Fig. 1e,f) (see Table S2).

At each grazing pressure intensity, herbivory depended on the sex and distance to unpalatable neighbours (Fig. 2). Within the exclosure, males exhibited higher herbivory by insect or small mammals than females growing at the same distance from *Stipa* (Fig. 2a) (Sex, $\chi^2 = 9.79$, $P = 0.0018$). At MG and IG sites, no differences in herbivory were found between sexes (Fig. 2b,c, respectively). Sheep grazed preferentially on plants growing far from *Stipa*, independently of the sex, both at the moderately grazed (Fig. 2b; Distance, $\chi^2 = 26.82$, $P < 0.0001$) and at the intensely grazed site (Fig. 2c; Distance, $\chi^2 = 13.52$, $P = 0.0002$) (see Table S3).

SPATIAL PATTERNS OF SEX DISTRIBUTION

Female and male associations with *Stipa* were different, especially in the absence of domestic herbivores. At two of the three UN studied, females showed slightly positive associations with *Stipa* at very short distances (< 4 cm). However, at intermediate distances (10–38 cm), strong negative departures from 99% confidence limits were observed in all the UN sites studied (Fig. 3). Consequently, females were spatially segregated from *Stipa* (Fig. 4a). In contrast, males showed strong positive departures from the 99% upper confidence limit at short distances (Fig. 3), resulting in positive associations with *Stipa* up to distances of 8 cm (Fig. 4b). At longer distances, a random pattern was observed in *Poa males-Stipa* associations (Fig. 4b).

Under MG intensity, both sexes showed stronger positive associations than in the UN sites at short distances (Fig. 4c, d). Positive departures from 99% confidence limits were observed up to 14–16 cm in all the sites studied (Figs 3 and 4c,d). However, segregated patterns between females and *Stipa* at intermediate distances (30–42 cm) remained significant in two of the three sites studied (Fig. 3).

Under IG, no significant departures from the null model were observed between *Poa* plants of either sex and *Stipa* plants (Fig. 4e,f). Only slight positive associations were found between plants at very short distances, especially for males (Fig. 3).

SEX DENSITY AND SEX RATIO

Overall *Poa* density decreased with increasing grazing intensity (G) (*G*, $F = 11.1$, $P = 0.0037$). At UN, the density of

