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Comparative pollinator limitation of two non-native shrubs: do mutualisms influence invasions?

Abstract While interactions between invaders and resident species have received a great deal of attention recently, the role of mutualists in facilitating or constraining invasions is rarely considered. We investigated the reproductive ecology of two closely related, woody legumes, *Cytisus scoparius* (Scotch broom) and *Genista monspessulana* (French broom), invading the same sites. Both species are considered noxious non-native weeds in California, and are considered to be ecologically similar, but *Genista* has much smaller flowers than *Cytisus*. Neither species showed appreciable levels of autogamous selfing. When experimentally self-pollinated, *Genista* demonstrated less depression of fruit set and seed set relative to outcrossed flowers than did *Cytisus*. At two sites on the Marin peninsula, Calif., *Genista* flowers were consistently less likely to be pollinated than *Cytisus* flowers. *Genista* was significantly pollen limited at both sites, while *Cytisus* was pollen limited at only the site with lower visitation rates. In the three populations with demonstrable pollen limitation, we found a significant relationship between fruit production and natural pollinator visitation at the level of the individual plant. However, we did not find that overall patterns of fecundity were strongly predicted by differences in pollen limitation between species or between sites. While a previous study found a tight link between patterns of pollinator visitation and patterns of reproduction in *Cytisus* in Washington State, we conclude that a more complex and variable environment (in terms of resources, herbivores, and florivores) on the Marin Peninsula de-coupled the relationship between pollinators and fruit production in these invaders. Our results suggest that the role of mutualisms in promoting or constraining invasions is likely to vary considerably among invaded communities.

Keywords Pollination · Invasion · Exotic species · Geographic variation

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Introduction

Interactions with resident species may influence the success or spread of an exotic invader. In one popular example, the theory of "biotic resistance" suggests that potential invaders may be outcompeted by similar species (Elton 1958; Simberloff 1986), or excluded by predators or pathogens (Strong et al. 1984; Mack 1996). While they have received less attention, beneficial interactions with residents may also influence invasions (Simberloff and Von Holle 1999; Richardson et al. 2000). When an outcrossing plant species arrives in a new region, it must either bring its pollinating mutualists with it, make do with resident species, or wait for the appropriate mutualist to be introduced independently. For example, from the early 1900s, clones of the fig *Ficus microcarpa* were planted for ornamental purposes in Florida. This species was restricted to artificial plantings until its particular pollinating fig wasp was introduced in the early 1970s; now the fig is found in self-sustaining naturalized populations (McKey and Kaufmann 1991; Nadel et al. 1992). Such dramatic stories are rare, in part because such specialized mutualisms are also rare (Schemske 1983; Thompson 1994; Waser et al. 1996). Nevertheless, limitation by mutualisms has been incorporated into commonly held beliefs about which species should be good invaders. It is often stated that non-clonal plant invaders should be self-fertilizing, to increase the probability of successful colonization and decrease dependence on varying pollinator populations (Allard 1965; Baker 1965; Brown and Marshall 1981).

Pollinators have long been recognized as an important factor limiting plant reproductive success (reviewed in Burd 1994). Pollen limitation can vary in time and space,

and may induce variability in plant fecundity (Campbell 1987; Johnston 1991; Dieringer 1992; Gomez 1993). The link between fluctuating pollination success and fluctuating reproduction may be mediated by the population dynamics of pollinators (Thomson 2001), by differences in the composition of particular pollinator assemblages (Schemske and Horvitz 1984; Herrera 1988; Thompson and Pellmyr 1992; Pellmyr and Thompson 1996), or by differences in the composition of plant species competing for pollinators (Campbell 1985; Ramsey 1995). However, background factors such as resource availability also influence reproduction, placing constraints on the role of pollinators (Stephenson 1981; Zimmerman and Aide 1989).

Cytisus scoparius (Scotch broom, Fabaceae) is a noxious invasive species that is strongly pollen limited in the glacial outwash prairies of the Pacific Northwest (Parker 1997). In Washington, *Cytisus* flowers early in the season relative to most native plants and relative to the peak activity of pollinators (I.M. Parker, unpublished data). We were led to ask whether pollen limitation is a general phenomenon for *Cytisus* across its introduced range, or rather if different assemblages of pollinators and flowering plants lead to very different plant-pollinator dynamics.

