

Phenotypic Sex Ratios of *Atriplex canescens* Shrubs in Relation to Cattle Browsing

Andrés F. Cibils
David M. Swift
Richard H. Hart

Abstract—Previous studies conducted at our research site on the shortgrass steppe in Colorado showed that phenotypic sex ratios of tetraploid fourwing saltbush (*Atriplex canescens* Pursh [Nutt]) shrubs were less female biased in grazed pastures than in adjacent exclosures. The potential effects of cattle browsing on shrub sex ratios were studied both in the field and in a greenhouse. In the field study, winter or late summer grazing resulted in higher utilization of female shrubs relative to males. In early spring, when shrubs were browsed the least, utilization of male and female shrubs was not different. Release from cattle browsing (in temporary exclosures) was related to sexual phenotype shifts toward femaleness that occurred mostly in shrubs with monoecious phenotypes. Such shifts, however, did not translate into detectable changes in overall phenotypic shrub sex ratios. In the greenhouse study, female clones of fourwing saltbush appeared to be more negatively affected by artificial defoliation than were male or monoecious clones. Sex biased herbivory, and (to a lesser extent) shrub gender specific responses to defoliation may have promoted higher mortality among female shrubs possibly leading to shrub sex ratio alteration at this site. Browsing-induced sex shifts are probably not an important factor affecting shrub sex ratios at this site.

Introduction

Sex ratios of dioecious shrubs can change across the landscape or through time in response to environmental stress (Freeman and others 1976; McArthur and Freeman 1982; Freeman and others 1993; Allen and Antos 1993). Gender-specific responses, both to availability of key resources and to a number of biotic and abiotic stressors, are responsible for such variations (Freeman and others 1980; Freeman and others 1984). There are at least two basic processes that can lead to sex ratio alterations, namely: (a) differential mortality of females and males (Williams and others 1978); or (b) phenotypic sex shifting in individuals

with sexually labile genotypes (Freeman and McArthur 1984; McArthur and others 1992). In stands of tetraploid fourwing saltbush (*Atriplex canescens*) on the shortgrass steppe in Colorado, Cibils and others (1997) found that female fourwing saltbush shrubs were more abundant inside exclosures than in adjacent grazed pastures.

Fourwing saltbush is fairly sensitive to defoliation (Buwai and Trlica 1977; Benjamin and others 1995). Seasonal rest periods are required to keep browsed populations viable (Pieper and Donart 1978; Price and others 1989). Gender-biased herbivory, that is common in a number of dioecious shrubs including some saltbushes (*Atriplex* sp.) (Watson 1995 and references therein; Maywald and others 1998; Maywald 1999), and/or gender-specific responses to browsing, could promote different mortality rates among male and female fourwing saltbush shrubs. Additionally, since close to 55 percent of tetraploid fourwing saltbush shrubs carry sexually labile genotypes and can shift toward maleness under stress (McArthur and others 1992), browsing could promote sex phenotype shifts in fourwing saltbush stands. Both these processes, potentially driven by cattle browsing, could be responsible for the sex ratio differences found by Cibils and others (1997) between exclosures and grazed pastures.

The objective of our work was to investigate whether cattle browsing could trigger processes that lead to sex ratio alterations. We addressed the following questions in a field and greenhouse study: (1) are female fourwing saltbush shrubs at our site browsed more intensely than are male shrubs? (2) does cattle browsing promote sex phenotype shifts toward maleness in fourwing saltbush at our site? and (3) are clones from female fourwing saltbush shrubs affected more negatively by defoliation than are clones from male or monoecious shrubs?

Materials and Methods

Field Study

We conducted the field study at the USDA-ARS Central Plains Experimental Range (CPER), approximately 60 km NE of Fort Collins, Colorado (40° 49' N 107° 47' W) at 1,650 m elevation. We set up the experiment at a shrub-dominated site, on a floodplain area with loamy to sandy loam soils. Fourwing saltbush, blue grama (*Bouteloua gracilis* [H.B.K.] Lag. Ex Griffiths), and western wheatgrass (*Agropyron smithii* Rydb.) are the dominant plant species at the site (Liang and others 1989). Mean annual precipitation is

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Andrés F. Cibils was Ph.D. Candidate in the Rangeland Ecosystem Science Department of Colorado State University at the time this study was conducted. He now does rangeland management research at INTA-EEA Santa Cruz, Casilla 332, (9400) Río Gallegos, Santa Cruz, Argentina. David M. Swift is a Senior Research Scientist at the Natural Resource Ecology Laboratory and Associate Professor in the Rangeland Ecosystem Science Department both at Colorado State University, Fort Collins, CO 80523. Richard H. Hart is a Senior Range Scientist at the High Plains Grasslands Research Station of the USDA-ARS, Cheyenne, WY 82009.