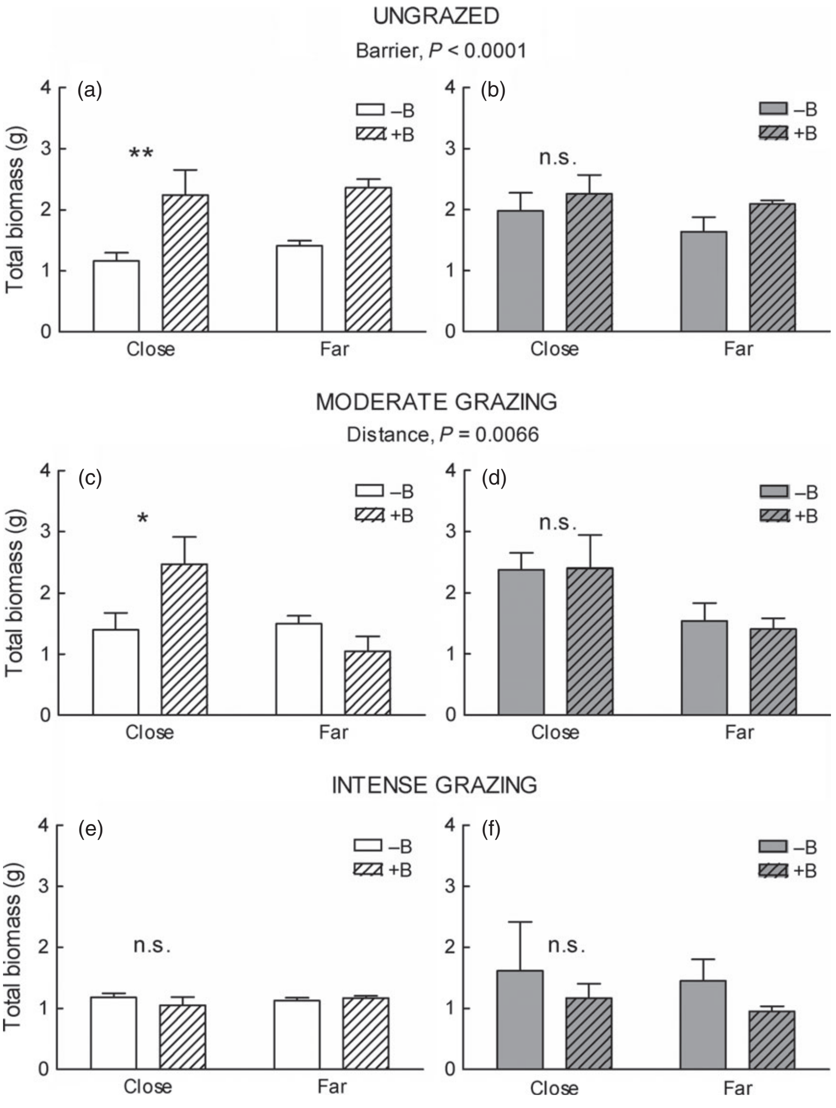

Fig. 1. Effects of distance and barrier to root competition manipulations on *Poa ligularis* female (left panels, white bars) and male plants (right panels, grey bars) total biomass, at each grazing intensity level. 'Close' and 'Far' indicate distance levels from *Stipa* neighbours (0 and 30 cm, respectively). Open and dashed bars represent without (−B) and with the barrier present (+B), respectively. Bars represent untransformed means ± SE. Asterisks above bars represent differences between treatments via *a priori* contrasts (with and without barrier, both at *Stipa* close proximity): $^{**}P \leq 0.001$ and $^{*}P \leq 0.05$.

female plants was higher than that of males (sex ratio 1.5 : 1), whereas in intensely grazed paddocks males were more dense than females (G × S, $F = 14.97$, $P = 0.0014$; sex ratio 1 : 2, Fig. 5a).

Males were more consumed in ungrazed sites (Fig. 5b, UN), despite their lower density. Females received more sheep bites than males in grazed sites, both at moderately grazed conditions (Fig. 5b, MG), where density of females and males was similar, and at the intensely grazed sites (Fig. 5b, IG), where density of females dropped by 70% (Fig. 5a) (see Table S4).

Discussion

As proposed by theory on dioecious species (Delph 1999; Geber, Dawson & Delph 1999; Obeso 2002; Cepeda-Cornejo & Dirzo 2010), *Poa* genders were dimorphic in traits related to resource allocation. As predicted (Prediction 1), females had lower vegetative growth rates (Table 1) but they were better defended against herbivores than males. Females produced more phenolic compounds (Table 1) and were also less palatable to herbivores than males. Preference of insects and small mammals for males, both under greenhouse (Table 1) and field conditions (Figs 3a and 5b), points towards differences between the sexes in their response to the trade-off between growth and defence. Gender dimorphism has been used to explain biased population sex ratios in several environments (reviewed by Delph 1999). However, for the first time, we were able to demonstrate further effects of this dimorphism. Females and males interacted differently with neighbouring species (Fig. 1a,b) promoting differential distribution patterns (Figs 3 and 4a,b). Proximity to neighbours controlled visibility to herbivores (and grazing damage) (Fig. 2b,c) and, in the end, changed population sex ratio towards male-biased as grazing intensified (Fig. 5a).

In general, it has been predicted that females should be more negatively affected by competition than males, as they have higher reproductive costs (Lloyd & Webb 1977). Nonetheless, to our knowledge, only two studies have explored the effects of gender dimorphism in traits on the