In addition to asking whether limitation by pollinating mutualists is a factor that can be generalized across the introduced range of a species, we would like to develop generalizations that hold across species of invaders as well. It has long been suggested that floral traits have evolved in response to interactions with pollinators (Darwin 1877; Van der Pijl 1961). There are general guidelines for predicting which species should be more attractive to potential pollinators and thus better at competing for visits in a novel biotic environment. Many observational studies and experimental manipulations have shown an inherent pollinator preference for large flowers or large flowering displays (Campbell 1989; Ohara and Higashi 1994; Schemske and Ågren 1995; Connor and Rush 1996). Visitation also increases with rewards such as nectar or pollen (e.g., Neiland and Wilcock 1998; Robertson et al. 1999). Therefore a large-flowered or highly rewarding species should be a better competitor for pollinators than an otherwise similar, small-flowered species.

In Marin County, California, *C. scoparius* coexists with the closely related *Genista monspessulana* (French broom, Fabaceae). These non-clonal European shrubs are very similar in appearance and are often lumped together in discussions of their ecological role as invaders (McClintock 1985; Hoshovsky 1986). Both vary greatly in size, and an individual plant of either species can have from several dozen to several thousand flowers. However, the flowers of the two species are quite different in terms of attractive structures and reward. *Genista* flowers are an order of magnitude smaller than *Cytisus* flowers, and produce one-fourth as many pollen grains (Parker et al., in press). Neither plant produces nectar, so pollen is the only reward for insect visitors. Based on the expectation that visitation should increase with floral allocation, we predicted that small-flowered *Genista* should receive fewer visits and experience more pollen limitation than large-flowered *Cytisus*.

On the other hand, small flowers are often part of an evolved syndrome that includes automatic selfing (autogamy) mechanisms that can ameliorate conditions of low pollinator availability (Piper et al. 1986; Rathcke and Real 1993; Parker et al. 1995; Brunet and Eckert 1998). Higher levels of autogamy could release the small-flowered *Genista* from pollen limitation.

Other factors are also in conflict with the prediction that pollinator limitation should decrease with increasing flower size. Some large, complex flowers may be more difficult to handle, preventing the access of certain insect species and leading to specialization on a narrower set of pollinators. Both *Cytisus* and *Genista* have papilionaceous flowers with fused keel petals. An insect visitor must push down on the keel and split the petals, releasing the style and anthers which then spring up to contact the pollinator in an explosive motion. "Tripped" flowers remain open and can be used to quantify visitation rates directly. In a companion study comparing *Cytisus* and *Genista*, *Cytisus* flowers required more than twice the force to open, and pollinators that tried to access a flower were significantly more likely to succeed with *Genista* than *Cytisus* (Parker et al., in press). Therefore, *Genista* could be released from pollen limitation by the ability to exploit a wider range of pollinators.

The primary objective of this study was to compare the importance of pollination in the reproductive success of these two invasive species, differentiating among the predictions outlined above. First, we examined the potential for pollinator control over reproduction in the two species: (1) Does *Genista* have methods of autogamous self-pollination that buffer it against low pollinator visitation more than *Cytisus*? (2) What is the relative effectiveness of self and outcross pollen in the two species? (3) As predicted by allocation to attraction and reward, are pollinator visitation rates higher in *Cytisus*?

Second, we examined the consequences of variation in pollination: (4) Are both *Cytisus* and *Genista* pollen limited? (5) Does variation in pollination among individuals translate directly into variation in fruit production? (6) Does variation in pollination also predict patterns of fecundity between species and between sites? In summary, what is the potential for these invaders to be limited by pollinator mutualists in coastal California?

