

INDIRECT EFFECTS OF AN INTRODUCED UNGULATE ON POLLINATION AND PLANT REPRODUCTION

DIEGO P. VÁZQUEZ¹ and DANIEL SIMBERLOFF

Department of Ecology and Evolutionary Biology, University of Tennessee, 569 Dabney Hall, Knoxville, Tennessee 37996-1610, USA

Abstract. Herbivores can affect plants not only directly through browsing and trampling, but also indirectly through other species. For example, herbivores could affect the interaction between plants and their pollinators. Because plant population density may affect plant–pollinator interactions and plant reproductive success, we hypothesized that herbivores could affect pollination and plant reproduction indirectly by modifying plant population density. Unlike previous hypotheses, which concerned individual-level effects on vegetative and reproductive traits, our hypothesis focuses on population-level effects and involves a feedback mechanism. To test this hypothesis, we conducted field studies in the temperate forest of the southern Andes, where introduced ungulates are a major source of anthropogenic alteration. For 10 animal-pollinated understory plants, we compared population density, pollinator visitation, pollen deposition in stigmas, and reproduction in four pairs of grazed and ungrazed sites. We found evidence of indirect effects of ungulates on pollination and reproduction only for the herb *Alstroemeria aurea* (Alstroemeriaceae). The general lack of evidence for indirect effects on most of the species may result from resistance to cattle browsing and trampling, or low statistical power. For *A. aurea*, we present additional evidence from trampling and hand-pollination experiments, plus path analyses of the effect of density on pollination and reproduction showing that: (1) cattle decrease the absolute and relative population density of this species through trampling; (2) density, particularly relative density, affects pollen deposition on stigmas; and (3) conspecific pollen deposition affects reproduction. Thus, our results indicate that, by directly reducing the population density of *A. aurea*, cattle are indirectly affecting its reproduction.

Key words: cattle; exotic species; grazing; herbivory; indirect effects; introduced ungulates; mutualism; path analysis; plant population density; plant reproduction; pollination; southern Andean temperate forest.

Given the ubiquitous presence of herbivores and their ability to utilize all the different plant tissues available, it is surprising that herbivory has not always been recognized as having a significant impact on the reproductive ecology of plants.

—S. D. Hendrix (1988:246)

INTRODUCTION

Herbivores affect plants in different ways, sometimes with dramatic ecological and evolutionary consequences for plant populations. For example, grazing animals can determine the relative abundance of different plant species in a community (Harper 1977, Huntly 1991). Classic examples are the control of the diversity of herbaceous species in British chalk grasslands by rabbits (Tansley and Adamson 1925) and the control of species diversity in intertidal algal communities by the snail *Littorina littorea* (Lubchenco 1978). The most obvious effect of herbivores on plants

is increased mortality from browsing and trampling. On the other hand, loss of vegetative tissues and resulting decreased growth may reduce plant fitness (Hendrix 1988). Furthermore, herbivores sometimes decrease plant fitness by directly consuming reproductive tissues (e.g., Gómez and Zamora 2000, Herrera 2000) or by preying on seeds (e.g., Louda 1982, Herrera 1993).

Herbivores also affect plants in subtler ways, for example through indirect effects mediated by one or more other species. An indirect effect occurs when one species affects another through a third one (Strauss 1991, Wootton 1994, 2002). One way herbivores can affect plants indirectly is by modifying interactions with animal mutualists such as pollinators and seed dispersers. Several studies have demonstrated that folivores and florivores indirectly affect both male and female fitness of plants. Folivory and florivory can affect floral morphology and physiological traits, which can, in turn, affect pollinator visit frequency and, ultimately, male and female reproductive success (e.g., Hendrix 1988, Strauss et al. 1996, Strauss 1997, Strauss and Agrawal 1999, Mothershead and Marquis 2000).

Modification of floral traits is not the only way herbivores can indirectly affect plant–pollinator interac-

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¹ Present address: National Center for Ecological Analysis and Synthesis, 735 State St., Suite 300, Santa Barbara, California 93101 USA. E-mail: vazquez@nceas.ucsb.edu

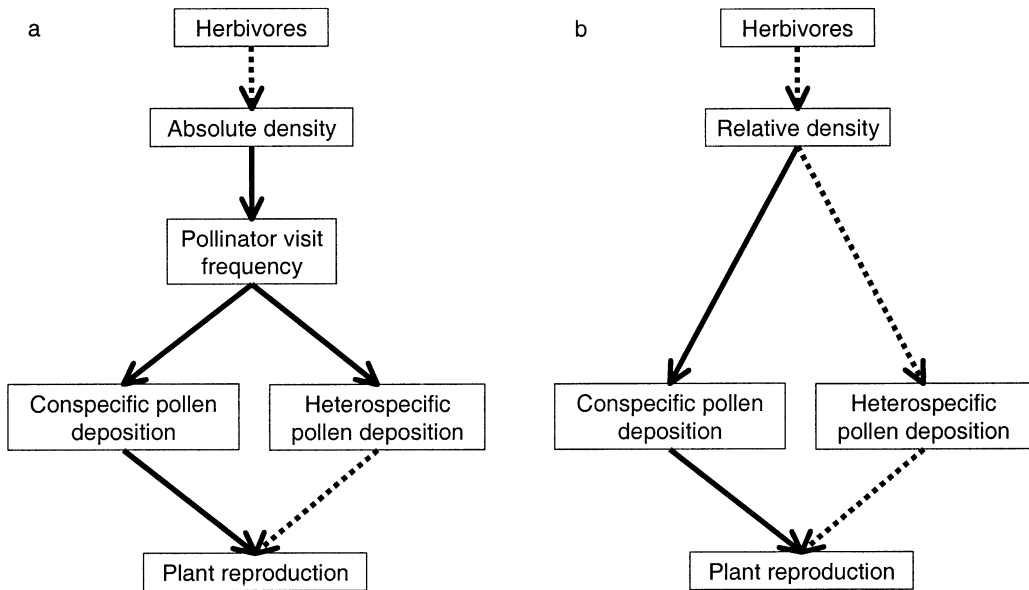


FIG. 1. Hypothetical effects of herbivores on plant reproduction through plant-pollinator interactions. Continuous lines represent positive effects; dashed lines represent negative effects. (a) Herbivores can reduce absolute population density (i.e., density of flowering conspecifics) directly through browsing or trampling. Decreased population density may result in lower pollinator visitation rates, especially by specialist pollinators that do not visit flowers of other plant species. Lower visitation can, in turn, result in lower pollen deposition in flower stigmas; both conspecific and heterospecific pollen deposition are expected to be affected, but the proportion of each is expected to remain constant. (b) Alternatively, herbivores can decrease relative population density, leading to decreased conspecific pollen deposition and increased heterospecific pollen deposition per pollinator visit (thus modifying the proportion of each kind of pollen). This effect of relative density on pollination is expected if pollinators are relatively generalized, visiting flowers of other plant species. Both chains of effects can result in decreased reproductive success.

tions, however. Plant population density may strongly influence plant-pollinator interactions, pollination, and plant reproductive success (Rathcke 1983, House 1992, Kunin 1993, 1997a, b, Bosch and Waser 2001). Thus, if herbivores modify plant population density directly (e.g., through browsing and trampling), this modification can in turn indirectly affect pollination and reproduction of individuals that have escaped herbivores, via a modified interaction with pollinators.

This density-mediated indirect effect of herbivores on plant reproduction can be brought about through at least two different chains of events. First, herbivores can indirectly affect pollination and reproduction through an effect on absolute population density (Fig. 1a). If we assume that plant population density is positively related to visit frequency and that pollinator visit frequency, in turn, positively affects pollen deposition (both conspecific and heterospecific), then a negative effect on absolute population density should result in an indirect, negative effect on pollen deposition. Notice that, although this can affect both conspecific and heterospecific pollen deposition, the relative proportion of each kind of pollen (a measure of the “quality” of pollination) should not be affected. Absolute density of conspecifics is expected to affect visitation rates more strongly when pollinators are specialists and do not visit other plant species in the community (Rathcke 1983, Kunin 1993, 1997a, b). Conversely, absolute

density of conspecifics will have little effect on visit frequency when pollinators behave as generalists (Rathcke 1983, Kunin 1993, Kunin and Iwasa 1996, Kunin 1997a). Decreased visit frequency at lower densities can occur in spite of increased proportions of flowers visited in small patches when a pollinator visits the patch (see Goulson 2000).

Second, herbivores can indirectly affect pollination and reproduction through an effect on relative population density (i.e., density of conspecifics relative to density of all species visited by pollinators; Fig. 1b). Reduced relative population density can lead to decreased conspecific and increased heterospecific pollen deposition in stigmas per pollinator visit (thus affecting the “quality” of pollination; see Rathcke 1983, Herrera 1987, Kunin 1993). This effect of relative population density is expected when pollinators are generalists, visiting flowers of other plant species in the community (Rathcke 1983, Feinsinger et al. 1991, Kunin 1993). Thus, even if visitation rates do not change, pollination and reproductive success can be affected because pollinators bring fewer conspecific and more heterospecific pollen grains per visit. Conversely, when pollinators are specialists, relative population density is not expected to affect pollination (Rathcke 1983, Feinsinger et al. 1991, Kunin 1993).

Lower conspecific and higher heterospecific pollen deposition can both translate into lower reproductive

success. The lower pollination level resulting from herbivore-induced reduction in population density may lead to lower reproductive success if reproduction is pollen-limited (Burd 1994), self-compatibility is low, and dependence on pollinators for reproduction is high (Feinsinger et al. 1991, Kunin 1993, 1997a, b, Bond 1995). In addition, reproductive success can suffer from high deposition of heterospecific pollen because of competition with conspecific pollen (Galen and Gregory 1989, Fishman and Wyatt 1999). Thus, by directly affecting population density, herbivores can also indirectly affect plant fitness through a modification of the interaction with pollinators.

An important consideration about this hypothesis is that an inverse relationship between plant population density and reproductive performance, via changes in pollinator behavior, can exist with extremely high plant population densities. Such effects can occur because the limited number of pollinators could result in competitive interactions among both conspecific and heterospecific plants (Rathcke 1983). In fact, several studies show that, although pollinators tend to visit more flowers and stay longer in larger floral patches, they tend to visit a smaller proportion of flowers there than in smaller patches (Totland and Matthews 1998, Comba 1999, Goulson 2000, Mustajärvi et al. 2001). A reduction in reproductive performance at high densities could also result from competition for resources other than pollinators (Mustajärvi et al. 2001). We assume that, at the low population densities resulting from herbivore impact, the potential for positive effects of density among conspecifics is far greater than the potential competition for pollinators and other resources.

The hypothesis that we are proposing for the effects of herbivores on plant reproduction is conceptually distinct from traditional models of indirect effects. Most examples of indirect effects have been classified in two broad categories: interaction chain and interaction modification (Wootton 1994, 2002). In interaction chain indirect effects, a species A indirectly affects a species C through a direct effect on the abundance of an intermediate species B. For example, a predator that reduces the abundance of an herbivore can benefit a plant species on which the herbivore feeds (i.e., a trophic cascade). Conversely, in interaction modification indirect effects, a species A indirectly affects a species C by modifying the per capita effect of a third species B on species C, but without an effect on the abundance of B. For example, an herbivore may affect the floral characteristics of a plant without affecting its population density, in turn modifying the interaction with a pollinator. Finally, the mechanism hypothesized to operate in this study involves a direct effect of a species A on the population density of another species C. This direct effect on C leads to a further indirect effect on C involving a feedback loop through a third species B; the latter effect can occur either by a modification of the interaction between B and C, or by indirectly af-

fecting B through an interaction chain indirect effect. This indirect effect on the B–C interaction further affects C (i.e., a doubly indirect effect of species A on C). Thus, the direct effect of A on C leads to a feedback involving a third species B, which results in further effects on C.

To our knowledge, previous studies of indirect effects of herbivores on plant reproduction have considered interaction modification indirect effects, involving effects of herbivores on individual floral traits such as flowering phenology (Brody 1997), flower number (Karban and Strauss 1993, Quesada et al. 1995, Mothershead and Marquis 2000), or flower size and pollinator rewards (Strauss et al. 1996, Strauss 1997, Mothershead and Marquis 2000). In contrast, our hypothesis requires no effect at the individual level on the plant's vegetative or reproductive traits. Rather, it entails an effect at the population level through a direct effect on mortality: browsing or trampling kills some individuals, and those that escape this direct effect suffer the indirect effect.

Objectives of the study

We tested the hypothesis that introduced ungulates affect pollination and plant reproduction by modifying plant population density through the mechanisms just described (Fig. 1). We took advantage of the presence of cattle introduced into the native forest of Nahuel Huapi National Park (Río Negro, Argentina). Introduced ungulates (both cattle and deer) are widespread throughout the region and are a major problem for the conservation of the temperate forest ecosystem in the southern Andes (Vázquez 2002b). We compared population density, plant–pollinator interactions, pollination, and reproduction for 11 plant species growing in the understory of pairs of sites with and without cattle in a forest dominated by *Nothofagus dombeyi*. Specifically, we asked: (1) Do cattle affect absolute and relative plant population density? (2) If absolute population density is affected, does this, in turn, result in further effects in the same direction on pollinator visitation, and on conspecific and heterospecific deposition? (3) If relative population density is affected, does this result in further effects in the same direction on conspecific pollen deposition, and in the opposite direction on heterospecific pollen deposition? (4) If pollen deposition is affected, does this affect plant reproductive success? Because of the limitations of our non-manipulative approach, for the only species for which we found evidence of indirect effects of herbivores on its pollination and reproduction, we looked for additional, independent evidence for the hypothesized mechanisms.

STUDY AREA

The study was conducted in Nahuel Huapi National Park and surrounding areas in Río Negro and Neuquén Provinces, Argentina. This park lies in the eastern

range of the temperate forest of the southern Andes, limited by the Patagonian steppe in the east; it encompasses a striking gradient of decreasing humidity from west to east (Dimitri 1962, Cabrera and Willink 1973).

Introduced vertebrate herbivores are a main source of anthropogenic forest alteration in this temperate forest. Cattle have been present for about two centuries (see references in Veblen and Alaback 1996, Vázquez 2002b). In Nahuel Huapi National Park, cattle are found today in 56% of the total area of the park suitable for them (i.e., excluding lakes and mountaintops; Lauría Sorge and Romero 1999). Red deer (*Cervus elaphus*) were introduced in the Nahuel Huapi area in the early 20th century (Veblen et al. 1992, Jaksic et al. 2002); they are currently the most widespread exotic deer in the region, ranging from Neuquén to Chubut provinces in Argentina (Bonino 1995) and between regions VII and XI in Chile (Jaksic 1998). Two other species of deer (fallow, *Dama dama*; axis, *Axis axis*) were introduced in the 1930s in Nahuel Huapi National Park but are not present in the study sites (Navas 1987, Bonino 1995). Cattle and deer have important effects on the native biota (Ramírez et al. 1981, Veblen et al. 1989, Veblen et al. 1992, Relva and Veblen 1998, Vázquez 2002b).