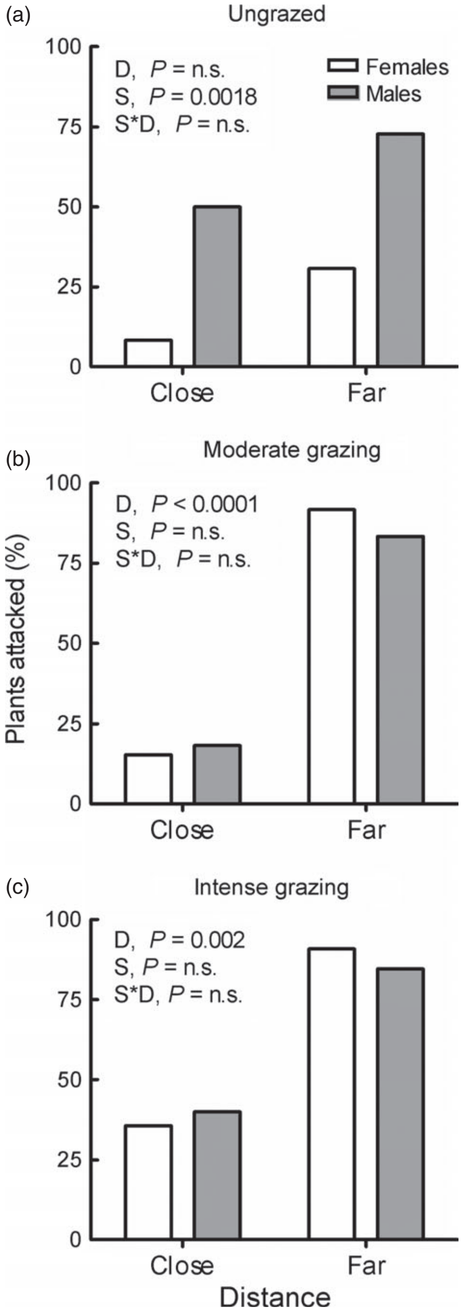

Fig. 2. Percentage of *Poa ligularis* female and male plants attacked, at two distances from the less palatable neighbour (0 and 30 cm), at each grazing intensity level. In the exclosure (ungrazed by domestic herbivores) damage to insects and small mammals was recorded, while in grazed sites only sheep bites were recorded. White and grey bars correspond to female and male plants, respectively. S, means sex and D, means distance.

interaction with other species (Sánchez-Vilas, Turner & Pannell 2010; Cranston *et al.* 2012). We showed that the effect of competition with *Stipa* was stronger on females than on males, as evidenced by a significant increase in total vegetative biomass (*c.* 50%) when their roots were experimentally isolated from the *Stipa* neighbours in a sheep exclosure (Fig. 1a). Spatial segregation of females at fine scales (Figs 3 and 4a) supported the inference about a lower competitive ability of females (Predictions 2 and 3). The distance at which females segregated from *Stipa* (in average, up to 30 cm; Fig. 4a) is consistent with observations that grass roots may extend horizontally up to 30–40 cm and occupy small bare-ground interspaces (Soriano, Golluscio & Satorre 1987). Since the scale of the segregated pattern in females matched root distribution patterns (Schurr *et al.* 2004), we are able to infer strong process-pattern relationships (McIntire & Fajardo 2009). We could not establish if those spatial patterns alternatively arose from differential germination or seedling survival for two reasons: seedlings of *Poa* are rarely observed in the field (Fernández *et al.* 2002), and it is currently not possible to determine the sex of the seedlings. That is why the sex ratio of seeds or seedlings has rarely been documented (Eppley 2001). However, regardless of the factors that initially generate patterns of spatial segregation of the sexes in adult plants, it appears that gender interactions with other species contribute to maintain them.

According to previous studies, distance to unpalatable species, rather than gender differences in palatability, controlled sheep consumption (Milchunas & Noy-Meir 2002; Baraza, Zamora & Hódar 2006; Graff, Aguiar & Chaneton 2007; Smit *et al.* 2007; Graff & Aguiar 2011). Experimental manipulation of distance of *Poa* transplants from *Stipa* indicated that males and females growing far from unpalatable neighbours were equally grazed by sheep (Fig. 2b,c) (Prediction 4). However, a female-biased herbivory was detected in mapped populations when distance to unpalatable grasses was not experimentally controlled (Fig. 5b) (Prediction 5). That female-biased herbivory is presumably a consequence of naturally established females occurring more distantly than males from their competitors (Fig. 4a); which could potentially serve as refuges from sheep. On the other hand, grazing increased positive associations at intermediate grazing intensities (Fig. 4c,d) as predicted (Prediction 7) (Graff & Aguiar 2011; Saiz & Alados 2012). Even though proximity to unpalatable species reduces the probability of herbivore attacks on palatable species via associational resistance (Milchunas & Noy-Meir 2002; Baraza, Zamora & Hódar 2006; Smit *et al.* 2007), it could also increase competitive interactions (Callaway 1998; Silvertown & Wilson 2000). We found that sheep grazing made neighbour interactions positive only for males (Fig. 1d). Instead, in the vicinity of the 'protector species', the net balance between competition and facilitation remained negative for females (Fig. 1c). The reduction in female density observed in moderately grazed sites (Fig. 5a) could be the result of the combined effect of the consumption of unprotected plants (those segregated from unpalatable grasses) and the cost of competition for females growing in the close vicinity of 'protector species'. Domestic herbivores disrupted the spatial pattern and the direct and indirect interactions balance, bringing a previously unreported trade-off or 'dilemma' for female plants: face competition to elude browsing.