Materials and methods

Study sites

Our study was conducted primarily at two sites approximately 12 km apart, on opposite sides of the Marin peninsula (Marin County, Calif., USA). The Mt. Tamalpais site is along the eastern edge of Mount Tamalpais State Park, on an exposed, west-facing slope. The site was dominated by non-native grasses, with patches of *Baccharis pilularis*, *Vinca major*, *Cotoneaster pannosa*, *Planta-*

go lanceolata, Rubus laciniatus, and *Conium maculatum*. *Cytisus* and *Genista* grew interleaved, with *Cytisus* individuals somewhat more sparse and spread out than *Genista*. Overall cover of *Cytisus* and *Genista* at the site was approximately 15% and 30%, respectively. Pollinators observed visiting the two focal species at this site were *Apis mellifera, Bombus vosnesenskii,* and *Xylocopa californica* (Parker et al., in press).

The China Camp site is located within a kilometer of China Camp State Park. The site includes a flat ravine, with a mixed evergreen woodland off to one side and a steep, NE-facing slope to the other side. *Cytisus* and *Genista* were growing in patches and as scattered individuals, with a total cover of approximately 15% and 35%, respectively. Other common species included *Heteromeles arbutifolia*, *Toxicodendron diversilobum*, *Cortaderia selloana*, *Baccharis pilularis*, *Carduus nutans*, and non-native grasses. Pollinators observed visiting the two focal species at this site were *Apis mellifera* and *Bombus vosnesenskii* (Parker et al., in press).

Additional replicates of the breeding system experiment on *Genista* were conducted in an old field on the campus of the University of California, Berkeley. At this site, *Genista* grew on a moderate slope among a mix of non-native herbs and grasses.

Autogamy rates and consequences of selfing

To gain information about the breeding system and fitness consequences of selfing for both species, we bagged flowering branches to estimate autogamous selfing and did a series of floral manipulations and hand-pollinations. In 1997, we selected 10 plants of *Genista* (5 in Berkeley, 5 at Mt. Tamalpais) and 10 plants of *Cytisus* (5 at China Camp, 5 at Mt. Tamalpais). Before any flowers had matured, we enclosed whole branches on each plant in bridal veil, counting the number of floral buds at the start of flowering. After flower senescence, we removed the bags from the developing fruits. We counted the number of fully developed fruits just before dehiscence. We bagged a total of 2,410 flowers of *Cytisus* and 2,790 flowers of *Genista*. In 1998, we chose 10 more plants of each species at Mt. Tamalpais, identified pairs of similar branches with immature flower buds on each plant, then randomly assigned the branches to bagged treatment (2,050 *Cytisus* and 2,140 *Genista* flowers in total) or open control (2,000 *Cytisus* and 1,740 *Genista* flowers in total). We counted the number of fully developed fruits, but were forced to terminate the experiment before fruits were completely dry. The proportion of buds resulting in a fruit was compared between bagged and control branches using a paired *t*-test. Two factors suggest that our estimate of fruit per flower was an upper bound: first, uncounted new buds may have appeared later in the season, and second, some flowers may have been tripped mechanically through interference from the bags.

For all analyses described in this study, proportions were arcsine square-root transformed to improve normality. Wherever the arcsine square-root transformation was used, we replaced $0/n$ with $1/(4n)$ and n/n with $1-1/(4n)$ to improve the transformation at the extremes of the range (Zar 1984).

In 1997, we also selected 10 plants of each species (same sites as in 1997 above) and haphazardly chose 40 flowers per plant. Ten flowers were assigned to each of four treatments: tripped with outcross pollen added, tripped with self pollen added, tripped with no pollen added, and untripped. Outcross pollen was collected from a mix of donors at least 3 m away to minimize biparental inbreeding, while self pollen was collected from other flowers on the same plant. The trip-only treatment was designed to determine whether mechanical disturbance of the flowers alone can effect pollination. For all tripped flowers, the banner and wing petals were cut away after the manipulation to eliminate secondary visits by pollinators. Unfortunately, several of the "untripped" *Cytisus* replicates were violated by pollinators; these were eliminated from the analysis. We tested for an effect of flower manipulation treatment on percent fruit production using ANOVA, with plants as blocks. We tested for a negative effect of self pollen on number of seeds per fruit by comparing selfed and outcrossed fruits paired within plants. A difference between self and outcross pollen could reflect either early-acting inbreeding depression or partial self-incompatibility; because both mechanisms predict a negative effect, we used one-tailed, paired *t*-tests.

