Node-by-node disassembly of a mutualistic interaction web driven by species introductions

Mariano A. Rodriguez-Cabala,b,1, M. Noelia Barrios-Gacraa,b, Guillermo C. Amico, Marcelo A. Aizenc, and Nathan J. Sandersa,d

Interaction webs summarize the diverse interactions among species in communities. The addition or loss of particular species and the alteration of key interactions can lead to the disassembly of the entire interaction web, although the nontrophic effects of species loss on interaction webs are poorly understood. We took advantage of ongoing invasions by a suite of exotic species to examine their impact in terms of the disassembly of an interaction web in Patagonia, Argentina. We found that the reduction of one species (a host of a keystone mistletoe species) resulted in diverse indirect effects that led to the disassembly of an interaction web through the loss of the mistletoe, two key seed-dispersers (a marsupial and a bird), and a pollinator (hummingbird). Our results demonstrate that the gains and losses of species are both consequences and drivers of global change that can lead to underappreciated cascading coextinctions through the disruption of mutualisms.

Significance

Examining the effects of species gains and losses is fundamental to understanding the assembly and disassembly of ecological communities in a changing world. However, field-based empirical studies that demonstrate the disassembly of mutualistic webs are exceedingly rare. In this study, we take advantage of an ongoing natural experiment that links the gain of invasive species (introduced large mammals and wasps), the loss of a native keystone species (a mistletoe), and the ensuing node-by-node disassembly of an interaction web in Patagonia, Argentina. Our results demonstrate that the gains and losses of species are both consequences and drivers of global change that can lead to underappreciated cascading coextinctions through the disruption of mutualisms.

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1To whom correspondence should be addressed. E-mail: rcabal@zoology.ubc.ca.

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alteration of the behavior of any of these species has the potential to disassemble this interaction web.

**Results and Discussion**

Cows (Bos taurus) were introduced to this system in the late 18th century (28), and game animals, such as red deer (Cervus elaphus) and fallow deer (Dama dama), were introduced in 1904 (29). These species are widespread (occupying >50% of the forests in Patagonia) and are significant sources of disturbance in these temperate forests (28, 30, 31). Exotic herbivores are known to modify native plant communities by directly affecting plant survival, growth, reproduction, and recruitment through browsing, grazing, and trampling, and indirectly by altering nutrient cycles, primary productivity, disturbance regimens, and by disrupting interactions with other herbivores, pollinators, and seed dispersers (32–34).

*A. chilensis* is a preferred forage for both deer and cows (28, 30, 35). As a result, *A. chilensis* is directly affected by the presence of exotic ungulates: the density of *A. chilensis* was 16-times lower in sites where these exotic species are present (hereafter, “invaded” sites) than where the species are absent (hereafter, “intact” sites) (Fig. 2C). We excluded deer for 1 mo and found that 99% of *A. chilensis* leaves outside of the deer exclosures were removed by deer (Fig. 2B) in invaded sites. Mistletoe plants appear to be specialists on *A. chilensis* (16): more than 75% of the plants parasitized by mistletoe are *A. chilensis*. Indeed, the density of mistletoes was 83-times lower in invaded sites than in intact sites (Fig. 2C). Thus, by preferentially browsing on *A. chilensis*, exotic ungulates may indirectly affect the persistence of the keystone mistletoe (30), on which many other species rely, thereby indirectly disassembling this interaction web.