320 mm, most of which occurs between the months of April and September.

We set up 14 half hectare experimental pastures in two blocks of seven pastures each, described in detail by Cibils (1999). We conducted three 2-year browsing experiments (in 1997 and 1998) that consisted of grazing four pastures with cattle for a period of 4 days in winter (January), early spring (April), and late summer (September). Two pastures were grazed moderately (four animals) and two were grazed heavily (12 animals). Two of the 14 pastures remained as exclosures. We tagged 20 female and 20 male shrubs in each experimental pasture before the onset of the experiments in 1996. In June 1997 we tagged an additional 20 monocious shrubs in each pasture. Tagged shrubs that had shifted phenotypic sex expression in 1997 or 1998 were replaced in order to keep numbers of male, female and monocious shrubs constant. Shortly before each experiment, we marked and measured the length of four stems on all tagged shrubs. We re-measured marked stems immediately after removing cattle, and thus calculated percent length removed by browsing. Utilization was expressed as a proportion of biomass removed from each marked stem, derived from length/mass equations described in detail by Cibils (1999). In June 1997 and 1998 we determined sex constancy on all tagged shrubs by comparing present and previous season's sexual phenotype. We also determined shrub sex ratios in each experimental pasture by recording phenotypic sex expression on 100 shrubs. To do this, we set up two transects per pasture and recorded sexual phenotype on the closest adult shrub to each 3 m interval.

Shrub utilization data from each experiment were analyzed separately using a mixed effects repeated measures analysis of variance. We studied the effects of shrub gender, stocking density, year and block (of experimental pastures) on shrub utilization. Utilization data were arcsin transformed and the overall level of significance was set at $P = 0.05$. SAS (1996) Proc GLM was used for the statistical computations.

Sex change on tagged shrubs was analyzed by grouping shrubs into one of three categories on the basis of their current season's floral phenotype, namely: 1) changing towards maleness ($\rightarrow\sigma$); 2) changing toward femaleness ($\rightarrow\phi$); and 3) constant sexual phenotype expression (constant). Nonflowering shrubs were not included in this analysis. We used a two-way contingency table to analyze these results by considering two classes of shrubs, namely: browsed or protected. We built separate tables for 1997 and 1998 and then related them by using the Cochran-Mantel-Haenzel test of general association. Level of significance was set at $P = 0.05$. SAS (1996) PROC FREQ was used for the statistical computations.

Sex ratios of experimental pastures were also collapsed into two groups (browsed or protected), and were compared to the 35 σ :55 ϕ :10[$\sigma\phi$] tetraploid *Atriplex canescens* sex ratio described by McArthur (1977) using χ^2 goodness-of-fit tests. We set the level of significance at $P = 0.05$ and used Statgraphics (1994) to perform the statistical computations.

Greenhouse Study

During the fall of 1996 we collected cuttings from 30 fourwing saltbush shrubs (10 female, 10 male, and 10 monocious) at five fairly distant sites within the CPER.

Stem-cutting collection, handling, and rooting techniques used in this study (described in detail by Cibils 1999) matched specifications proposed by Wiesner and Johnson (1977) and McArthur and others (1984). Overall, close to 30 percent of clippings rooted and survived through the first growing season (1997). We were therefore able to use clones taken from 18 parent fourwing saltbush shrubs in an 8-month experiment beginning in December 1997, designed to measure the effects of winter defoliation and water stress on several plant productivity-related variables.

We imposed three defoliation treatments (control, moderate and heavy), and two watering treatments (control and water stress). The former consisted of one clipping event at the beginning of the experiment that removed either 40 percent (moderate) or 70 percent (heavy) of the length of all stems 5 cm or longer. Water stress was imposed by varying watering intervals. Water in the control pots was replenished when soil water potential reached a value of -1.3 bars. In the water stressed pots, water was replenished when soil water potential reached a value of -6.5 bars. The experiment included a total of 108 clones. Treatments were randomized within parental origin (hereafter referred to as genotype). Surviving clones did not flower either before or during the experiment. This frustrated the corroboration of clone gender by floral phenotype observation. Therefore, clone gender in this paper must be interpreted as meaning "clones taken from a donor shrub exhibiting that gender."