METHODS

Study sites and organisms

We selected four pairs of forested sites for the study, each pair consisting of one site with cattle and one without them. Site area was 6–12 ha. All sites were dominated by coihue (*Nothofagus dombeyi*), usually accompanied by Chilean cypress (*Austrocedrus chilensis*); these two tree species are wind pollinated. In contrast, many understory and ground cover species are insect pollinated. Because we could not find comparable paired sites with and without deer, all of our grazed sites have cattle only. The four pairs of sites (Fig. 2) are: (1) Llao Llao (nc, no cattle)–Cerro López (c, with cattle); (2) Safariland (nc)–Arroyo Goye (c); (3) Lago Mascaradi (nc)–Lago Mascaradi (c); and (4) Quetrihué (nc)–Quetrihué (c). The paired sites are contiguous and separated by a fence in two sets (i.e., Mascaradi and Quetrihué) and separated by 2–3 km in the other two. We worked with pairs of sites instead of unmatched replicates because of the strong longitudinal humidity gradient that characterizes the area. To be comparable, all sites should be located at the same longitude; because it was impossible to find a set of sites that met this criterion, we used paired sites differing only in the presence of cattle. We conducted all of the studies described here under the forest canopy, to avoid confounding the effect of tree cover with that of cattle.

We selected 11 animal-pollinated plants growing in the understory and ground cover of our sites. These constituted all animal-pollinated species abundant

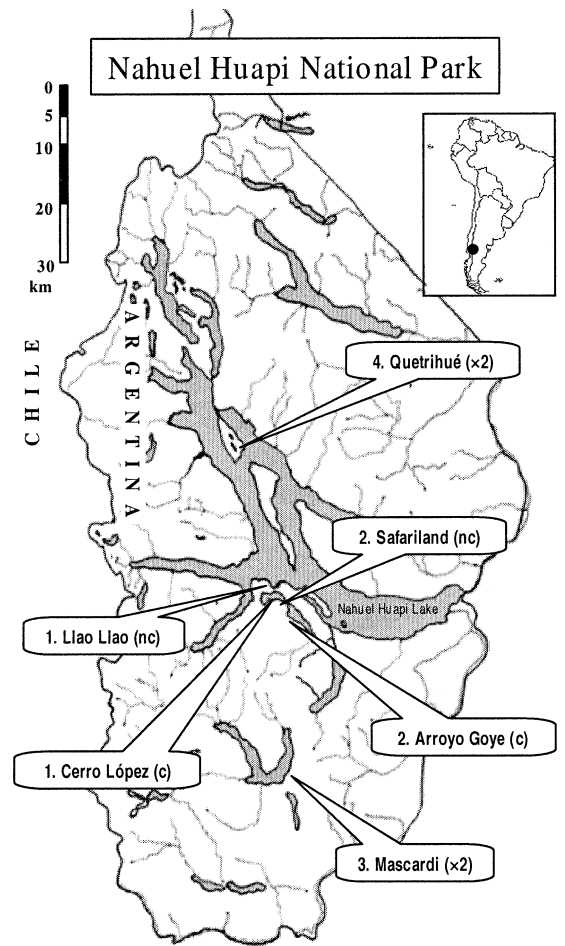


FIG. 2. Four pairs of sites: (1) Llao Llao (without cattle, nc) and Cerro López (with cattle, c); (2) Safariland (nc) and Arroyo Goye (c); (3) Lago Mascaradi (nc) and Lago Mascaradi (c); (4) Península Quetrihué (nc) and Península Quetrihué (c). Paired sites in pairs 1 and 2 were separated by a few kilometers; sites in pairs 3 and 4 were contiguous (indicated as “×2”). Gray shading indicates lakes.

enough in at least three pairs of sites to allow replication, and on which we could measure interaction with pollinators and fruit or seed set. Plant species are listed in Table 1.

Field and laboratory methods: comparison between grazed and ungrazed sites

Estimation of plant population density.—To estimate plant density, we examined 30 rectangular quadrats of 2×10 m separated by a fixed distance along transects in each site, counting the number of individuals of each species per quadrat. Because understory vegetation was too dense to permit walking through it at times (especially in sites without cattle), we used forest walking paths as transects. Each quadrat was divided in two halves (2×5 m²), and each half was placed at the sides of a walking path, at a distance of 1–2 m from the trail edge. Transect length varied according to site size,

TABLE 1. Plant species included in the study and their life forms, origins, and reproductive characteristics.

Scientific name	Family	Common name	Life form	Origin	Breeding system	Dependence on pollinators	Ref.†
<i>Alstroemeria aurea</i>	Alstroemeriaceae	amancay	herb	native	self compatible, protandrous‡	high	1
<i>Aristolelia chilensis</i>	Eleocarpaceae	maqui	small tree	native	dioecious	high	2
<i>Berberis buxifolia</i>	Berberidaceae	calafate	shrub	native	self incompatible	high	2
<i>Berberis darwinii</i>	Berberidaceae	michai	shrub	native	self incompatible	high	2,3
<i>Calceolaria crenatiflora</i>	Scrophulariaceae	topa-topa	herb	native	self compatible	possibly low	2
<i>Cynanchum diemii</i>	Asclepiadaceae		vine	native	unknown	possibly high	
<i>Gavilea odoratissima</i>	Ochidaceae	orquídea	herb	native	self compatible	high	3
<i>Rosa eglanteria</i>	Rosaceae	rosa mosqueta	shrub	introduced	self compatible	low	4
<i>Ribes magellanicum</i>	Saxifragaceae	parrilla	shrub	native	unknown	unknown	
<i>Schinus molle</i>	Anacardiaceae	laura	small tree	native	dioecious	high	3
<i>Vicia nigricans</i>	Leguminosae	arvejilla	vine	native	self compatible	high	3

† References for breeding systems and dependence on pollinators: 1, Aizen and Basilio (1995); 2, Riveros et al. (1996); 3, this study (see Appendix A); 4, Hansen (1985).

‡ Flowers of this species are synchronized within ramets and among neighboring ramets, which makes selfing opportunity virtually nil (Aizen and Basilio 1995).

ranging between ~300 m (Quetrihué nc) and ~700 m (Llao Llao); therefore, interquadrat distance also varied (~10–23 m). Because we used pre-existing forest trails as transects, transect shape also varied among sites, ranging from one nearly linear transect to irregular, sigmoidal transects. Because all of our plant species were perennials, we assumed that their population abundance would not change greatly between years. Thus, although we studied pollinator visitation, pollination, and reproduction in two consecutive flowering seasons (1999–2000 and 2000–2001), we estimated plant abundances only in the first field season (1999–2000). We used mean density per species per quadrat as an estimate of absolute population density. To estimate relative population density per site, we divided absolute abundance of a species by the abundance of all species with which it shared pollinators (including itself) and whose flowering period overlapped (see Fig. 3).

An important caveat of our methods is that the use of forest paths as transects could have influenced the estimates of plant population density. This effect could occur if other variables, such as cattle impact or light availability, were correlated with distance to the trail edge. Such correlation could result, for example, if cattle used trails and adjacent areas more intensely than other areas, or if forest canopy were less dense on trails than away from them, allowing more light to reach the understory. However, cattle graze over large areas and are not restricted to trails; in fact, seldom did we see cattle on trails; furthermore, trails are narrow enough to have an insignificant effect on the establishment of

dominant tree species, and it is thus unlikely that canopy cover is affected by the presence of trails. We are thus confident in our estimates of plant population density.

Pollinator visitation.—Pollinator visitation observations were conducted on plants flowering throughout each of the eight sites. Individual plants to be sampled were selected haphazardly; we tried to sample as many individuals in as many locations within each site as possible, so as to minimize spatial autocorrelation among samples. Pollinators visiting flowers of each species were sampled in 10-min periods. Paired sites were always simultaneously sampled. In total, 2710 observation periods were conducted for all sites and plant species in 1999–2000, and 1039 observation periods were conducted for six plant species in 2000–2001. These numbers varied across species and sites, because species differed in their abundance, duration of flowering period, and flowering time overlap with other species (see Appendix C). For each visiting pollinator, we recorded species name, number of flowers visited, and whether it contacted flower reproductive parts (anthers or stigmas). When the pollinator species was unknown, we collected the specimen and identified it in the laboratory. A reference collection from the collected material has been deposited in the Laboratorio Ecotono at the Universidad Nacional del Comahue in Bariloche, Río Negro, Argentina (some bee specimens were also placed in the Museo Argentino de Ciencias Naturales in Buenos Aires). Vázquez (2002a) gives a complete list of species.

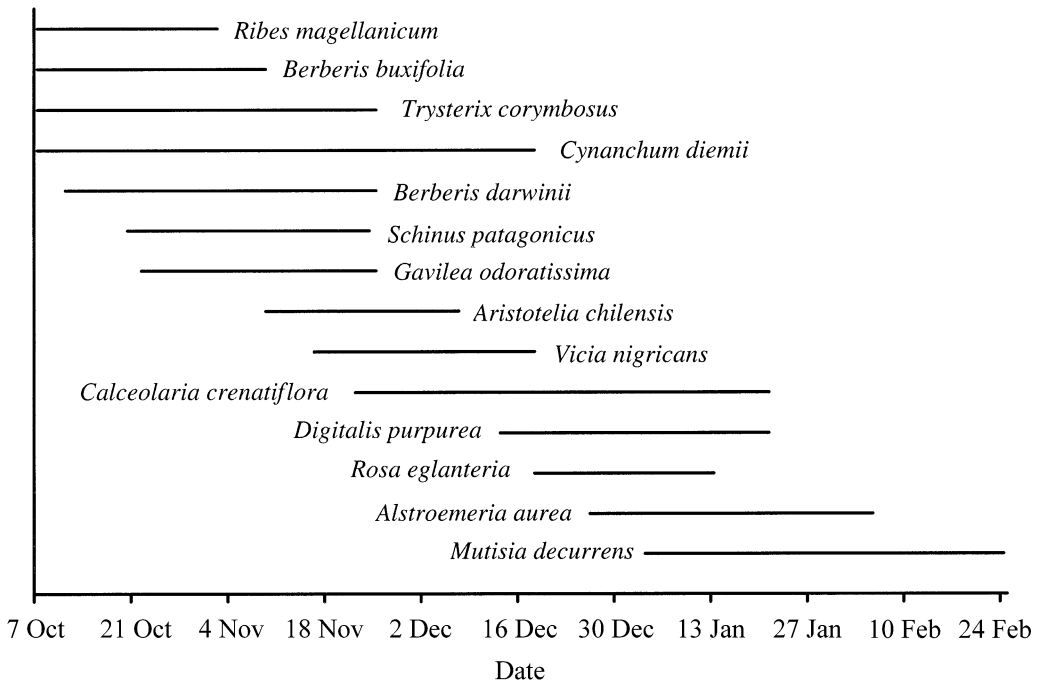


FIG. 3. Phenology of insect-pollinated plants in the study sites. Horizontal bars represent periods during which plants were recorded bearing flowers at any study site during the 1999–2000 field season. Four species not included in the study were included in the graph because they were abundant at some study sites and were visited by pollinator species visiting plant species included in study.

The number of flowers observed per observation period varied across periods, depending on the species, the total number of flowers on the plant, and their spatial arrangement (see Appendix C). In each sampling period, we selected a priori a subset of flowers small enough to allow recording of visits by pollinators to any of the flowers under observation. Following Vázquez and Simberloff (2002), we estimated the number of flowers of plant species j effectively visited by each individual pollinator of species i as

$$e_{ij} = p_{ij} t_{ij} \quad (1)$$

where t_{ij} is the total (uncorrected) number of visits made by an individual pollinator of species i to plant species j during a given observation period, and p_{ij} is the overall proportion of visits (across all observation periods) in which pollinators of species i contacted flower reproductive parts of plant species j (our estimate of pollinator effectiveness). We assume p_{ij} to be constant for each combination of plant and flower visitor species. We used the 1999–2000 data for the calculation of e_{ij} and assumed p_{ij} values to be the same in 2000–2001. Because it was impossible to calculate p_{ij} for unidentified insects that could not be captured, we assumed p_{ij} based on what was known for species of similar body size in the same family or order. However, these unidentified species were usually rare and poorly represented in our samples; thus, overall results are unlikely to be affected by the latter assumption.

Because our statistical unit was each observation period, we summed e values obtained for each individual visitor within each sampling period to calculate V , the total number of visits per flower of plant species j observed in a given observation period, as

$$V_j = \sum_{i=1}^n e_{ij}/o \quad (2)$$

where n is the number of individual flower visitors recorded in the sampling period and o is the number of flowers observed. Thus V_j estimates all of the effective visits made by flower visitors of any species per flower in 10 minutes.

Pollination.—We studied pollination performance of plants by estimating the number of conspecific and heterospecific pollen grains deposited on flower stigmas. Individual plants to be sampled were selected haphazardly; we attempted to sample as many individuals in as many locations within each site as possible, so as to minimize spatial autocorrelation among samples. We collected pistils throughout the flowering season of each species, each time a site was visited (usually once a week). Flower pistils of most species were collected and immediately placed in vials with 70% ethanol. Although this storage method may wash off pollen grains not attached to the stigmatic surface, centrifugation of the ethanol solution after removal of pistils indicated that such loss was minimal. Furthermore, the grains

remaining after preservation may provide a good estimate of the amount of germinating pollen received on stigmas. Stigmas were excised, placed on microscope slides, and stained with Alexander's stain (Kearns and Inouye 1993); we counted the number of conspecific and heterospecific pollen grains under a compound microscope. We usually collected five pistils per individual and used the average number of conspecific and heterospecific pollen grains per individual for statistical analyses; sample sizes per species and site used for statistical analyses are reported in Appendix C. For the asclepiad *Cynanchum diemii*, we observed virtually no pollinia inserted in the flower stigmatic surfaces; therefore, we used the number of pollinia removed as a surrogate for pollination success. For *Alstroemeria aurea*, only primary flowers were considered for the analysis, because secondary flowers usually do not contribute significantly to reproduction (Ladio and Aizen 1999).

Plant reproduction.—We tagged individuals of all plant species in all sites where they were present, recording the number of flowers per individual (herbs) or per branch (shrubs, trees, and vines). Individual plants to be tagged were selected haphazardly; we attempted to sample as many individuals in as many locations within each site as possible, so as to minimize spatial autocorrelation among samples. Sample sizes per species and site are reported in Appendix C. We estimated three reproductive variables: fruit set (the proportion of tagged flowers that set fruit), seeds per fruit, and seeds per flower (the mean number of seeds produced per tagged flower).

Because the degree of self-compatibility and of dependence on pollinators for reproduction may be important for determining the indirect effects of herbivores on plant reproduction, we compiled the available information on breeding systems for the species included in the study (Table 1). For those species for which we could find no published studies on breeding systems, we conducted hand-pollination experiments to study the degree of self-compatibility and of dependence on pollinators for reproduction. A description of the methods and results of these experiments is given in Appendix A.

Evaluation of browsing and soil compaction by cattle.—We checked all individuals of all species within each quadrat used to estimate density for signs of browsing. We calculated browsing indices for each plant species as the average proportion of browsed individuals per quadrat, i.e.,

$$B_i = \left(\sum_{j=1}^n b_{ij} \right) / n \quad (3)$$

where B_i is the browsing index for species i , b_{ij} is the proportion of individuals of species i browsed in quadrat j , and n is the number of quadrats sampled ($n = 30$ in all cases).