It has been proposed that the protective role of the benefactor unpalatable species on their *protégés* will decline with

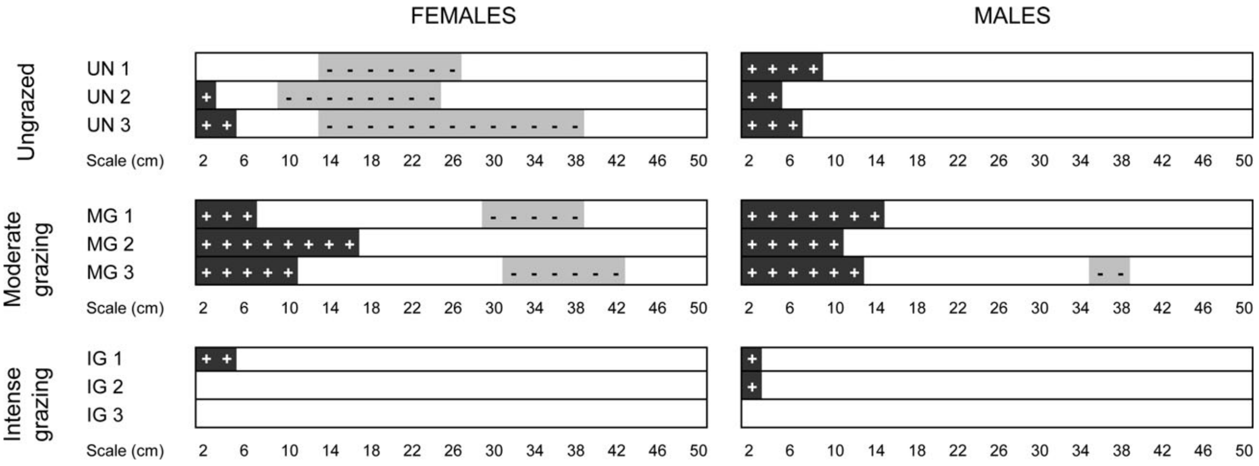

Fig. 3. Summary of the patterns observed for female (left panels) and male (right panels) individuals of *Poa ligularis* in relation to the distance from *Stipa* along the three grazing intensities [ungrazed (UN), moderate grazing (MG) and intense grazing (IG), $n = 3$]. Eight maps were combined into a single weighted O-ring statistic per grazing site (e.g. UN 1), resulting in three patterns (e.g. UN 1, UN 2, UN 3) per each grazing intensity level (e.g. UN). Positive signs represent positive significant deviations from the null model (i.e. aggregation) at specific distances from *Stipa* ($P < 0.001$). Negative signs represent negative significant deviations from the null model (i.e. segregation or repulsion) at specific distances from *Stipa* ($P < 0.001$). Absence of signs indicates that male and female plants distribute as null model from *Stipa* (i.e. random).