Visitation rates

In 1997, we initiated a study to quantify visitation rates and pollinator limitation for the two species at China Camp and Mount Tamalpais. Thirty focal plants of each species at each site were selected using random transects, then each plant was paired with a second individual of similar size. On all plants, we measured diameter, stem number, height, and total fruits produced. For the pollen limitation experiment described below, the two plants in each pair were randomly assigned to either hand-pollination or unmanipulated control. However, for the analysis of visitation rates, all plants were combined (total *n*=60 per species per site).

A branch on each plant was selected at the beginning of the season, after buds had formed but before they opened. To quantify rates of pollinator visitation, we counted untripped and tripped flowers per branch through the flowering season (9 March–17 June). Regular census intervals could not be used reliably in this study, because floral longevity varied among sites, plants, and dates (unpublished data), and because flowers of *Genista* closed within 1 day of being tripped, which would have caused an underestimation of the tripping rate for this species. Therefore we marked every censused flower with Wite-Out and did not count previously marked flowers. We did a separate experiment to test whether Wite-Out had a negative effect on fruit production; we found no significant effect on fruit per flower (Mann-Whitney U, all *P*>0.6) for either *Cytisus* (mean±SD, 0.24±0.08 marked, 0.24± 0.18 control, *n*=5 plants, 22–90 flowers per plant) or *Genista* (0.05±0.10 marked, 0.05±0.11 control, *n*=22 plants, 8–18 flowers per plant). Some marked flowers may have been visited by a pollinator after being counted as untripped; this unavoidable problem occasionally led to ratios of greater than one for fruit/tripped flower. We adjusted the timing of our censuses throughout the season to minimize the number of flowers missed (e.g., senescent but unmarked). There were 12 censuses at China Camp and 15 censuses at Mt. Tamalpais.

What is referred to here as "pollinator visitation rate" is the per-branch proportion of flowers that were tripped, calculated by dividing the sum of tripped flowers over all censuses by the sum of total flowers over all censuses. All proportions were arcsine square-root transformed for analysis. Some branches or whole plants were lost to senescence, herbivory, or other factors during the study; some branches failed to make flowers and others had zero tripped flowers. All of these factors affected the final sample size in each analysis (e.g., the same individual might have a value for fruit/flower but be undefined for fruit/tripped flower). We used a two-way ANOVA to test for significant differences in visitation between the two species and the two sites. For all analyses, we treated species and site as fixed effects. To test for differences between the species at each site, and between the sites within each species, we used orthogonal contrasts.

Pollinator limitation: experimental assessment

Within each pair of plants described above, we chose two branches of similar size on the treatment plant and randomly assigned them to either hand-pollination treatment or unmanipulated control. We pollinated all flowers on the treatment branch using glass slides containing pollen from three or more donors collected at least 3 m away from the recipient plant, in order to minimize both donor-recipient interactions and bi-parental inbreeding. We tripped flowers by hand, then dragged the stigmatic surface across the slide of pollen. In total, we hand-pollinated 4,260 *Cytisus* and 6,540 *Genista* flowers at the two sites. We censused developing fruits through the summer until they were mature and ready to dehisce. All analyses were done on final fruit counts.

We tested for pollinator limitation using paired *t*-tests on the number of fruits per flower of experimental versus control branch-

es in each species at each site. Paired *t*-tests were used in lieu of an orthogonal contrasts approach in order to take advantage of the power of the paired design of the experiment. *P*-values were adjusted across the four tests using the sequential Bonferroni technique (Sokal and Rohlf 1995). We also estimated the mean increase in fruit production under full pollination by taking the ratio of fruit number on the experimental branch to that on the control branch, adding 1 to both numerator and denominator so that no replicates would be undefined.