In fact, the ungulates affected more than just *A. chilensis*; they affected the entire plant community (28). Total plant cover in the understory was 35-times lower (*t*<sub>25</sub> = 7.63, *P* < 0.0001) and habitat complexity was 20-times lower (*t*<sub>25</sub> = 13.99, *P* < 0.0001) in invaded than in intact sites. Habitat characteristics, such as the abundance and diversity of food resources, nest sites, and distance to shelter can influence the distribution of many vertebrate frugivores (36), and in turn fruit-bearing plants. We found that the density of fruiting plants was three-times lower in invaded than in intact sites (Fig. 2D). The changes in habitat structure and food availability also affected the marsupial (25). In 52 trapping nights, we never captured marsupials at any of the invaded sites, although they occurred at each of the 13 sampled intact sites. Because mistletoe seeds must pass through the gut of the marsupial to germinate and be deposited on branches of *A. chilensis* to establish, the local extinction of marsupials undoubtedly affects mistletoe recruitment (37). Indeed, this was the case: we did not detect any mistletoe seed dispersed by marsupials.
The effect size of exotic species ($\ln(N_{W + N_{E}})$) on the different response variables measured in this study. Error bars are 95% confidence intervals. The effect of exotic species is significant if the confidence intervals do not overlap zero (dashed line). Violet indicates indirect effects, red depicts herbivory on $A. \text{chilensis}$ by exotic ungulates, yellow shows the effect of German wasps on fruit removal, and green shows number of fruiting plants dispersed by the marsupial and $E. \text{albiceps}$. Because we used presence/absence data to evaluate the indirect effects of exotic ungulates on the marsupial, we cannot calculate an effect size. However, we did not find marsupials at any of the invaded sites, but marsupials were present in all of the intact sites.
Material and Methods
Study Area and Natural History. We conducted our research in Nahuel Huapi National Park (705,000 ha) and Arrayanes National Park (1,753 ha) in northwestern Patagonia, Argentina. The native forest vegetation in the study area is a temperate subantarctic biogeographic region, which is distinctive from the Neotropical biomes of the rest of South America (42). This temperate forest is known for its high endemic flora and number of plant-animal mutualisms (43). For example, more than 50% of the woody plants produce fleshy fruits and their seeds are dispersed by vertebrates (43).

We selected 26 sites for the study. Exotic ungulates are present at 13 of the sites, 7 have only deer and 6 have cows and deer (invaded sites); the other 13 sites harbor no exotic ungulates (intact sites). Sites were 25 × 25 m and were chosen haphazardly to incorporate the range of habitats found in each treatment. Sites were separated by at least 1–2 km. The most common trees, at all sites, are the evergreen southern beech Nothofagus dombyei and the conifer Austrocedrus chilensis. The understory is dominated by the shrub Aristotelia chilensis and the bamboo Chusquea culeou. The two forest layers are well differentiated, with tree canopy reaching up to 40 m in height and understory reaching up to 5 m in height.

Exotic Ungulates. In the Austral summers of 2010 and 2011, we examined the direct effect of exotic ungulates on A. chilensis and their indirect effects on the interaction between the hummingbird, the mistletoe, and the marsupial. We measured the following set of biotic and physical variables in haphazardly selected sites at each of 60 A. chilensis trees. At each site, we counted the number of contacts with a vertical pole (3-m height) for habitat structure. As a measure of habitat complexity within each plot, we counted the number of contacts with a vertical pole (3-m height) for branches 5–10 cm in diameter and oriented <45° relative to the ground and number of fruiting plants.

To quantify the effects of browsing by exotic ungulates on A. chilensis, we conducted an enclosure experiment. In 2010, we planted 12 pairs of A. chilensis at three sites, each pair consisting of one A. chilensis fenced with 1.2-m-high woven wire to prevent browsing by exotic ungulates, and the other A. chilensis open to allow browsing (control). The A. chilensis used in the exclusion experiment were generally the same sizes as those that are parasitized by mistletoe in these forests. We counted the number of leaves in each A. chilensis at the beginning of the experiment and after 30 d. Additionally, using standard dendrochronological techniques, we found that the age structure of the potential hosts for the mistletoe differed significantly between intact and invaded sites (Fig. S2).

To facilitate rapid surveys on the indirect effects of exotic ungulates on the presence or absence of the marsupial, the 26 study sites were grouped by presence or absence of mistletoe (see ref. 25 for a detailed explanation). At sites with mistletoe, we concluded that marsupials were present if we found recently dispersed mistletoe seeds (recall that this marsupial is the only seed disperser of mistletoe in these forests). If no dispersed seeds were found at the site, we used trapping to confirm the absence of the marsupials. We also trapped in sites without mistletoe. At each site, we placed a 5 × 5 grid of Tomahawk-style traps –5 m apart. Each trap was placed 1–2 m above ground in the shrub closest to the sample point. Traps were baited with apples and bananas. Because the objective was to document presence, once at least one marsupial was trapped, we stopped trapping at the site; we were not trying to estimate population sizes. We continued trapping for four nights if no marsupials were captured. Based on previous capture data for marsupials in this same system (25), and following procedures in ref. 44, the probability of capture was 99.6% in four nights if marsupials were present. Thus, we are confident that marsupials were absent after four nights of trapping if no marsupials were captured.