Trampling by cattle can affect plants directly through damage of tissues or indirectly through changed soil properties. We used soil compaction (bulk density) as a measure of the intensity of trampling by cattle. Increased soil compaction as a result of trampling by ungulates is well-documented in many systems (e.g., Yates et al. 2000). We took 30 soil samples per site using a 442-cm³ corer. Sample locations were separated by a fixed distance along transects, as for estimation of plant population density. Samples in all sites were taken during three days in January 2000. Summer is the dry season in the area, and thus water content in all soil samples was low. Soil samples were subsequently dried at 105°C for 24 h and were then weighed immediately.

Field and laboratory methods: further studies on Alstroemeria aurea

For *Alstroemeria aurea*, the only species for which we found evidence of indirect effects of cattle on pollination and reproduction in the comparisons between grazed and ungrazed sites, we sought additional evidence for the hypothesized mechanisms. To this end, we conducted trampling and hand-pollination experiments and a spatial analysis of the effect of floral neighborhood density on pollinator visitation, pollination, and reproduction.

Trampling experiments.—We studied the effect of trampling on survival of *A. aurea*, which we suspected to be highly susceptible to trampling (D. P. Vázquez, *personal observation*). For this purpose, we selected a high-density patch of *A. aurea* in an ungrazed area in Quetrichué. We set up four pairs of 1-m² plots, to which we assigned one of two treatments: trampled and control (not trampled). In the “trampled” plots, the first author walked once over the entire surface of the plot; control plots were left untouched. We counted the number of live *A. aurea* ramets before trampling and three weeks later.

Hand-pollination experiments.—To evaluate pollen limitation in *A. aurea*, we performed hand-pollination in three of the grazed sites (we were unable to hand-pollinate in Mascardi because of the small number of flowering ramets). *A. aurea* is clonal, and it is usually difficult to determine in situ which ramets belong to the same genet without destroying the plants. Because it has been shown that the degree of genetic relatedness falls sharply after 10 m (Souto et al. 2002), we took pollen from individual ramets >20 m apart from the individual to be hand-pollinated to ensure cross-pollination, usually mixing pollen from anthers collected from individuals at different locations within each site. To avoid the confounding effect of resource reallocation within an individual plant (see Zimmerman and Pyke 1988), we hand-pollinated all flowers within a ramet. However, the clonal nature of *A. aurea*, and the difficulty of knowing relatedness of ramets within a patch without destructive sampling, do not allow us to

exclude the possibility of resource reallocation among ramets of the same genet. Pollen supplementation was done on open-pollinated (unbagged) flowers. To compare the reproductive performance between hand-pollinated and naturally pollinated ramets, we selected unmanipulated ramets close to, and at the same floral phase as, the hand-pollinated ramets. Eight hand-pollinated and eight unmanipulated ramets were selected in each site; however, some ramets were lost (presumably to trampling by cattle or tourists), which reduced the final number of experimental and control pairs to six in Arroyo Goye, six in Cerro López, and four in Quetrihué.

Spatial analysis of floral neighborhood density effects on pollination and reproduction of A. aurea.—To test for the effect of population density on pollinator visitation, pollination, and reproduction, we conducted a spatial analysis in the four grazed sites. The rationale behind this test was that, if the observed effect of cattle on pollination and reproduction operated through density, variation in floral density around individuals should explain variation in pollination and reproduction within sites. To this end, we mapped all flowering ramets of *A. aurea* in the four grazed sites. Because we did not control the density of *A. aurea* and that of other species, both density of conspecifics and relative density covaried at the local neighborhood. This observation agrees with the site-level observation that both density measures are highly correlated ($r = 0.84$; $P = 0.0088$). Thus, we use only one measure of neighborhood density as a surrogate for absolute and relative density.

Maps of the location of flowering ramets were drawn by taking the linear distance with a measuring tape and the angular deviation from north with a hand compass from known points taken from a previously drawn topographic map. Density of the floral neighborhood was calculated as described in Appendix B. Because we included all flowering ramets in each of the four grazed sites, sample sizes for the path analysis varied among sites. Total numbers of individual ramets mapped at the sites were: Cerro López, $n = 139$; Arroyo Goye, $n = 220$; Mascardi (c), $n = 34$; Quetrihué (c), $n = 228$.

We estimated pollinator visit frequency as previously described, throughout the flowering period of *A. aurea* in 2000–2001. Because we attempted to obtain a sample size as large as possible, and because pollinator visit frequency varies highly in time and space, we sampled some ramets repeatedly on different days; we assumed that those observations were independent. Thus, each individual ramet had several associated pollinator visitation observations, but only one associated observation for each of the other variables (neighborhood density, pollen deposition, and reproductive variables). We used the average V_j (see Eq. 2) for analyses. Pollen deposition and plant reproduction were studied as we have described for the comparison between ungrazed and grazed sites.

Statistical analyses and inference

Comparisons between ungrazed and grazed sites.—We used a mixed-ANOVA model to test for effect of herbivory on each of the independent variables. The model was:

$$y_{ijk} = \mu_{...} + G_{i..} + P_{.j.} + G \times P_{ij.} + \varepsilon_{ijk} \quad (4)$$

where $\mu_{...}$ is the overall mean, $G_{i..}$ is the difference in the mean caused by presence of cattle (“grazing,” a fixed factor with two levels, grazed and ungrazed), $P_{.j.}$ is the difference in the mean due to the effect of “pair” (a random factor with four levels), $G \times P_{ij.}$ is the interaction between grazing and site, ε_{ijk} is an independent error term drawn from a normal distribution with mean 0 and variance σ^2 , and y_{ijk} is the response of the dependent variable to the factors and their interaction. Tests were performed with the MIXED procedure in SAS (SAS Institute 1999).

A mixed linear model is a generalization of standard linear models, the generalization being that the data are permitted to exhibit correlation and nonconstant variability. The parameters of the mean model are referred to as fixed-effects parameters, and the parameters of the variance–covariance model are referred to as covariance parameters. The fixed-effects parameters are associated with known explanatory variables, as in the standard linear model. However, the covariance parameters distinguish the mixed linear model from the standard linear model. One common situation in which the need for covariance parameters arises is when experimental units can be grouped into clusters and data from a common cluster are correlated (Littell et al. 1996). In our design, data were clustered in sites, and each pair of sites represented a level of the random factor, pair. Pair was considered a random rather than a fixed factor because the particular sites used in this study were chosen from a universe of possible sites; our intention was to test for the effect of grazing in general (rather than answering the more restricted question of whether grazing had an effect in these particular four pairs), so it was appropriate to consider “pair” to be a random factor (see Underwood 1997). Because in most cases data did not meet the assumption of normality, we performed nonparametric tests using ranks in the mixed procedure just described, as recommended by Conover and Iman (1981).

Because of the small number of replicates (i.e., four pairs of sites), the statistical power of our analyses was rather low. Therefore, we used a critical probability level of $\alpha = 0.1$ for all ANOVAs to avoid an excessive probability of committing Type II error (i.e., accepting a false null hypothesis). However, in order to accept the observed effects as evidence of indirect effects of cattle on pollination and plant reproduction, we also devised a “congruence criterion,” requiring that significant effects be detected for all of the variables hypothesized in the chain of effects leading to indirect

effects (Fig. 1) in at least one of the two study years. The probability of getting significant results in all variables by chance is $\theta = \alpha^n$, where n is the number of variables and θ is the overall probability value. For example, if we set $\alpha = 0.1$ and $n = 3$ (e.g., population density, conspecific pollen deposition, and fruit set), the overall probability of getting significant results in the three variables by chance is $\theta = 0.001$. Thus, although our α value is rather high compared to the conventional $\alpha = 0.05$, our congruence criterion makes our test very conservative. A caveat in our analysis is that we performed multiple tests for each plant species and dependent variable. By doing so, we increased the probability of getting congruent effects by chance in at least one of the species. A procedure frequently used to correct α to account for multiple tests is the sequential Bonferroni correction (Rice 1989). Using this correction, for $\alpha = 0.05$, the overall significance level for the lowest P value becomes $\theta = 0.05/11 = 0.0045$, a value still higher than the 0.001 that we got by fixing α at the 0.1 level and requiring congruence among different effects.

When evaluating how grazing affects the dependent variable, we took into account both the main effect of grazing and the grazing \times pair interaction. When the interaction was significant, contrasts were conducted between grazed and ungrazed sites in each pair, as recommended by Underwood (1997); we used the *estimate* option in the MIXED procedure to construct the contrasts. We considered the effect of grazing on the dependent variable to be significant when there was a significant main effect of grazing and a nonsignificant interaction, or when there was a significant interaction and all paired differences were significant and in the same direction. We considered the effect of grazing to be partially significant when there was a significant interaction, paired differences were in the same direction in all pairs, and the contrasts were significant in at least half of the pairs in which comparisons were made.

Power analysis of mixed-model ANOVA.—We used Monte Carlo simulations to conduct a power analysis for our mixed-model ANOVA. In particular, we asked (1) how high the power of our analysis was to detect effect sizes of different magnitudes, (2) how much power is affected by the number of samples per site, and (3) whether the power of the analysis would have increased substantially had we been able to include more pairs of sites in the study. Although the limit to the number of site pairs included was set by logistical constraints rather than by sample size considerations (i.e., we used all appropriate sites located within a distance that allowed daily travel among sites), it is still useful to conduct a power analysis to assess the likelihood that negative results are due to Type II errors rather than to true lack of effect.

Data used in the mixed-model analysis were unbalanced for all dependent variables except absolute den-

sity, soil compaction, and irradiance, for which we sampled 30 quadrats in each site. We were unable to find an appropriate method to calculate power for such an unbalanced design; thus, we present power estimates for balanced data only. However, our analysis may give a general idea of the power attained by a particular effect size, number of sites, and average number of samples per site. For the power analysis, we generated random data for two levels of a fixed factor (i.e., grazing), 4–10 levels of a random factor (i.e., pairs of sites), and 30 and 60 observations per fixed and random factor level combination (i.e., samples per site). For simplicity, we assumed no interaction between fixed and random factors. We used four different magnitudes of random errors for data generation, so as to obtain contrasting effect sizes. Data were then transformed into ranks (as we did in our analyses), and the ANOVA calculations were performed by least square methods. We calculated effect size for the fixed factor as $d = |\mu_{nc} - \mu_c|/\sqrt{MS_{G \times P}}$, where μ_{nc} is the mean across all ungrazed sites, μ_c is the mean across grazed sites, and $MS_{G \times P}$ are the means of squares for the interaction term in Eq. 4, which is the correct pooled variance estimate for effect size calculations in a mixed-model ANOVA (Zar 1999). It is important to bear in mind that, even though we used specific effect sizes to create the data (by specifying the parametric mean and the random error of each level of grazing), the resulting effect size for each randomized data set varied, owing to the stochastic nature of the process; for this reason, we report the mean effect size with its confidence intervals. Power was calculated as the proportion of F statistics calculated for the randomized data that exceeded the critical value of $F_{\alpha,a,b}$ for $\alpha = 0.1$ (the probability value used in our analyses), $a = 1$ degree of freedom for the fixed factor, and $b = \text{number of levels} - 1$ as the degrees of freedom for the random factor. All calculations were done in Matlab (MathWorks 1999).

Results of the power analysis are presented in Fig. 4, together with the resulting effect sizes measured upon data ranks. Power with four pairs of sites (i.e., the replication used in our study) was strongly influenced by effect size; although mean effects sizes of 0.75 or higher (as observed for total density for *Aristotelia chilensis*; see Appendix D) had a relatively high probability of being detected, the statistical power for smaller effect sizes (the situation for all other species) was considerably lower. Thus, some nonsignificant results may actually be Type II errors.

As expected, increased sample size per site leads to increased power. For example, doubling the sample size per site increases power (compare lines of equivalent effect size in Fig. 4a and f). Increasing the number of pairs of sites also would have increased the statistical power of our analyses. For example, doubling the number of pairs of sites from four to eight would have led to roughly a twofold increase in power (Fig. 4). The effect on power of an increased number of sites is stron-

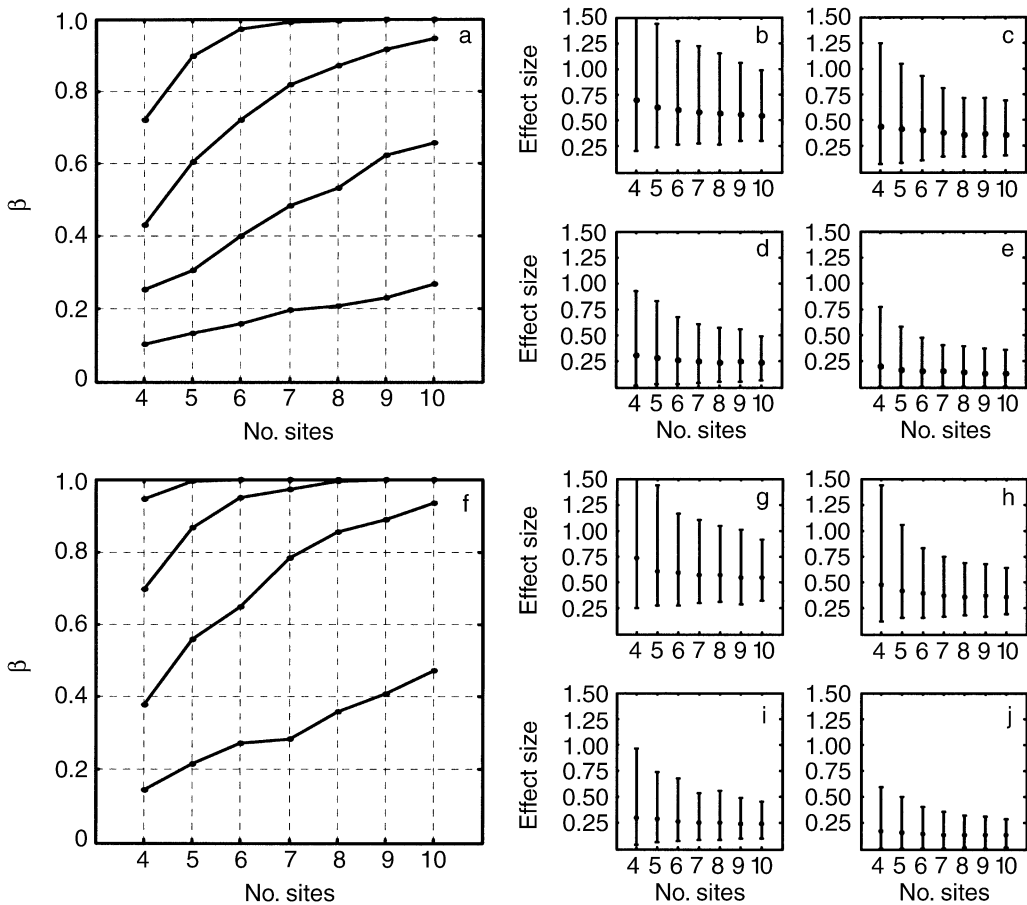


FIG. 4. Results of power analysis of mixed-model ANOVA: (a) power (β) as a function of the number of sites (i.e., levels of random factor), for four effect sizes (larger power always corresponds to larger effect size) and 30 samples per site; (b–e) resulting effect sizes calculated for ranks, for 30 samples per site; (f–j) as for (a–e), with 60 samples per site.

ger than that of an increase of similar magnitude in the number of samples per site. Regrettably, as we have explained, it was not possible to include more sites in the study; thus we strove to maximize the number of samples per site.