In mid August 1998 we finalized the experiment and harvested aboveground biomass from all 108 clones, and root biomass from a subset of 54 clones. Aboveground biomass was harvested, oven-dried and weighed. Roots were washed and separated from pot soil (using 10 mm, 2 mm, and 1 mm sieves), oven-dried and weighed. In this paper we discuss the response to the experiment treatments in terms of total above- and belowground biomass of the subset of 54 clones. Aboveground biomass of defoliated clones included the biomass removed by the clipping treatments that had been oven-dried and weighed immediately after defoliation.

We used a mixed effects analysis of variance to analyze the impact of shrub gender, defoliation regime, watering regime, and genotype (nested within gender) on total above and belowground biomass. Significance level was set at $P = 0.05$. Means were compared using the LSM method controlling for comparisonwise error rates. We used SAS (1996) proc GLM and proc MIXED to perform the statistical computations.

Results

Field Study

In January and September percent biomass removed by cattle from marked leaders on tagged female shrubs was significantly higher than that removed from male shrubs (fig. 1). In both of these experiments female-biased browsing occurred in both years and across a three-fold increase in cattle density. In April we did not observe any significant differences in the levels of utilization of male and female shrubs (fig. 1).

Sex change in tagged shrubs was not significantly associated with browsing when monocious shrubs were excluded

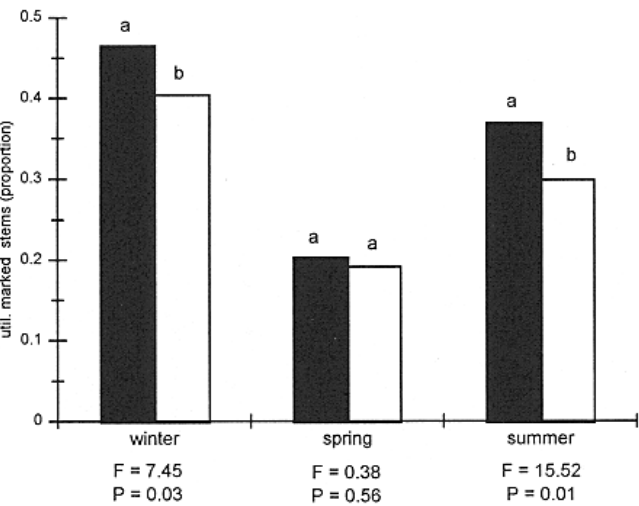


Figure 1—Mean utilization of tagged female (solid bars) and male (open bars) shrubs in the January, April and September experiments. F and P values for the “shrub gender” factor in the repeated measures ANOVAS are given below each pair of bars. Different letters indicate significant differences. Comparisons were made within experiments.

from the analysis (table 1). However, in 1998 when monocious shrubs were included, the association was highly significant (table 1). Whereas sex shifts towards maleness were the most common, only shifting toward femaleness (that occurred much more frequently in exclosures) was significantly associated with cattle browsing (table 1). Shrub sex ratios in both browsed and protected pastures did not differ significantly from the 35♂:55♀:10[♂♀] phenotypic frequency, empirically derived for tetraploid fourwing saltbush (McArthur 1977).

Greenhouse Study

Total above- and belowground biomass was significantly affected by the defoliation-by-gender interaction (F = 2.713; P = 0.049). Whereas control female, monocious, and male clones produced similar amounts of total biomass, defoliated female clones consistently produced less biomass than clones of the other genders (fig. 2). Under heavy defoliation,

Table 1—Contingency table with counts of shrubs that either changed sex phenotype or exhibited constant sex phenotype in 1997 and 1998 under cattle browsing or in exclosures.

Year	Treatment	Shrub counts			P
		→♀	→♂	Constant	
1997 ([♂♀] excluded)	browsed	1	9	90	0.672
	protected	1	13	86	
1998 (including [♂♀])	browsed	1	30	69	0.001**
	protected	13	20	67	
Overall	(C.H.M. Test)				0.001**