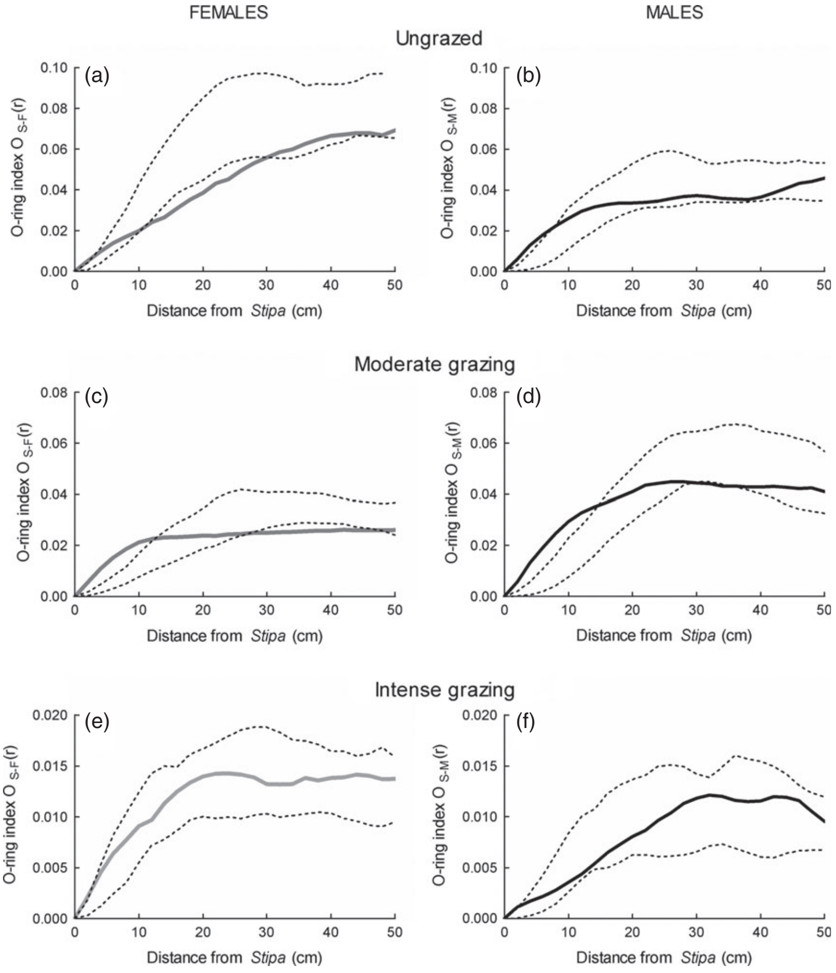

Fig. 4. Average distribution patterns of female (left panels) and male (right panels) individuals of *Poa ligularis* in relation to distance from *Stipa* along the three grazing intensity levels: ungrazed (a, b), moderate grazing (c, d) and intense grazing (e, f). The solid line represents the association O-ring index calculated for the different sexes (F or M) and *Stipa* (S). Dotted lines represent the confidence interval (99%). O-ring index values above the confidence interval indicate aggregation, whereas O-ring index values below the confidence interval indicate repulsion or segregation.

increasing grazing intensity (Smit, Rietkerk & Wassen 2009; Graff & Aguiar 2011). Thus, physical aggregation between species will also decline (Graff & Aguiar 2011; Saiz & Alados 2012).We have confirmed that positive effects of *Stipa* on males were no longer present at IG (Fig. 1f), and physical aggregation between species decline for both sexes (Figs 3 and 4e,f) (Predictions 6 and 7). When grazing pressure increases, palatable plants growing in the close vicinity of