We investigated patterns in the degree of pollen limitation by creating a metric from the difference between the fruit/flower ratios for experimental and control branches on the same plant. We then performed a two-way ANOVA on this pollen limitation metric, and orthogonal contrasts were used to test for differences between the species at each site, and between the sites within each species.

We also counted the number of seeds per fruit for a haphazard (blind) subsample of five fruits per branch (or all fruits if <5). The number of plants used to calculate seed number per fruit (Mount Tamalpais: *Cytisus*=24, *Genista*=19; China Camp: *Cytisus*=4, *Genista*=17) was limited to those plants that produced at least three fruits on both experimental and control branches, and those that did not dehisce, senesce or get predated early in the season. A paired *t*-test was performed on mean experimental versus control seed number per fruit at each site separately for the two species.

Reallocation of resources between branches on a plant may cause the appearance of pollen limitation on a single branch, even when the plant as a whole is limited by resources rather than by pollination (McCall and Primack 1985; Zimmerman and Pyke 1988). This can occur if a plant responds to heavy pollination of some flowers by shunting resources to those flowers at the expense of others, resulting in abnormally low fruiting success in branches that do not receive supplemental pollination. Therefore, we included a second control for each experimental branch, a similar branch on the unmanipulated plant of each pair. If reallocation is important, the control branch on the unmanipulated plant should have a higher rate of fruit maturation than the control branch on the manipulated plant. We tested for this reallocation using a paired *t*-test of the ratio of fruits to tripped flowers (arcsine square-root transformed), comparing the control branch on the manipulated plant to the "outside control" branch on the unmanipulated plant. Because reallocation might become detectable only when the manipulated branch constitutes a large proportion of the total reproduction, we also tested whether the difference between the two control branches in ratio of fruits to tripped flowers increased with this proportion (fruits on experimental branch/total fruits per plant).

Linking pollinators to fecundity:
patterns in fruit production at different scales

To put our experimental results in the context of natural patterns, we examined the relationship between pollination and fruit production among individuals. Using data from both sets of control branches (from manipulated and unmanipulated plants), we tested for an association between the proportion of flowers visited by pollinators and the proportion of those flowers producing fruits. Regressions were done for each species and each site separately, on arcsine-transformed fruit/flower ratios versus arcsine-transformed proportion of tripped flowers.

Pollination should interact with other factors such as resources to determine overall reproductive output in different environments or sites. We investigated natural patterns in fruit production between the two sites and between species, using two-way ANOVA and orthogonal contrasts on the fruit/flower of control branches.
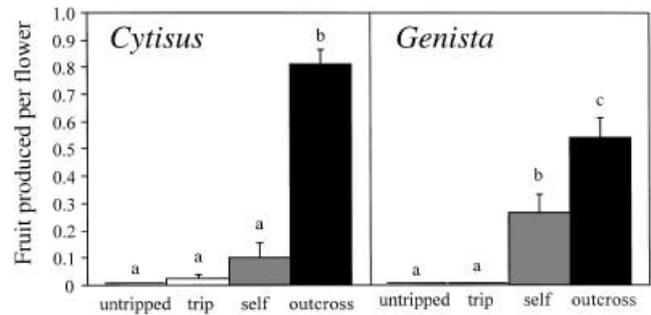

Fig. 1 Number of fruits per flower for *Cytisus scoparius* and *Genista monspessulana* in a hand-pollination experiment. Treatments were: untripped (unvisited) flowers, flowers tripped open but without pollen added, flowers pollinated with self pollen, flowers pollinated with a mix of outcross pollen. For each treatment, *n*=10 plants (except *Cytisus* untripped, 5 plants), 10 flowers per plant. Treatments that share the same letter are not significantly different by the Bonferroni-Dunn post hoc test; differences were significant at *P* <0.001. Error *bars* represent 1 SE

Results

Autogamy rates and consequences of selfing

The bagging experiments, which included approximately 4,500 *Cytisus* and 5,000 *Genista* flowers over the 2 years, revealed a very low, but non-zero, rate of unaided self-fertilization for both species. In 1997, fruit production per flower on bagged branches (mean±SD) was 0.013±0.018 for *Cytisus* and 0.012±0.018 for *Genista*. In 1998, *Cytisus* fruit production was 0.011±0.017 for bagged branches compared to 0.115±0.08 for open controls (t=5.4, P<0.0001). *Genista* fruit production was 0.012±0.009 for bagged branches compared to 0.229±0.22 for controls (t=4.3, P=0.0005).