Trampling and hand-pollination experiments for A. aurea.—Data from trampling experiments were analyzed with a paired-sample *t* test, with the TTEST procedure in SAS (SAS Institute 1999). Data from the hand-pollination experiment were analyzed with a two-way, model I ANOVA, in which type of pollination (hand or natural) and site (Cerro López, Arroyo Goye, Quetrihué) were fixed factors. Unlike in the comparisons between grazed and ungrazed sites, sites were treated as a fixed rather than random factor in this analysis because our intent was to test whether our results in these particular populations could result from pollen limitation. ANOVA tests were conducted in the GLM procedure in SAS; we used Type III sums of squares, which are appropriate for unbalanced, complete designs.

Path analysis of the effect of density on pollinator visitation, pollination, and reproduction of A. aurea.—

We used path analysis to study the effect of floral neighborhood density on pollinator visitation, pollination, and reproduction of *A. aurea* (the only species for which we found evidence of indirect effects in the comparison between paired sites), using the spatial data described previously. This technique allows us to evaluate the structure of causal relationships among a group of variables; it has been used in many previous studies involving pollination and plant reproduction (Schemske and Horvitz 1988, Mitchell 1992, 2001, Herrera 1993, Brody 1997, Gómez and Zamora 2000, Mothershead and Marquis 2000).

We used a general path model including six variables of interest: neighborhood density, pollinator visit frequency, conspecific pollen deposition, heterospecific pollen deposition, fruit set, and seeds per fruit (Fig. 5). We did not include seeds per flower in the model because this variable is simply a linear combination of fruit set and seeds per fruit; such inclusion would have artificially inflated the goodness of fit of the model to the data. Because the observational nature of our study made it impossible to obtain independent measures of absolute and relative density at the neighborhood scale

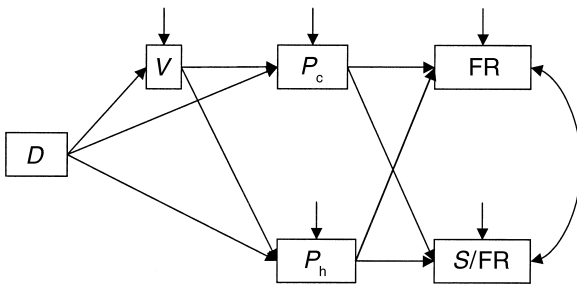


FIG. 5. Full causal model used to relate floral neighborhood density, pollinator visitation, pollination, and reproduction of *Alstroemeria aurea*. One-headed arrows represent directional effects; two-headed arrows represent correlations between pairs of variables. Conspecific and heterospecific pollen deposition were log-transformed. Variables included in the full model are: our estimate of floral neighborhood density (D ; see Appendix B); pollinator visitation rate (V); number of conspecific (P_c) and heterospecific (P_h) pollen grains deposited in stigmas; and two reproductive variables, number of fruits per flower (FR) and number of seeds per fruit (S/FR). Unexplained variability is indicated with vertical arrows above each endogenous variable.

(see *Spatial analysis . . .*), we assumed that they were positively correlated and used a single measure as a surrogate for both (see Appendix B for calculation details). In our conceptual model (Fig. 1), total density directly affects visitation frequency and relative density directly affects conspecific and heterospecific pollen deposition; thus, we included paths connecting our single measure of neighborhood density to visit frequency and conspecific and heterospecific pollen deposition (Fig. 5). Although this convention makes it difficult to tease apart the two chains of events outlined in our hypothesis (Fig. 1), it is still possible to identify alternative outcomes that would support either one of the two mechanisms. For example, if neighborhood density positively affected visit frequency, and this in turn had positive effects of similar magnitude on conspecific and heterospecific pollen deposition, the mechanism outlined in Fig. 1a would be supported; conversely, if density did not significantly affect visit frequency, visit frequency had no detectable effect on pollen deposition, and density had a direct, positive effect on conspecific pollen deposition and a direct, negative effect on heterospecific pollen deposition, this would support the mechanism outlined in Fig. 1b.

We used structural equation modeling (SEM) procedures for the estimation of standardized path coefficients and the assessment of model fit. We first computed the covariance matrix for the variables using the CORR procedure in SAS and then used the covariance matrix as input in the CALIS procedure. Because we could not measure all variables for all flowering ramets in the population, we dealt with missing observations by applying "pairwise deletion" to our data set, which is the default option in SAS; that is, we calculated the covariances between pairs of variables with all of the available observations. It is important to mention that

results of path analyses can be sensitive to missing data, and thus the use of pairwise deletion can be misleading if missing data are common, especially if the missing data are from a nonrandom subset of the subjects. To make sure that the use of pairwise deletion did not bias our results, we also conducted the analyses using "listwise deletion"; results were generally consistent with those obtained when using pairwise deletion.

To evaluate the fit of a structural model to a set of data, CALIS uses the specified model to generate a predicted covariance matrix, and then compares this predicted matrix with the observed matrix given as input. To assess the goodness of fit between predicted and observed covariance matrices, we used the maximum likelihood χ^2 statistic; a statistically significant χ^2 value suggests poor fit between both matrices. However, a significant χ^2 can also result from violation of several assumptions, and failure to reject a model (a nonsignificant χ^2) may result from inadequate statistical power at low sample sizes (Hatcher 1994). Therefore, we also report the Normed Fit Index (NFI), which has been proposed by Bentler and Bonett (1980) as a more robust alternative to the χ^2 test. NFI ranges between 0 and 1, with values >0.9 usually considered as an indication of good agreement between predicted and observed covariances (Hatcher 1994). When the general model (Fig. 5) did not provide a good fit to the observed covariance structure, we constrained nonsignificant paths to zero (i.e., we removed the arrows from the graph) and re-ran the CALIS procedure with the significant paths only. In all cases, this resulted in a model whose predicted covariance structure did not differ significantly from the observed one. We assessed significance of individual path coefficients using the t statistic calculated by CALIS for each coefficient estimate. We tested the significance of the improvement in fit by testing for the significance of the difference between χ^2 statistics calculated for the full and for the reduced models; the degrees of freedom for the test are obtained by subtracting those of the revised model from those of the original model (Hatcher 1994).

A separate path analysis was conducted to summarize relationships among studied variables observed among sites. We conducted this analysis for heuristic reasons, as a way of summarizing relationships among the variables observed. The idea behind this analysis was that the variability in site means should be explained, at least in part, by the hypothesized causal scheme imposed by our hypothesis. We thus constructed a path model that matched the hypothesized causal relationships presented in Fig. 1. We used mean browsing index for all species per site (Table 2) as a summary of the overall intensity of the impact of cattle. The small sample size ($n = 8$ sites) prevented use of the SEM procedures that we have described for path coefficient estimation and testing of model fit (most model selection criteria used in structural equation modeling are sensitive to sample size, becoming unreliable at low

TABLE 2. Browsing indices: mean percentages of browsed individuals per quadrat for each site (c, with cattle; nc, no cattle) and species.

Site	Plant species										Total	
	A.a.	A.c.	B.b.	B.d.	C.d.	C.c.	G.o.	R.e.	R.m.	S.p.		V.n.
Llao Llao	0	0		0	0	0	0	0	0	0	0	0
Cerro López	0	86		94	0		0	0	45	98	0	36
Safariland	0	10		4	0		0	0	2	18	0	4
Arroyo Goye	0	96	73	70	23		33		93	98	4	54
Mascardi (nc)	0	0		2			2	0	12	17	0	4
Mascardi (c)	0	100	100	100			57	0	67	100	0	58
Quetrihué (nc)	0	28	0	50	10			0	27	47	0	18
Quetrihué (c)	1	89	70	93	39	0	0	0	85	89	0	42
Average (nc)	0	9	0	14	3	0	1	0	10	20	0	5
Average (c)	0	93	81	89	21	0	23	0	72	96	1	43

Notes: Empty cells indicate that the browsing index could not be estimated because the species was rare. Plant species are indicated by initials of genus and species names (see Table 1 for full names).

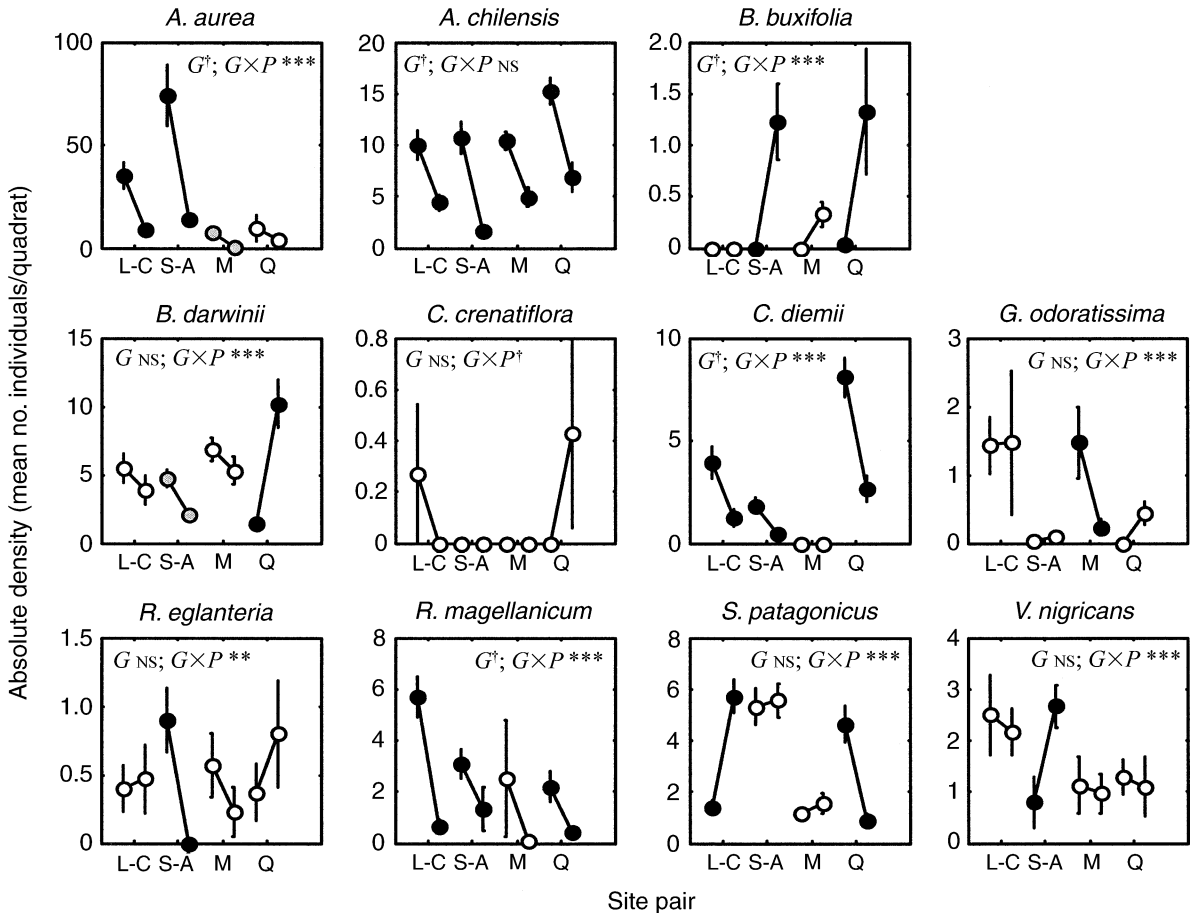


FIG. 6. Mean plant species density per quadrat per site, averaged over 30 quadrats. Error bars represent standard error of the mean. Paired sites are joined by lines, with the ungrazed site on left and the grazed site on right: x-axis paired sites are as follows: L-C, Llao Llao–Cerro López; S-A, Safariland–Arroyo Goye; M, Mascardi (nc)–Mascardi (c); Q, Quetrihué (nc)–Quetrihué (c). Statistical significance of main grazing effect (G) and grazing–pair interaction ($G \times P$) in nonparametric (rank) mixed-model ANOVA is indicated as follows: $\dagger P < 0.1$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ (see Appendix D for ANOVA tables). Circle fills indicate the statistical significance of paired contrasts: black, $P < 0.05$; gray, $P < 0.1$; white, $P \geq 0.1$.

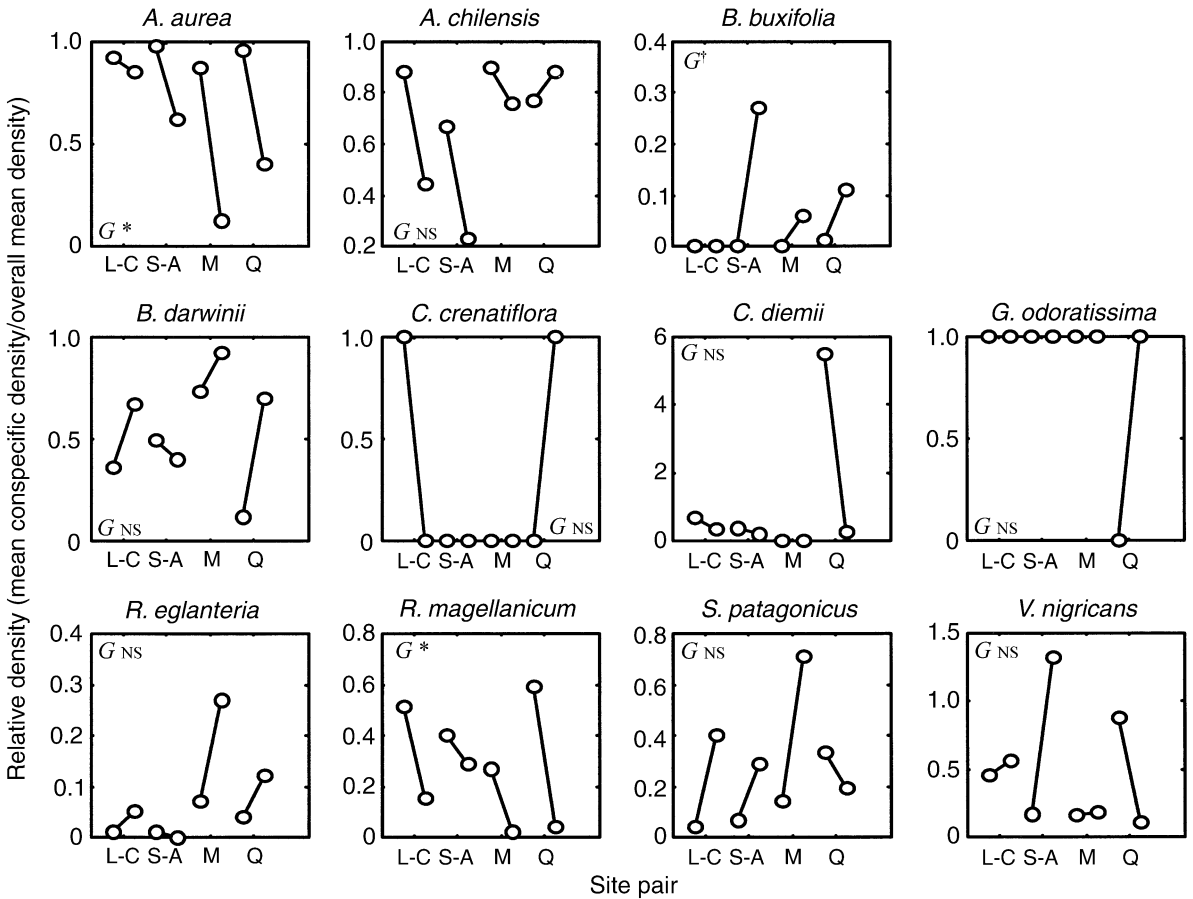


FIG. 7. Relative population density per site, calculated as mean density of conspecifics over the sum of the mean density of all species with overlapping flowering period. Paired sites are joined by lines, with the ungrazed site on the left and the grazed site on the right (see Fig. 6). Statistical significance for main grazing effect (G) in nonparametric (rank) mixed-model ANOVA is indicated as follows: † $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (see Appendix D for ANOVA tables).

sample sizes; Shipley 2000). However, path coefficients can also be calculated as standardized regression coefficients with conventional least squares regression procedures. Although this technique does not allow evaluation of the overall goodness of fit of the covariance structure of the model to the data, it is useful to summarize relationships among a set of variables in a coherent way. We used the REG procedure in SAS to calculate path coefficients, which are obtained as standardized regression coefficients using the *stb* option in the *model* statement. The statistical significance of the path coefficients was tested by the t tests for the parameter estimates in the regression analysis.