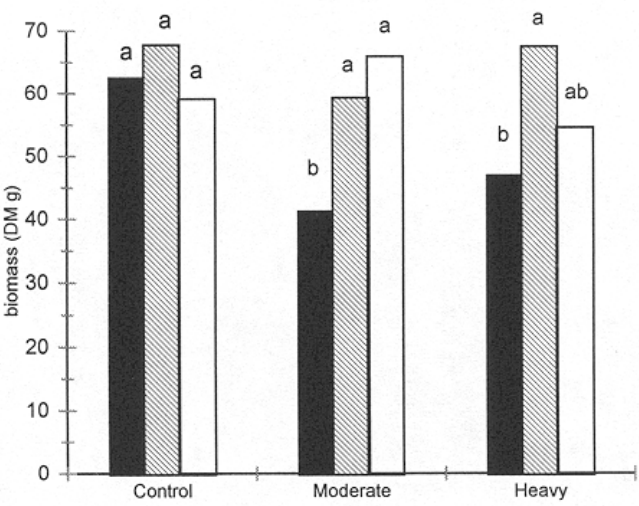


Figure 2—Overall means of total above- and below-ground biomass for female (solid bars), monocious (slashed bars), and male (open bars) clones under 3 defoliation levels. Different letters indicate significant differences. Comparisons were made among genders within levels of defoliation.

however, differences between males and females were not statistically significant (fig. 2). Whereas the water stress treatment we imposed had an overall significant effect on total above- and belowground biomass (F = 6.846; P = 0.014), its effect did not depend on the gender of the clone (shrub gender X water stress: F = 0.886; P = 0.423). It did have, however, different effects depending on the level of defoliation considered (water stress X defoliation: F = 5.455; P = 0.01). Water-stressed non defoliated clones produced less than the controls, however such differences disappeared with defoliation. We found a considerable amount of genotypic variation within genders (F = 2.332; P = 0.057).

Discussion and Conclusions

In January and September female shrubs were browsed more heavily than were males. In April, when overall shrub utilization was lowest, we detected no gender bias in utilization. Our results are only partially consistent with sheep-browsing patterns observed by Maywald and others (1998) in a fourwing saltbush transplant garden. Sheep preferred to browse male fourwing saltbush shrubs in June, and showed no gender-related preferences in March, a date that is comparable to our April experiment. While we did not find any instances of significant male-biased browsing, patterns involving greater use of males relative to females did occur in some plots of the April experiment in 1997. Our results tend to match the pattern observed in sheep browsing bladder saltbush (*Atriplex vesicaria* Heward ex Benth). Sheep preferentially browsed female shrubs irrespective of season and phenological stage (Maywald 1998). In bladder saltbush stands, female-biased herbivory by sheep is thought to cause greater mortality among female shrubs thus altering overall shrub sex ratios (Williams and others 1978). This may also be the case in the stands of fourwing saltbush that

we studied. In a previous study of crown and stem dimensions of browsed and protected fourwing saltbush shrubs at this site, Cibils (1999) found that whereas female shrubs were apparently younger than males under grazing, in exclosures such apparent age differences were absent. The gender-biased browsing pattern we observed could be causing higher mortality of female shrubs at our site as well. This could ultimately lead to situations of sex ratio alterations.

Experimental pastures that were protected from cattle browsing exhibited a larger number of tagged shrubs that shifted toward femaleness than did the pastures that were browsed. If browsing is in fact a source of considerable stress to fourwing saltbush shrubs, then our results are consistent with the trend described by McArthur and Freeman (1982). In that study, release from stress caused labile shrubs that had previously shifted toward male sex expression under stress to return to female flowering status. However, sex phenotype shifts observed at the level of tagged shrubs did not translate into detectable deviations of overall shrub sex ratios at the level of experimental pastures. Labile shrubs may have been over-represented in our sample of tagged shrubs. This could account for the apparent lack of consistency between results obtained at the level of tagged individuals and the level of experimental pastures. Browsing-induced sex shifting may not be an important source of sex ratio alteration in fourwing saltbush stands at our site.

Defoliated female clones produced less above- and belowground biomass than either male or monoecious clones. Whereas defoliation experiments conducted with dioecious species have not shown a consistent pattern of gender-specific response (Elmqvist and Gardfjell 1988; Oyama and Mendoza 1990; Delph and others 1993) female plants are generally assumed to grow more slowly than their male counterparts (Jing and Coley 1990). Should this be the case with fourwing saltbush, it is reasonable to speculate that female clones were not able to compensate the biomass removed by clipping in a growing season. This could explain differences in biomass production between genders observed in our greenhouse experiments. Our results suggest that defoliation may put female shrubs at a competitive disadvantage.

Overall, differences in sex ratios between exclosures and adjacent grazed pastures at our site reported by Cibils and others (1997) may have been the result of greater mortality rates in female shrubs. Both female-biased browsing by cattle, and possibly higher susceptibility to defoliation in female fourwing saltbush shrubs may have promoted differences in mortality rates of males and females. Browsing-triggered sex phenotype shifts of labile shrubs are possibly not important in driving alterations in phenotypic sex ratios of fourwing saltbush at our site.

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