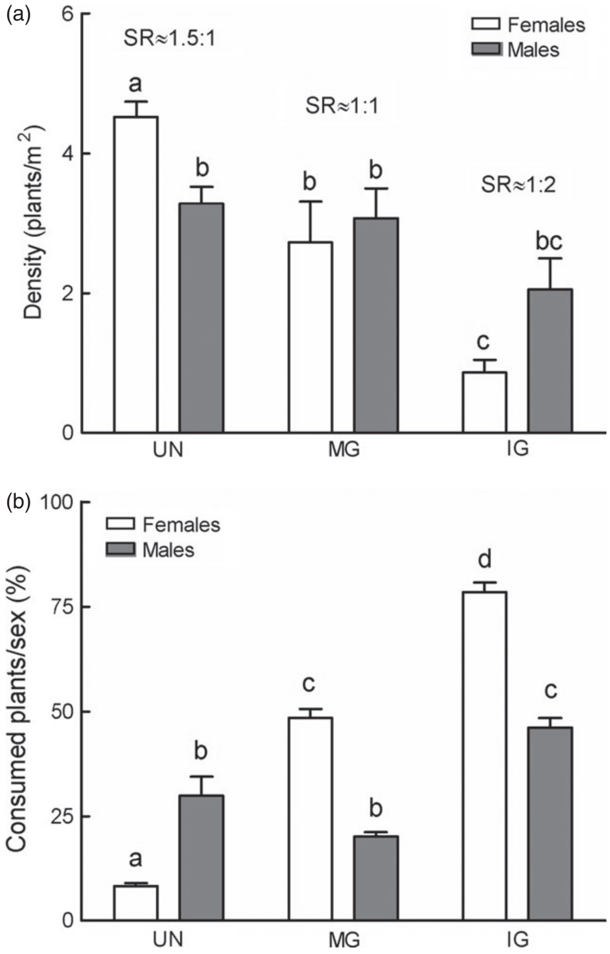

Fig. 5. Variation in density (a) and herbivory on naturally established plants (b) along three levels of grazing intensity: ungrazed (UN), moderate grazing (MG) and intense grazing (IG). Sex ratio is indicated for each consumer pressure level (SR). In the exclosure (UN), insect and small mammal's damage was recorded, while in grazed paddocks (MG and IG) only sheep bites were recorded. White and grey bars correspond to female and male plants, respectively. Bars labelled with different letters are significantly different (Tukey *post hoc* tests, $P < 0.05$).

unpalatable species are frequently encountered by consumers (Graff, Aguiar & Chaneton 2007; Graff & Aguiar 2011). In that situation, females are more vulnerable to defoliation than males (De Jong 1995). Since females generally grow slower than their male counterparts, they may need more time than males to recover from a defoliation event (Elmqvist & Gardfjell 1988). With increasing consumer pressure (intensity and frequency of herbivory), it takes longer before plants could compensate defoliation (Ferraro & Oesterheld 2002). An increase of plant reliance on reserves could result in increased stress levels, and ultimately, in plant death (Elmqvist & Gardfjell 1988). Therefore, it would be reasonable to expect higher mortality of females than males, and, in the end, a strong male-biased population sex ratio (Fig. 5a).

Understanding the mechanisms governing skewed sex ratios is vital for evaluating their ecological implications. Our results showed that the reasons for male-biased sex ratios in some arid environments are far from being explained solely by sex differences in traits and the response to abiotic stress (Delph 1999; Obeso 2002). Male-biased sex ratios seemed to be related to the combined effects of differential vegetative growth, response to competitors, spatial segregation of sexes at small scales and consumer pressure. A reduced density of females directly affects the quantity of seeds produced and may accordingly reduce seedling recruitment within a given population. The reduction of female density by 70% in intensely grazed sites (Fig. 5a) resulted in an 83% reduction of flowering stems per m^2 (data not shown). Even though *Poa* possesses small and light seeds which can be wind dispersed (Fernández *et al.* 2002), seed dispersal from UN sites (potential seed sources) did not seem to compensate for individuals lost in grazed sites through mass effects. The presence of long sticky hairs probably facilitates the rapid attachment of seeds to other plants, soil particles or litter, preventing seed dispersal to long distances (Pazos, Bisigato & Bertiller 2007; Pazos & Bertiller 2008). Moreover, seed germination and seedling survival is extremely low in this steppe (Fernández *et al.* 2002; Rotundo 2005), and recruitment chances are restricted to infrequent years (Rotundo 2005). Taking into account all these constraints, it seems that increases in male ratios strongly affect *Poa* population growth rates and persistence. This could explain the significant reductions of *Poa* populations in the last decades (Perelman, Leon & Bussacca 1997), which have compromised the sustainability of sheep production in the Patagonian region (Texeira & Paruelo 2006).

Conclusion

Plant trade-offs set limits to the range of ecological conditions that species can tolerate and therefore control species abundance and distribution at regional and local scales. Previous studies with dioecious species demonstrated that small variation between genders in certain traits could result in spatial segregation of the sexes (associated with physical stress), a different competitive ability of genders, and different palatability or response to consumers. However, in the 'real world' these three components act together and determine population sex ratios. Domestic grazers disrupt this balance by shifting gender interactions and their spatial organization. Changes in the sex ratio with grazing intensity are far from being predicted directly by gender-specific traits.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Split plot ANOVA to test the effect of grazing intensity and sex on plant size (cm^2) under field conditions.

Table S2. (a) ANOVAS tests of the effects of distance and root-barrier manipulations on females and males' total biomass at each grazing intensity level. (b) *A priori* orthogonal contrasts to compare the effect of below-ground competition exerted by *Stipa* closest neighbours on genders at each grazing intensity level.

Table S3. Effects of distance and gender on herbivore consumption at each grazing intensity level.

Table S4. Split plot ANOVA tables to test the effect of grazing intensity and sex on the density of *Poa ligularis* plants and the number of consumed plants per sex.