RESULTS

Testing the hypothesis for the 11 species: comparison between grazed and ungrazed sites

Plant population density.—For five species, there were significant main effects of grazing on absolute population density. For only one of these species (*Aristotelia chilensis*) was there no significant interaction between grazing and pair (Fig. 6; Appendix D). Of the

species with both significant main grazing and interaction effects, *Alstroemeria aurea*, *Cynanchum diemii* and *Ribes magellanicum* showed a trend toward decreased density in grazed sites in all pairs in which they were present, but with significant contrasts in only a subset of pairs (Fig. 6). Conversely, *Berberis buxifolia* showed a trend toward increased absolute density in grazed sites, with significant contrasts in two pairs (Fig. 6). The remaining species, all of which had significant interactions between grazing and pair, did not show consistent patterns of density in relation to grazing.

There were significant, negative effects of the presence of cattle on relative density for *A. aurea* and *R. magellanicum*, and a positive effect for *B. buxifolia* (Fig. 7; Appendix D). Because our measure of relative density was unreplicated (i.e., only one measurement per site), no test of the grazing \times pair interaction is possible.

Because our hypothesis requires an effect on either absolute or relative population density, the remaining comparisons between grazed and ungrazed sites are

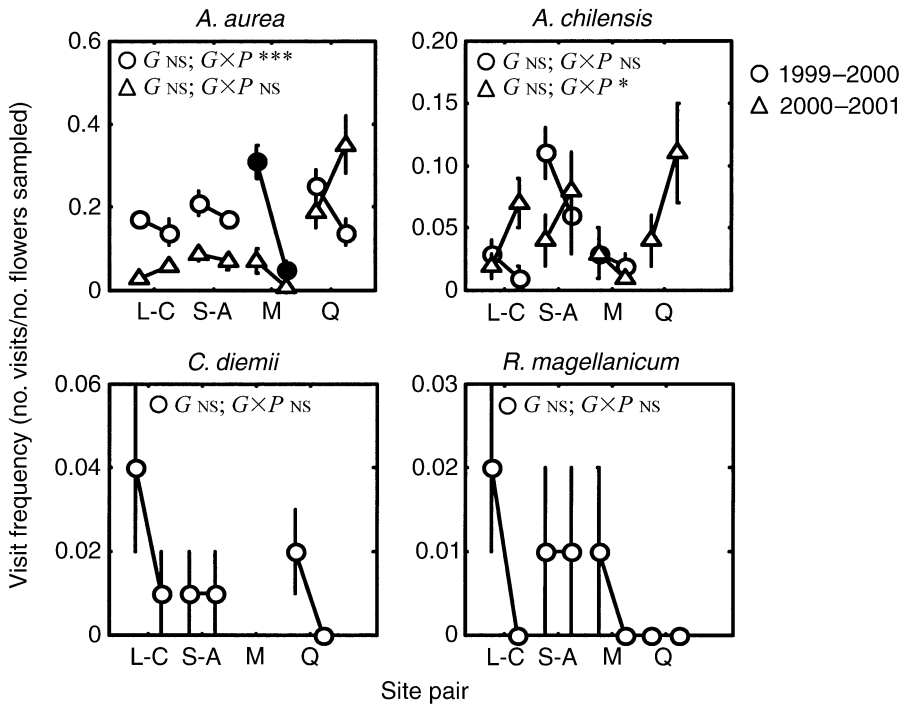


FIG. 8. Pollinator visit frequency per 10-min sampling period for four species with density negatively affected by cattle. Sites with no marker indicate that there were no observation periods, owing to the scarcity of flowering individuals. Conventions are as in Fig. 6; see Appendix E for ANOVA tables.

reported only for the species that showed at least partial evidence of such effects. However, no tests of the effect of grazing on pollinator visitation, pollination, and reproduction were possible for *B. buxifolia*, because individuals of this species virtually failed to flower in ungrazed sites. Thus, the remainder of this section deals only with *A. aurea*, *A. chilensis*, *C. diemii*, and *R. magellanicum*.

Pollinator visitation.—No significant main effects of grazing were found for any of the four species (Fig. 8; Appendix E). Significant interactions were detected for *A. aurea* in 1999–2000 and for *A. chilensis* in 2000–2001; a significant contrast was found only for one pair of sites for *A. aurea* (Fig. 8). Thus, cattle presence does not affect pollinator visit frequency for any of the four species.

Pollination.—There was a significant main effect of grazing on conspecific pollen deposition for *A. aurea* in 2000–2001 but not in 1999–2000; in both seasons, significant interaction effects were also detected. Contrasts revealed that conspecific pollen deposition was significantly lower in grazed sites in all pairs and seasons except Safariland-Arroyo Goye in 1999–2000 (Fig. 9; Appendix F). *A. aurea* also showed a significant main effect of grazing on heterospecific pollen deposition in 1999–2000 but not in 2000–2001, and significant interaction effects were found in both years; contrasts revealed higher deposition of heterospecific pollen in grazed sites in all pairs and seasons except

Llao Llao-Cerro López in 2000–2001 (Fig. 9; Appendix F). Finally, significant main effects of grazing on proportion of conspecific pollen were found for *A. aurea* in both seasons, with a significant interaction in 1999–2000 but not in 2000–2001; contrasts revealed that proportion of conspecific pollen was significantly lower in grazed sites in all pairs and seasons (Fig. 9; Appendix F).

No main effects of grazing on any of the three dependent pollination variables were detected for *A. chilensis*; we found a significant interaction only for conspecific pollen, but no contrasts were statistically significant. No main or interaction effects were detected for pollinia removal of *C. diemii* (Fig. 9; Appendix F).

For *R. magellanicum*, we found a significant main effect of grazing for heterospecific pollen deposition and for the proportion of conspecific pollen in 2000–2001, and significant interactions for conspecific pollen deposition and proportion of conspecific pollen; however, in only one pair and year (Mascardi, 2000–2001) were the contrasts statistically significant (Fig. 9; Appendix F). Furthermore, the number of heterospecific pollen grains deposited in flower stigmas of this species was much lower than the number of conspecific pollen grains, which minimizes chances of competition for pollination.

Fruit and seed set.—*A. aurea* exhibited significant main effects of grazing on the number of fruits per flower in 1999–2000 and the number of seeds per fruit

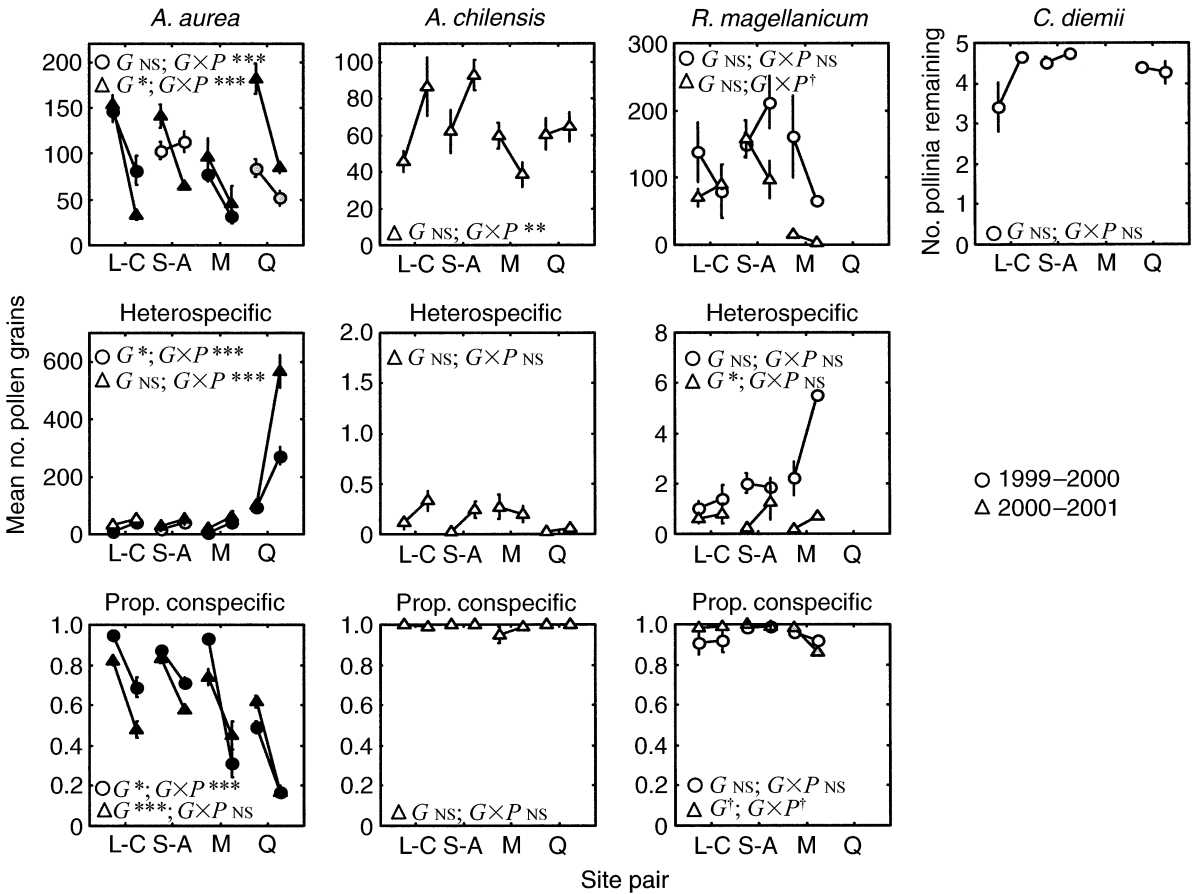


FIG. 9. Conspecific and heterospecific pollen deposition, and the proportion (prop.) of conspecific pollen deposition on stigmas for four species with density negatively affected by cattle. Sites with no marker indicate cases in which no observations were conducted. Conventions are as in Fig. 6; see Appendix F for ANOVA tables.

and seeds per flower in 2000–2001; we detected significant interaction effects for the three reproductive variables in both seasons. Contrasts revealed negative effects of grazing for the three reproductive variables in at least one season in all pairs except Safariland-Arroyo Goye; in the latter case, we found significantly higher fruit set and lower seeds per fruit in the grazed site in 2000–2001, and no significant contrasts for the remaining combinations of reproductive variable and season (Fig. 10; Appendix G).

Significant effects of grazing on the number of fruits per flower were found for *A. chilensis* in 1999–2000, with a trend toward higher fruit set in grazed sites; this effect was not observed in 2000–2001. We detected a significant interaction for the number of seeds per fruit, and contrasts revealed significantly fewer seeds per fruit in the grazed site of one pair and the opposite result in another pair (Fig. 10, Appendix G). No significant effects were found for the number of seeds per flower (seed set was quantified for this species only in 2000–2001).

No significant main effects were found for *C. diemii*; significant interactions were found for this species for

number of fruits per flower and seeds per fruit (seed set was quantified for this species only in 2000–2001); only one significant contrast was observed, which revealed higher seeds per fruit with grazing (Fig. 10; Appendix G). Finally, no significant effects on fruits per flower were detected for *R. magellanicum* (seed set could not be quantified for this species).

Summary of comparisons between grazed and ungrazed sites.—Results of the comparisons between grazed and ungrazed sites for the 11 plant species are summarized in Table 3. For most species, we found no density-mediated indirect effects of cattle on pollination and reproduction. In six cases, there were no significant effects of grazing on either absolute or relative population density, which precludes further analysis of effects on plant–pollinator interactions, pollination, and reproduction; a seventh species for which we detected positive effects of grazing on density, *B. buxifolia*, failed to produce flowers in ungrazed sites, which precluded further analyses of other dependent variables. Of the remaining four species for which we found at least partial evidence of negative effects of grazing on absolute or relative population density, only *A. au-*

TABLE 3. Summary of results of comparisons between grazed and ungrazed sites for the 11 species. Cells with symbols indicate at least partial evidence for effects of grazing on a particular species, dependent variable, and season.

Plant species	Density		Pollinator visitation		Pollination†			
	Absolute	Relative	1	2§	Conspecific		Heterospecific	
	1§	1			1	2	1	2
<i>Alstroemeria aurea</i>	(-)	-	NS	NS	(-)	-	+	(+)
<i>Aristotelia chilensis</i>	-	NS	NS	NS		NS		NS
<i>Berberis buxifolia</i>	(+)	+						
<i>Berberis darwinii</i>	NS	NS						
<i>Calceolaria crenatiflora</i>	NS	NS						
<i>Cynanchum diemii</i>	(-)	NS	NS		NS			
<i>Gavilea odoratissima</i>	NS	NS						
<i>Rosa eglanteria</i>	NS	NS						
<i>Ribes magellanicum</i>	(-)	-	NS		NS	NS	NS	+
<i>Schinus patagonicus</i>	NS	NS						
<i>Vicia nigricans</i>	NS	NS						

Notes: Symbols indicate the following: +, -, significant main effect of grazing with no significant interaction, or significant interaction with all contrasts significant, thus providing strong evidence of positive or negative effects, respectively; (+), (-), significant interaction with at least half of the contrasts significant and with effects in the same direction, thus providing weak evidence of positive or negative effects, respectively. Cells with NS are statistically insignificant results; empty cells indicate unmeasured or unreported effects.

† Results are summarized for deposition of conspecific pollen, heterospecific pollen, and percentage of conspecific pollen.

‡ Reproductive variables: fruits per flower, seeds per fruit, and seeds per flower.

§ Fieldwork seasons are indicated as follows: 1, 1999–2000; 2, 2000–2001.

|| No comparisons were possible for this species for pollinator visitation, pollination, and reproductive variables because it flowered only in grazed sites.