Finally, during the Austral winter of 2011 flowering season of the mistletoe (March–November) (21), the number of hummingbirds (visually and acoustically) was recorded using fixed-radius point counts (45) at eight sites. Because of the eruption of the volcano Puyehue-Cordon Caulle in the fall of 2011, we could not use the same set of sites as these were heavily affected by ash fall. Consequently we selected eight new sites. Additionally, because sites with a long history of disturbance by exotic ungulates do not maintain mistletoe populations, we selected four sites in places were exotic ungulates were present but in very low densities. We paired those four sites with sites where exotic ungulate were absent (intact sites). At each site, point counts were conducted during the morning on days without rain and wind. The distance between points was always >300 m. Hummingbirds were counted at three 25-m fixed radius plots during a 10-min period (46).

Exotic Wasps. To evaluate the impact of German wasps on the mutualism between A. chilensis and seed dispersers, we marked 20 immature fruits with small paper tags on each of 60 plants haphazardly chosen in three different treatments in the Arrayanes National Park. Twenty A. chilensis plants were marked in wasp-excluded sites (poison treatment), 20 in invaded sites (nonpoison treatments), and 20 in overabundance sites (protein + sugar treatment). The sites were separated by at least 1 km, because German wasps travel 20 kg above the nest during foraging (47). We reduced the abundance of German wasps using toxic baits (poison treatment) prepared with 0.1% Fipronil mixed with 20 g of raw ground beef (47). The poison treatment was developed to control only German wasps and does not affect the native fauna. The baits were placed in feeding stations made of 500-mL plastic bottles with both ends cut off. To increase the abundance of German wasps locally (protein + sugar treatment), we added baits containing 20 g of fish-scented cat food bait (Whiskas, Kal Kan Foods) (48), 20 g of raw ground beef, and 20 g of honey. In the two treatments, feeding stations were hung between 1 and 2 m above ground in a shrub on a 25 × 25-m grid. Poison and protein + sugar treatments were carried out before the fruits were ripe and maintained throughout the entire fruiting season. To determine the abundance of German wasps, we hung in each of the 60 A. chilensis a 20 × 20-cm sticky trap. At monthly intervals during the fruiting season, we counted the number of German wasps in each sticky trap. Sticky traps were replaced monthly. As a result, the activity of German wasps was significantly lower in plots in which we applied poison treatments (1.95 ± 1.82 mean ± SD per 20 × 20-cm sticky trap) relative to sites where we experimentally increased wasp activity (11.65 ± 5.14 protein + sugar baits treatment), and to unmanipulated sites with natural abundance (7.75 ± 5.54 in nonpoison treatments).

At weekly intervals, we recorded the status of each marked fruit and scored it as a developing fruit, ripe fruit, removed fruit, senescent fruit, and eaten fruit. Eaten fruits are those for which the pulp had been consumed without detaching the fruit/seed from the peduncle. We predicted that the German wasp, by feeding on fruits and being aggressive toward native birds, would reduce the number of fruits removed by seed dispersers. To test the impact of German wasps on frugivorous birds, we conducted a biweekly bird census at intact, invaded, and protein + sugar addition sites.

Overall Direct and Indirect Effect of Exotic Species. We compiled the results of each experiment and observational study conducted here and measured the direct and indirect effect of the exotic ungulates and the German wasp. We estimated the effect of these exotic species as the log-response ratio (ln(N/Ne)) of the mean of the response variable in the presence of the exotic species (N) divided by the mean of the response in the absence of the exotic species (Ne) (49, 50). The effect of exotic species is significant if the confidence intervals do not overlap zero.

Data Analysis. To examine the impacts of these exotic species on a suite of response variables, we log- or square-root transformed the response variables as needed to improve normality and reduce heteroscedasticity (see Table S1 for residual diagnosis tests). When data transformation did not meet the assumptions of normality, we used Wilcoxon signed rank tests for analysis continuous data and generalized linear models using a Poisson distribution with a log-link function for count data with zero-inflation. For clarity, we show the untransformed values in figures. In cases when multiple measurements were taken at a particular site over a period of days or weeks, we used the average of all of the observations as the particular response variable in the analyses. Sites were the unit of replication when we investigated the impacts of exotic ungulates. However, we used individual A. chilensis as replicates only when we examined the relationship between the direct and indirect effect of the exotic ungulates and the German wasp. We estimated the effect of these exotic species as the log-response ratio (ln(N/Ne)) of the mean of the response variable in the presence of the exotic species (N) divided by the mean of the response in the absence of the exotic species (Ne) (49, 50). The effect of exotic species is significant if the confidence intervals do not overlap zero.

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