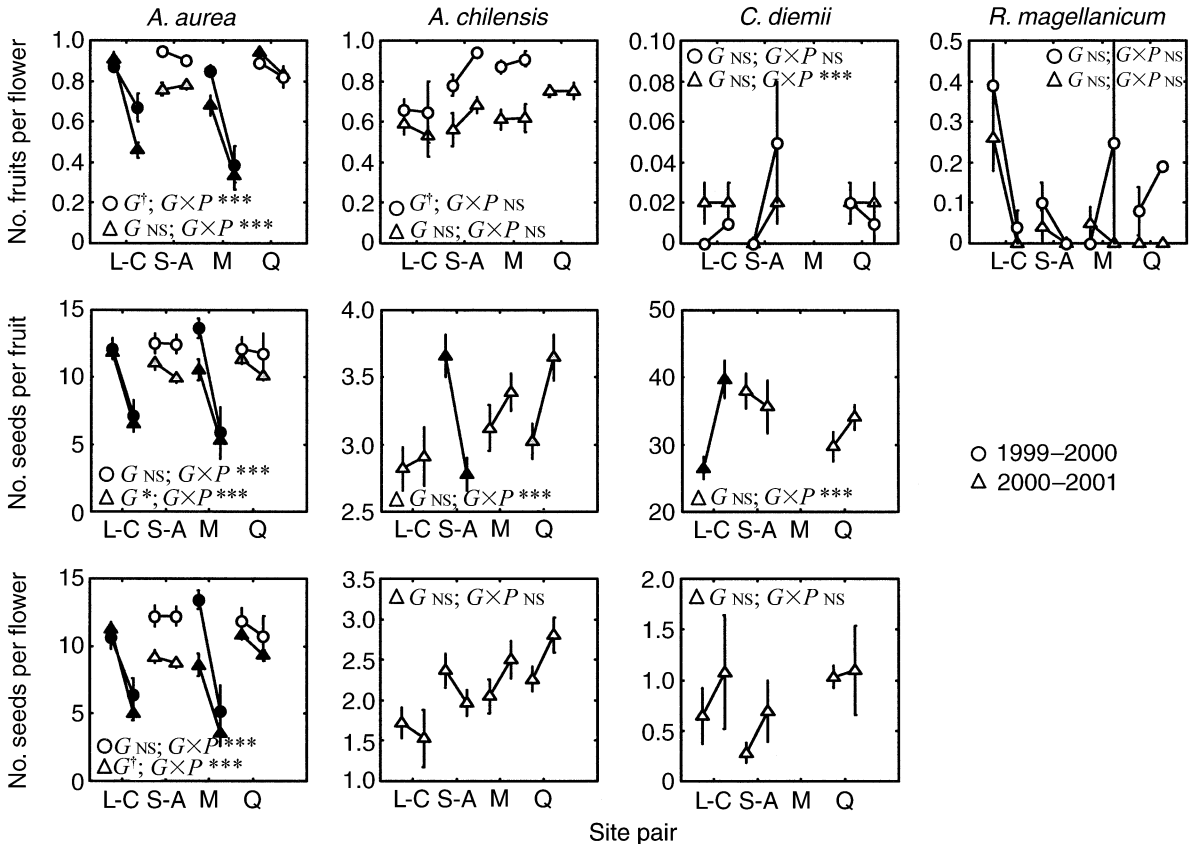


FIG. 10. Fruit set, number of seeds per fruit, and number of seeds per flower for four species with density negatively affected by cattle. Sites with no marker indicate that no observations were conducted. Conventions are as in Fig. 6; see Appendix G for ANOVA tables.

TABLE 3. Extended.

Pollination†		Reproduction‡					
Conspecific %		Fruits/flower		Seeds/fruit		Seeds/flower	
1	2	1	2	1	2	1	2
–	– NS	(–) +	(–) NS	(–)	(–) NS	(–)	(–) NS
		NS	NS		NS		NS
NS	(–)	NS	NS		NS		NS

rea showed effects on pollination and reproduction that were consistent with the hypothesis. Thus, our congruence criterion is met for *A. aurea*: we found significant effects for all of the variables involved in our hypothesis in at least one of the two years. Furthermore, stronger evidence was found for an effect through relative density (Fig. 1b) than through absolute density and pollinator visit frequency (Fig. 1a). Therefore, we

take these results as evidence for indirect effects of cattle on *A. aurea* pollination and reproduction.

A closer look at Alstroemeria aurea

Are these effects on *A. aurea* really due to the mechanisms hypothesized in Fig. 1? Do cattle truly affect the population density of *A. aurea*, and does population density affect pollination and reproductive success? To

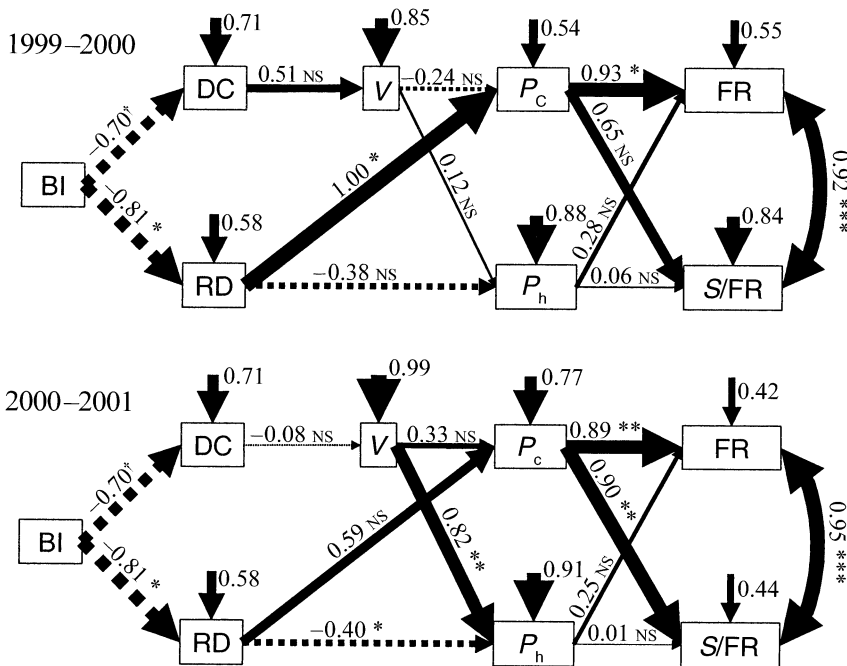


FIG. 11. Among-site path analysis of causal relationship among variables hypothesized to be involved in indirect effects of cattle on *Alstroemeria aurea* pollination and reproduction. One-headed arrows represent direct causal effects; two-headed arrows represent correlational effects. For each effect path, coefficients are given and are also represented by arrow line-thickness. Continuous lines indicate positive effects; dashed lines indicate negative effects. Significance of the path coefficients is indicated as follows: † $P < 0.1$; * $P < 0.05$; ** $P < 0.01$. Data for browsing and density are from the 1999–2000 period and assumed to be the same for 2000–2001 (see *Methods*). See Appendix J for covariance matrix. Variables included in the model are: browsing index (BI); absolute (DC) and relative (RD) plant population density; pollinator visitation frequency (V); conspecific (P_c) and heterospecific (P_h) pollen deposition; fruits per flower (FR); and seeds per fruit (S/FR). Unexplained variability is indicated with vertical arrows above each endogenous variable.

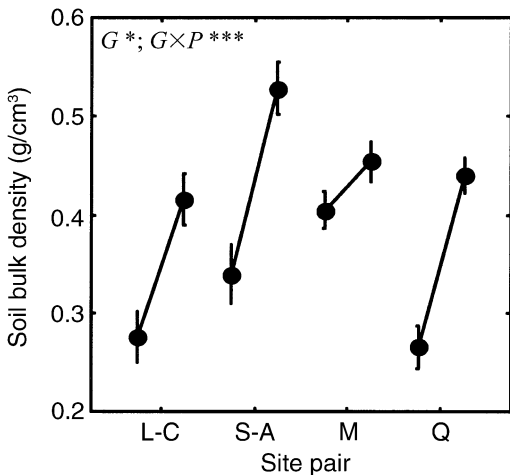


FIG. 12. Mean soil dry mass in grazed and ungrazed sites ($n = 30$ samples/site); error bars represent ± 1 SE. Mixed-model ANOVA results are: main fixed effect (grazing), $F_{1,3} = 19.0$, $P = 0.0223$; interaction effect (grazing \times pair), $F_{3,232} = 3.90$, $P = 0.0096$. Other conventions are as in Fig. 6.

further evaluate this question, we first present a path analysis summarizing the relationships among the different variables among sites, and then the results of further studies to test the hypothesized mechanisms involved in the indirect effect of cattle on *A. aurea* pollination and reproduction.

Explaining intersite variation in pollination and reproduction of A. aurea: among-site path analysis.—If the hypothesized causal relationships among cattle trampling, population density, pollination, and reproduction obtain, among-site variability of these variables should be explained by the causal scheme proposed in Fig. 1. We used path analysis as a heuristic tool to summarize relationships among means of these variables per site.

Browsing index (a measure of the overall impact of cattle) has a significant, positive effect on mean absolute population density per site and a negative effect

on relative density (Fig. 11). In turn, relative density affects conspecific pollen deposition positively and heterospecific pollen deposition negatively (although these effects were significant in only one season). In turn, conspecific pollen deposition positively affects the two reproductive variables; conversely, the effect of heterospecific pollen on reproduction is weak and nonsignificant (Fig. 11). This model is consistent with an effect of cattle on pollination and reproduction through relative population density (Fig. 1b), but not through absolute population density and pollinator visitation (Fig. 1a).

Direct effect of cattle on the population density of A. aurea.—A requirement of our hypothesis is that cattle directly reduce the population density of *A. aurea*. *A. aurea* is indeed consistently sparser in grazed sites. How does the effect of cattle on *A. aurea* arise? *A. aurea* is virtually unbrowsed by cattle (Table 2). However, observational evidence indicated that *A. aurea* is highly susceptible to trampling (D. P. Vázquez, *personal observation*). Furthermore, soil dry mass per unit volume (an indirect measure of trampling intensity) is significantly higher in grazed than in ungrazed sites (Fig. 12). This finding led us to formulate the following prediction: if trampling leads to decreased density of *A. aurea*, then simulating this effect should also lead to decreased density owing to increased ramet mortality. To test this prediction, we conducted trampling experiments as previously described (see *Methods*). There was significantly higher ramet mortality in trampled plots ($67.9 \pm 9.2\%$, mean ± 1 SD) compared to control (untrampled) plots ($12.3 \pm 3.8\%$; $t_{0.05(2),3} = 10.64$; $P = 0.0018$). Taken together, this evidence supports the hypothesis that cattle trampling can directly decrease the population density of *A. aurea*.

Pollen limitation.—Receiving fewer pollen grains can lead to lower reproductive success if reproduction is pollen-limited. Thus, increasing pollen loads in *A. aurea* populations in grazed sites should lead to higher reproductive success. Our hand-pollination experi-

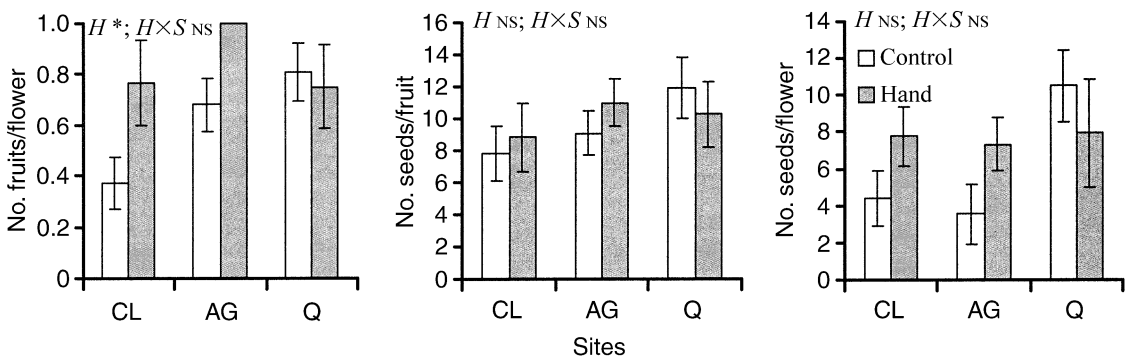


FIG. 13. Results of hand-pollination experiments on fruit set (mean ± 1 SE) in *Alstroemeria aurea*. Sites are: CL, Cerro López; AG, Arroyo Goye; Q, Quetrihué. Statistical significance of ANOVA is indicated for the pollination type (H) and the interaction between pollination type and site ($H \times S$; see Appendix H for the ANOVA table). Statistical significance is indicated as: $*P < 0.05$; NS, $P \geq 0.05$.

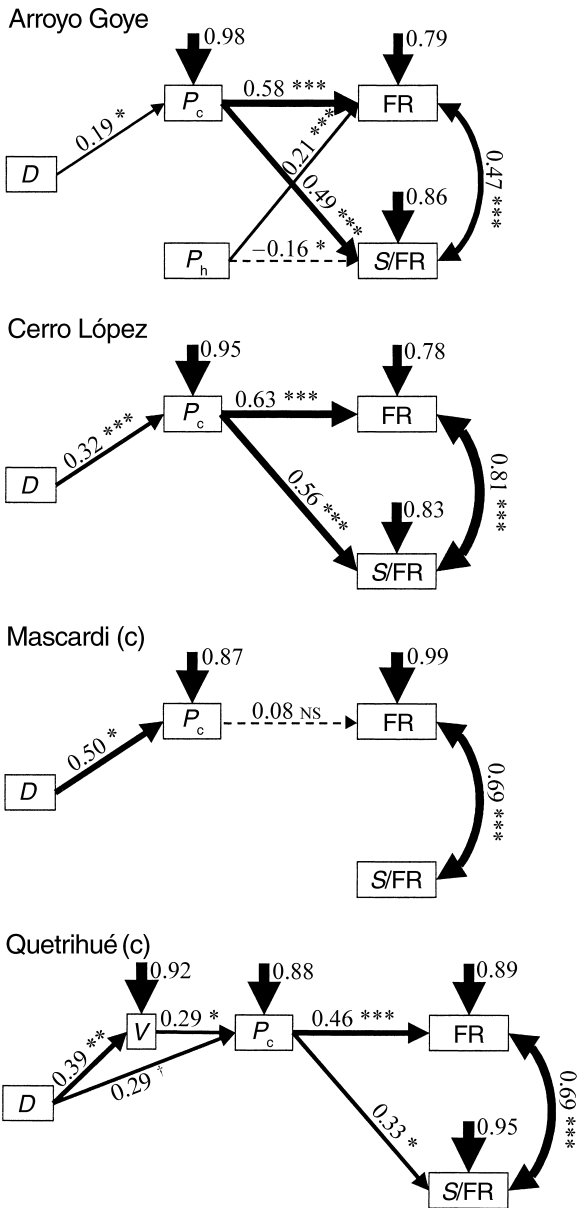


FIG. 14. Within-site path analysis of the effects of floral neighborhood density on pollinator visit frequency, pollination, and reproduction of *Alstroemeria aurea*. Best-fitting structural equations models are given for four grazed sites. Arrows represent directional effects; numbers above arrows represent the magnitude of the path coefficients, also represented by line thickness. Continuous lines indicate positive effects; dashed lines indicate negative effects. All flowering ramets within each site were included. Variables are: estimate of floral neighborhood density (D ; see Appendix B); pollinator visitation frequency (V); conspecific (P_c) and heterospecific (P_h) pollen deposition; fruits per flower (FR); and seeds per fruit (S/FR). P_c and P_h were log-transformed. Statistical significance of path coefficients is indicated as follows: † $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. See Appendix I for fit statistics of full and best-fitting models and Appendix K for observed covariance matrices.

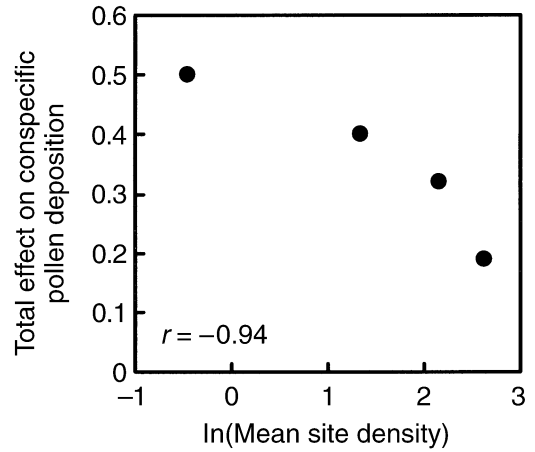


FIG. 15. Relationship between the total effect of floral neighborhood density and site density on conspecific pollen deposition in *A. aurea*, calculated from path analyses (see Fig. 14). Sites are, from right to left, Mascardi (c), Quetrihué (c), Cerro López, and Arroyo Goye.

ments provide some support for this prediction. Hand-pollination resulted in significantly higher fruit set compared to open-pollinated ramets; however, no significant effects were observed on the number of seeds per fruit and seeds per flower (Fig. 13; Appendix H).

Effect of floral neighborhood density on pollination and reproduction: within-site path analysis.—In the four grazed sites, the full path model (Fig. 5) contained nonsignificant paths, and in three sites (the exception being Arroyo Goye) the model did not provide a good fit for the observed covariance matrix (Appendix I). We removed the nonsignificant paths and tested the fit of the reduced models again. Such model simplification substantially improved the fit between predicted and observed covariance structures in all sites except Arroyo Goye, where the fit of the two models did not differ significantly (Appendix I). However, it is important to note that the NFI calculated for the simplified model in Mascardi was substantially lower than the recommended 0.9 (Appendix I).

Although details of the simplified models varied among sites, they had several common features (Fig. 14). First, in all cases there was a direct, significant effect of neighborhood density on conspecific pollen deposition; although the strength of this effect differed among sites, the amount of unexplained variability of pollen deposition was generally high. Second, pollinator visit frequency did not significantly affect pollen deposition in three of the four sites; the exception was Quetrihué, where neighborhood density significantly affected conspecific pollen deposition both directly and indirectly through pollinator visitation. Third, conspecific pollen deposition significantly affected both reproductive variables in all sites except Mascardi. Fourth, in only one case (number of fruits per flower in Arroyo Goye) did heterospecific pollen deposition have a significant, negative effect on reproduction; in

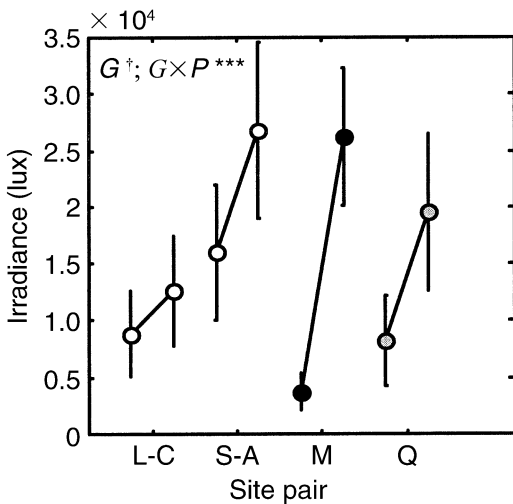


FIG. 16. Irradiance (mean \pm 1 SE) reaching the ground in the understory of each of eight sites. Results of mixed-model ANOVA are as follows: fixed effect of grazing, $F_{1,3} = 7.35$, $P = 0.0731$; grazing \times pair interaction, $F_{3,232} = 3.94$, $P = 0.0091$. Other conventions are as in Fig. 6.

the remaining cases, the effect was either nonsignificant or positive (the latter case was probably a spurious effect resulting from the correlation between conspecific and heterospecific pollen deposition). It is noteworthy that even though the reduced-path models did not include heterospecific pollen, in the full model this variable was always negatively, albeit nonsignificantly, affected by neighborhood density (not shown). Finally, the two reproductive variables were positively correlated. In summary, results in the four sites suggest there is a significant effect of density on conspecific pollen deposition, and this effect is translated into a significant effect on reproduction in three of the four sites. Results are generally more consistent with an effect through relative density (Fig. 1b) than through absolute density and pollinator visit frequency (Fig. 1a).

An important point in the above results is that the magnitude of the total effect of density on pollen deposition decreased with overall site density (Fig. 15). In three cases (Arroyo Goye, Cerro López, and Mascardi), the path structure does not include indirect effects of density on conspecific pollen deposition, so the total effect is simply the direct effect of density on conspecific pollen deposition. In the fourth site (Quetrihué), total effect does involve an indirect effect, calculated as the sum of that direct effect plus the sum of the product of the effects on density of visits and of visits on pollen deposition in Quetrihué (Mitchell 2001). This effect was strongest in Mascardi, the site with the lowest site density, and was lowest in Arroyo Goye, the site with the highest density.

DISCUSSION

Effects of cattle on plant population density

Our hypothesis (see Fig. 1) requires that cattle affect (either negatively or positively) plant absolute or rel-

ative population density. The population density of six of the 11 species showed no association with the presence of cattle. Thus, the first part of the mechanism hypothesized to link cattle indirectly with pollination and plant reproduction is not observed for these species. For only five species did we find significant effects of cattle on absolute population density, and for only three of these five species did we also find significant effects on relative density.

How do cattle affect absolute population density of these five species? Two species, *Aristotelia chilensis* and *Ribes magellanicum*, were heavily browsed (Table 2), and this direct effect is the more parsimonious explanation for their decreased abundance in grazed sites. Dimitri (1962), Veblen et al. (1989, 1992), and Relva and Caldiz (1998) previously observed heavy browsing on these species.

Browsing appeared to be less important for the remaining affected species. Browsing on *Cynanchum diemii* seemed to be not as strong as on *A. chilensis* and *R. magellanicum*, and there was no browsing in one grazed site (Table 2), even though density was lower than in the paired ungrazed site. Thus, we have no clear explanation for the decline of these species in the presence of cattle.

Alstroemeria aurea was virtually unbrowsed by cattle (Table 2), but, as our experiments demonstrate, trampling negatively affects ramet survival. However, our experiments do not allow us to evaluate the alternative effect that could result directly from soil compaction. An evaluation of such an effect would require experiments in which individual plants are grown under different levels of artificially manipulated soil compaction; logistic constraints prevented us from conducting such experiments.

Only *Berberis buxifolia* had higher density in grazed than in ungrazed sites. The abundance of this species increases despite heavy browsing (Table 2). A similar effect has been observed in other studies (Veblen et al. 1989, 1992, De Pietri 1992, Relva and Veblen 1998), although Raffaele and Veblen (2001) found the opposite effect in postfire *Austrocedrus chilensis*–*Maytenus boaria* matorral, perhaps because grazing can inhibit recolonization by *B. buxifolia* after fire. An explanation of the increase of this species with grazing may be that low browsing levels increase growth rates, as has been observed for some other species (Belsky 1986, Agrawal 2000). However, higher growth may also occur because of reduced competition for light in grazed areas. Light availability tends to be lower in the understory of ungrazed sites (Fig. 16). *B. buxifolia* is usually found in forest gaps and seems unable to reproduce in the shady understory of ungrazed *Nothofagus dombeyi* forests: virtually no *B. buxifolia* individuals produced flowers in ungrazed sites, and those that did had extremely low fruit and seed set, whereas flowering individuals were present in all grazed sites, and most reproduced successfully.

An important point about the response of plant species density to cattle is that in most cases there was a significant interaction between grazing and geographic location. For the five affected species, the effect of cattle was always in the same direction for all pairs of sites, but the magnitude of the effect varied among pairs (i.e., a “synergistic” interaction). A possible explanation for the existence of this interaction is that at low population densities individual plants are more difficult for cattle to find (or to trample), and thus the per capita impact of cattle on a susceptible species would be stronger in areas where the species naturally occurs at high densities. For example, absolute density of *A. aurea* in the ungrazed sites at Mascardi and Quetrihué is lower than at Safariland or Llao Llao; we can therefore expect that the effect of cattle in the former two sites is weaker than in the latter, an expectation supported by the data (Fig. 6). A similar argument can be made for *C. diemii* and *R. magellanicum*.

Contrary to previous studies (Ramírez et al. 1981, Veblen et al. 1989, 1992), we found no positive effect of cattle on abundance of *Berberis darwinii*. In our study sites, *B. darwinii* was actually more abundant in ungrazed sites of three of the four pairs, but the overall difference was not significant. *B. darwinii* reproduces successfully under the canopy, and, although it showed a trend to decreased reproductive performance in ungrazed sites, that trend was not significant. Studies of *B. darwinii* in its introduced range in New Zealand, where it escaped in 1946 from a garden, also suggest that this species is highly shade-tolerant (Allen 1991).

Also surprising was the lack of invasion by *Rosa eglanteria* in grazed sites. This spiny shrub commonly invades open areas (Damascos and Gallopin 1992), where it usually forms large thickets virtually un browsed by cattle (see Table 2). However, we found no significant effect of cattle on *R. eglanteria* abundance, perhaps because we worked under the tree canopy to avoid the confounding effect of tree cover with that of cattle. Thickets of this species were more common in open areas of grazed sites than in similar areas of ungrazed sites (D. P. Vázquez, *personal observation*). Thus, once the canopy is removed, grazing may indeed enhance invasion by this species.

Indirect effects of cattle on pollination and plant reproduction

Lack of effect in most species: possible explanations.—Cattle seem to have no indirect effects on pollination and reproduction of most plant species in our study. Only for the herb *Alstroemeria aurea* did we find evidence for such effects.

One possible reason why we found no indirect effects for six species is that they showed no association with cattle; thus, the first part of the hypothesized mechanism (see Fig. 1) is not met. Furthermore, we could not test for indirect effects on pollination and reproduction of *B. buxifolia*, because this species failed to

flower in ungrazed sites. This left four species with population density lower in grazed than in ungrazed sites and for which we could compare pollinator visitation, pollination, and reproduction between grazed and ungrazed sites.

Another factor that may have contributed to the lack of effects for these species is that sample sizes per site were considerably smaller than for *A. aurea* (Appendix C), which we know decreases the statistical power of the analyses (Fig. 4). Smaller sample sizes were obtained for these species because they were substantially rarer than *A. aurea*.

Among the species for which we did observe an effect on population density, the most striking lack of indirect effects involves *Aristotelia chilensis*. This species showed the strongest decline in response to cattle. It is dioecious, which should exacerbate Allee effects resulting from low population density (House 1992, 1993, Kunin 1997a). Furthermore, it is pollinated almost exclusively by a single bee species, the colletid *Cadeguala albopilosa*. However, this bee is not restricted to *A. chilensis*: only ~60% of visits in our sites were to *A. chilensis*, whereas the remaining 40% were to *Schinus patagonicus* (Vázquez 2002a). *C. albopilosa* was also recorded visiting flowers of seven species in Chiloé Island, Chile (Aizen et al. 2002). Although at low population densities specialization on a generalist pollinator may be beneficial (because it increases the likelihood of receiving visits if other, coflowering species can support the pollinator population), it can also be problematic because of the increased probability of improper pollen transfer (Rathcke 1983, Kunin 1993, Kunin and Iwasa 1996). However, *C. albopilosa* behaved as a specialist during the second year of study, because *S. patagonicus* failed to flower that year; the change from generalist to specialist behavior of *A. chilensis*' main pollinator made no difference for its reproductive success. We can thus provide no good explanation (besides lack of power) for the lack of indirect effects of cattle on pollination and reproduction of this species.

Also striking is the apparent lack of indirect effects on pollination and reproduction of *Cynanchum diemii* and *Ribes magellanicum*, in spite of their decreased density in grazed sites. There is no obvious explanation. A possibility for *C. diemii* is that the natural low reproductive output of this species (a characteristic of most species in the Asclepiadaceae; Wyatt and Broyles 1994) makes it harder to detect differences between grazed and ungrazed sites.

Indirect effects of cattle on the pollination and reproduction of A. aurea.—Our results suggest that cattle decrease the population density of *A. aurea* through trampling and that this decreased abundance leads to decreased pollination and reproductive performance.

What specific mechanism leads to this apparent indirect effect? In particular, which of the two chains of events proposed in Fig. 1 has the stronger support? Our

comparisons between grazed and ungrazed sites and the among-site path analysis suggest that both relative and absolute density of *A. aurea* are negatively affected by cattle. However, these analyses also suggest that lower absolute density does not result in a detectable decrease of pollinator visit frequency, nor do changes in pollinator visit frequency significantly affect pollen deposition. Thus, these results provide little support for the mechanism hypothesized in Fig. 1a. Conversely, the comparisons between paired sites and the among-site path analysis suggest that the significant, negative effect of cattle on relative population density translates into a significant decrease of conspecific pollen deposition, a significant increase in heterospecific pollen deposition, and a decrease in the relative proportion of conspecific pollen. These results are thus consistent with the mechanism hypothesized in Fig. 1b.

The results of the within-site path analysis are also generally consistent with the mechanism proposed in Fig. 1b. Although *D*, our measure of neighborhood density, does not allow us to separate relative and absolute density, in three of the four sites *D* affected pollen deposition directly and not indirectly through pollinator visitation. In Quetrihué, however, *D* affected pollen deposition both directly and indirectly through pollinator visitation, which suggests that in this population both chains of effects may have been important. As we have pointed out, it is noteworthy that, even though the reduced-path models did not include heterospecific pollen, in the full model this variable was always negatively, albeit nonsignificantly, affected by *D*, which is also consistent with the mechanism proposed in Fig. 1b, but not with that proposed in Fig. 1a.

An effect through relative population density requires that the number of conspecific pollen grains and their proportion relative to heterospecific pollen grains deposited by pollinators per visit decrease with relative density. Such an effect is expected to be greater when pollinators are generalists (Rathcke 1983, Kunin 1993), which is true for the main pollinator of *A. aurea*, *Bombus dahlbomii* (Vázquez and Simberloff 2002). Although our results are consistent with this hypothesis, we are unable to demonstrate that pollinators are, in fact, bringing fewer conspecific pollen grains per visit. Such a demonstration would require quantifying pollen deposition by individual pollinators in each site.

An alternative explanation for these findings is that high error variance for pollinator visit frequency makes it impossible to detect an effect of density on visit frequency, and of visit frequency on pollen deposition, even if such effects exist (e.g., compare mean and standard deviation for visit frequency and for other variables in Appendix K). Although such a scenario is certainly possible, we cannot test it with the available data. Thus, we must accept an effect through relative density as the best explanation of observed patterns.

Another necessary condition for the hypothesized mechanism is that *A. aurea* be pollen-limited. If it is,

then lower pollen receipt should result in lower reproductive success. In our among-site path analysis, there was a positive effect of conspecific pollen deposition on fruits per flower and seeds per fruit, although this effect was not significant for seeds per fruit and seeds per flower in 1999–2000. The same positive effect of conspecific pollen loads on reproduction is observed in our within-site path analysis in three of the four grazed sites. Hand-pollination results also suggest that pollen deposition could be limiting reproduction, at least fruit set.

Our finding that population density affects pollination and reproductive success of *A. aurea* contrasts with the results of Aizen (1997) in the same region. Aizen analyzed the impact of local floral neighborhood density on pollination and reproduction of *A. aurea* in three populations in Nahuel Huapi National Park. Our study involved analyses of the effect of density at two different geographic scales: comparisons between means per site (a scale of 5–12 ha), and studies of floral neighborhood at smaller scales (meters). The latter scale is comparable to that of Aizen's study, yet our results differ strikingly: whereas Aizen failed to find significant effects of density on pollination and reproduction, we found significant effects of density on conspecific pollen deposition, and of conspecific pollen deposition on reproduction. We offer two possible explanations for this apparent contradiction. First, Aizen's measure of floral neighborhood density differed from ours. Aizen used nearest neighbor distances (distance to the nearest neighbor, distance to the fifth nearest neighbor, and the geometric mean of the distances to the five nearest neighbors), whereas we used the square roots of the actual densities in concentric bands around the focal ramet. As we show in Appendix B, our measure is more likely to detect contrasting densities, especially for populations with a clumped spatial distribution.

Our second explanation of the contrasting results between our and Aizen's (1997) studies has to do with the scale at which pollinators respond to variations in floral neighborhood density. Some pollinators respond to large-scale variations in floral density but not variation at smaller scales. For example, Thomson (1981) found that bumblebees respond to variation in floral density in patches of >500 m² but not in smaller patches. As we have noted, bumblebees are the main pollinators of *A. aurea*. Can this scale variance in bumblebee perception of floral density explain the contradiction between Aizen's and our results? The overall densities at each of Aizen's (1997) sites seem to be higher than the densities at our grazed sites, as can be inferred from the range of nearest neighbor distances that he observed. In Aizen's study, distance to the nearest neighbor varied between 0.1–1.1 m in the site with the highest density (Challhuaco in 1993) and 0.1–15 m in the site with the lowest density (Cerro Otto). In contrast, distance to the nearest neighbor varied between 0.05–19 m in Arroyo Goye and 0.01–53 m in

Quettrihué. As we have shown (Fig. 15), the magnitude of the effect of neighborhood density on conspecific pollen deposition decreases with increasing overall site density. Thus, it is in the grazed site with the lowest mean density (Mascardi) that the relationship between neighborhood density and conspecific pollen deposition is strongest, and it is in the grazed site with the highest mean density (Arroyo Goye) that this relationship is weakest. We propose that the effect of local floral density on pollination and reproduction depends on the larger scale density at entire sites and that only at low overall densities will floral neighborhood density affect pollination and reproduction.

Although our results support the hypothesis that cattle indirectly affect pollination and reproduction of *A. aurea*, our data do not allow us to assess the relative importance of direct and indirect effects in causing the decline of this species. Evaluating the relative importance of direct and indirect effects is important for a broader understanding of population dynamics of *A. aurea*, in particular, and of flowering plants in general. A possible scenario is that decreased pollination and reproductive performance are simply by-products of the direct effect of cattle, with no added input to the population decline. Alternatively, there could be a positive feedback between population density and pollination and reproductive performance, thus accelerating population decline. Our results showing that the effect of density on pollination is stronger when overall site density is extremely low suggest that such a scenario is possible. However, an assessment of the relative importance of direct and indirect effects would require other kinds of information currently not available for this species, particularly on demography.

Conservation implications

Previous studies of the effects of introduced ungulates in the southern Andean temperate forest have dealt mainly with direct effects on forest dynamics and understory vegetation. These studies, along with the results presented here, have shown that introduced ungulates have important effects on some understory species (Vázquez 2002b). Our study demonstrates that subtler indirect impacts can also occur.

Although our study dealt only with the impact of cattle, it is likely that other introduced ungulates (particularly deer) have similar effects. For example, on Isla Victoria, where introduced deer have important impacts on the forest understory vegetation (Veblen et al. 1989), *A. aurea* is also extremely rare, restricted to areas relatively inaccessible to deer (e.g., among fallen trees; D. P. Vázquez and D. Simberloff, *personal observation*). Likewise, the observed effects on *A. aurea* could be occurring beyond the limited geographic range of this study, because introduced ungulates are widespread in the region (Navas 1987, Bonino 1995, Jaksic 1998, Jaksic et al. 2002).

Effects of introduced ungulates could cascade to other species in the system. For example, animal pollinators could be affected, either by the decreased abundance of their floral resources or by the direct effect of trampling (Sudgen 1985). Some plant species negatively affected by introduced ungulates are likely to be important resources for the guild of flower-visiting insects. *A. aurea* seems to be particularly important: it is the species in our study with the highest number of flower visitors (Aizen et al. 2002, Vázquez and Simberloff 2002). *A. aurea* is virtually the only insect-pollinated plant flowering in the summer in the *Nothofagus dombeyi* forest, and many insect species recorded visiting *A. aurea* seem not to visit other species. Thus, the decreased abundance of *A. aurea* in grazed sites could negatively affect the assemblage of flower visitors.

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APPENDIX A

STUDY OF SELF-COMPATIBILITY AND DEPENDENCE ON POLLINATORS FOR REPRODUCTION

Self-compatibility and dependence were studied in *Berberis darwinii*, *Gavilea odoratissima*, *Ribes magellanicum*, *Schinus patagonicus*, and *Vicia nigricans*. Because *S. patagonicus* is dioecious, no pollen additions were made to study self-compatibility. We could not study self-compatibility and dependence on pollinators for *C. diemii* because we were unable to insert pollinia in the stigmatic surfaces.

Plants selected for experiments were tagged and the following treatments were applied: (1) unbagged flowers with no pollen addition (natural); (2) bagged flowers with no pollen addition (bagged, no pollen); (3) bagged flowers with self hand-pollination (bag, self); and (4) bagged flowers with cross hand-pollination (bag, cross). For *Schinus patagonicus*, no bagged flowers were hand-pollinated. Bags were removed after flower senescence to minimize the negative effect of bagging on fruit development. Proportion of fruit set was the reproductive variable used for comparisons. We always applied treatments to branches in different individuals to minimize the effect of resource reallocation between branches.

B. darwinii had the same proportion of fruits per tagged flower in the natural and cross-pollinated treatments (Table A1). The proportion of fruit set by self-pollinated flowers was lower than for natural- and cross-pollinated flowers, but still higher than for bagged flowers with no pollen addition, indicating a relatively high degree of self-incompatibility and dependence on pollinators for reproduction (Riveros et al. 1996).

G. odoratissima had low fruit set in the naturally pollinated flowers (Table A1). However, fruit set for these flowers was

still over four times higher than for bagged flowers with no pollen addition. Conversely, bagged flowers with either self- or crossed-hand-pollination had high fruit sets. Fruit set for self-pollinated flowers was higher than for cross-pollinated flowers. Thus, our results indicate a high dependence on pollinators for reproduction and high degree of self-compatibility.

R. magellanicum produced no fruits in any bagged treatment. This result indicates a negative effect of bagging on reproduction. These results cannot be used to infer the degree of self-compatibility and the degree of dependence on pollinators. Riveros (1996) mentions this species as being dioecious. This observation is incorrect, because this species develops hermaphroditic flowers with functional gynoecea and androecea.

Bagged flowers in *S. patagonicus* produced almost three times fewer fruits than naturally pollinated flowers. This species is dioecious, and thus this result suggests dependence on pollinators (i.e., absence of agamospermy). However, this lower fruit production in bagged flowers could also be due, in part, to an effect of bagging, as described in *R. magellanicum*. Thus, our results for this species should be interpreted with caution.

Finally, self- and cross-pollinated bagged flowers of *Vicia nigricans* had similar fruit sets, producing a higher fruit set than bagged flowers with no pollen addition but still lower than the naturally pollinated flowers. Thus, our results indicate a high degree of self-compatibility, a high degree of dependence on pollinators for reproduction, and a negative effect of bagging on reproduction in this species.

TABLE A1. Results of hand-pollination experiments to study the self-compatibility of some species included in the study.

Species	Natural			Bag, no pollen			Bag, self			Bag, cross		
	Mean	1 SE	<i>n</i>	Mean	1 SE	<i>n</i>	Mean	1 SE	<i>n</i>	Mean	1 SE	<i>n</i>
<i>Berberis darwinii</i>	0.59	0.05	51	0.15	0.04	27	0.24	0.06	19	0.59	0.05	27
<i>Gavilea odoratissima</i>	0.09	0.02	72	0.02	0.02	18	0.84	0.06	15	0.67	0.10	15
<i>Ribes magellanicum</i>	0.10	0.05	26	0.00	0.00	5	0.00	0.00	4	0.00	0.00	4
<i>Schinus patagonicus</i> †	0.59	0.04	56	0.21	0.04	11						
<i>Vicia nigricans</i>	0.35	0.02	108	0.01	0.01	25	0.14	0.04	15	0.15	0.09	10

Note: Treatments were: unbagged flowers with no pollen addition (natural); bagged flowers with no pollen addition (bagged, no pollen); bagged flowers with self-hand-pollination (bag, self); and bagged flowers crossed-hand-pollination (bag, cross).

† For this species, no bagged flowers were hand-pollinated.

APPENDIX B

CALCULATION OF FLORAL NEIGHBORHOOD DENSITY

We used an algorithm implemented in Matlab to calculate the density of the floral neighborhood around each ramet in a given population from maps of flowering ramets of *Alstroemeria aurea*. For each flowering ramet, our algorithm added the square root of the density of flowering neighbors in concentric bands of 1 m width at increasing distances of 1 m from the ramet, according to the following equation:

$$D = \sum_{i=1}^x \sqrt{r_i/a_i} \quad (\text{B.1})$$

where D estimates density of flowering neighbors within radius x from the ramet, r_i is the number of ramets in band i , and a_i is the area of band i . The area of each band, defined by two concentric circles of radii i and $i - 1$, is given by a_i

$= \pi[i^2 - (i - 1)^2]$, which simplifies to $a_i = \pi(2i - 1)$; thus, the area of each band increases linearly with distance to the center of the circle.

Several properties of D are worth noting. Because area increases with distance to the center, less weight is given to each individual ramet as radius increases. In a randomly distributed population, density of neighbors is expected to increase proportionally to band area as distance increases, and thus r_i/a_i should remain constant. In contrast, in an aggregated distribution, density of neighbors at short distances is expected to be high and to decrease gradually with increasing density.

In the patchily distributed *A. aurea*, density dropped rapidly after a few meters. Therefore, we chose $x = 20$ m, because

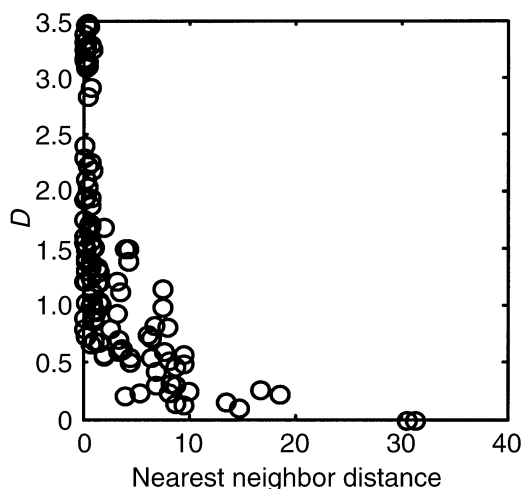


FIG. B1. D , estimate of floral neighborhood density vs. distance to the nearest neighbor for *A. aurea* at Cerro López.

densities at greater distances from the focal ramet were extremely low and did not change the value of D significantly. To avoid underestimating density of neighbors in the edges of the distribution of individual ramets, we used a "buffer zone" in our distributional maps. Individuals in the buffer zones were used to estimate densities but not as focal individuals for the analysis (see Dale 1999).

Our method for estimating neighborhood density resembles that of Galiano (1982). It has the disadvantage of being rather computationally intensive and requiring detailed maps of the location of each flowering individual. However, it has an advantage over more traditional nearest neighbor distance methods in that it allows a finer resolution at high and moderate densities, especially for species with clumped distributions. Under a highly clumped distribution, most individuals will have a close neighbor, yet neighborhood densities may differ markedly. Thus, the use of nearest neighbor estimates will probably not detect this density difference. An example is provided in Fig. B1. We plotted D (obtained from Eq. B.1) vs. distance to nearest neighbor for each flowering ramet of *A. aurea* in the Cerro López population. Most ramets in this population have their nearest neighbor at short distances (2–3 m); however, the densities of their floral neighborhoods, as estimated by D , show a great spread. Thus, using nearest neighbor distance as a surrogate for density would have led to assuming wrongly that very different densities were similar.

APPENDIX C

Sample sizes per site for pollinator visit frequency, pollen deposition, and reproduction for the four species that were significantly less dense in grazed sites are available in ESA's Electronic Data Archive: *Ecological Archives* M074-006-A1.

APPENDIX D

The ANOVA table of mixed-model analysis of the effect of cattle on absolute and relative population density is available in ESA's Electronic Data Archive: *Ecological Archives* M074-006-A2.

APPENDIX E

The ANOVA table of mixed-model analysis of the effect of cattle on pollinator visit frequency for four species whose density was significantly lower in grazed sites is available in ESA's Electronic Data Archive: *Ecological Archives* M074-006-A3.

APPENDIX F

The ANOVA table of mixed-model analysis of the effect of cattle on pollen deposition for four species whose density was significantly lower in grazed sites is available in ESA's Electronic Data Archive: *Ecological Archives* M074-006-A4.

APPENDIX G

The ANOVA table of mixed-model analysis of the effect of cattle on plant reproduction for four species whose density was significantly lower in grazed sites is available in ESA's Electronic Data Archive: *Ecological Archives* M074-006-A5.

APPENDIX H

The ANOVA table for pollination experiments is available in ESA's Electronic Data Archive: *Ecological Archives* M074-006-A6.

APPENDIX I

Fit statistics for structural equation models used to study relationship between floral neighborhood density, pollinator visitation, pollination, and reproduction of *Alstroemeria aurea* in grazed sites are available in ESA's Electronic Data Archive: *Ecological Archives* M074-006-A7.

APPENDIX J

Observed covariance matrices used for among-site path analysis of *Alstroemeria aurea* are available in ESA's Electronic Data Archive: *Ecological Archives* M074-006-A8.

APPENDIX K

Observed covariance matrices used for within-site path analysis of effect of floral neighborhood density on pollinator visitation, pollination, and reproduction of *Alstroemeria aurea* in grazed sites are available in ESA's Electronic Data Archive: *Ecological Archives* M074-006-A9.