For *Cytisus*, fruit production differed significantly among treatments of untripped, tripped, selfed, and outcrossed flowers (ANOVA, df=3,22, F=84.1, P<0.0001). While tripped (with no pollen added) and self-fertilized flowers did occasionally produce fruit, neither of these treatments was significantly different from the untripped control (Fig. 1). Outcrossed flowers had more than a 5-fold higher probability of producing a fruit than did selfed flowers, and this treatment was significantly different from all other treatments. The mean number of seeds per fruit for selfed flowers was half that for outcrossed flowers (Fig. 2). This difference was marginally significant (paired t, t=2.1, df=3, P=0.06), although six of the ten replicate plants made no selfed fruits and were left out of the analysis, reducing the power of the test.

For small-flowered *Genista*, fruit production also differed among treatments (ANOVA, df=3,27, F=40.1, P<0.0001). Unlike for *Cytisus*, selfed flowers produced significantly more fruits than did untripped and tripped-only flowers (Fig. 1). Flowers that were tripped-only did not produce more fruits than untripped flowers; therefore augmentation by secondary insect visitors cannot explain the fruit production of selfed flowers. Outcrossed flow-
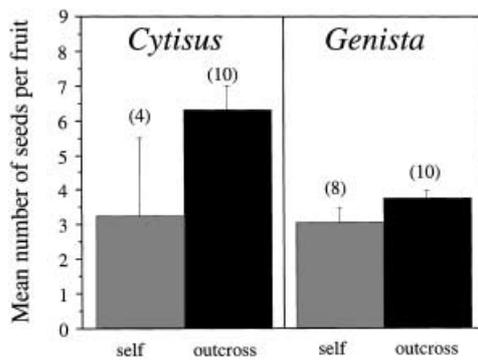
Fig. 2 Mean number of seeds per fruit for selfed versus outcrossed flowers of *C. scoparius* and *G. monspessulana*. Fruits were produced by hand-pollinating 10 flowers on 10 plants of each species; the number above each bar is the number of individuals upon which the estimate is based (i.e. which produced at least one fruit). *Error bars* represent 1 SE. Fruits from outcrossed flowers contained marginally significantly more seeds in *Cytisus* (df=3, t=2.1, P=0.06) but not in *Genista* (df=7, t=1.2, P=0.13)

ers also produced more fruits than selfed flowers. Mean number of seeds per fruit was not significantly different between outcrossed flowers and selfed flowers of *Genista* (paired t, t=1.2, df=7, P=0.13; Fig. 2).

Visitation rates

For both *Cytisus* and *Genista*, pollinator visitation varied from 0% to 100% in the 88 (*Cytisus*) and 120 (*Genista*) individuals that produced at least some mature flowers. Mean visitation rates showed interesting differences between species and also between sites (Fig. 3, Table 1). At both sites, the large-flowered *Cytisus* received significantly more visits per flower than did small-flowered *Genista* (orthogonal contrasts, Mt. Tamalpais, F=16.0, P=0.0001; China Camp, F=168.5, P=0.0001). Within each species, there were differences between the sites but in opposite directions, resulting in a significant population × species interaction effect (Table 1). For *Cytisus*, plants at China Camp experienced significantly higher proportional visitation (orthogonal contrast, F=35.8, P<0.0001), while for *Genista*, plants had higher visitation at the Mt. Tamalpais site (F=13.0, P<0.0004).

Pollinator limitation: experimental assessment

We found no evidence for reallocation of resources away from under-pollinated to fully pollinated branches. The outside control branch on the unmanipulated plant did not produce significantly more fruits per tripped flower than the paired control (on the manipulated plant) in either *Cytisus* (mean±SD, 0.29±0.29 vs 0.43±0.38; paired t, df=36, t=1.22, P=0.23) or *Genista* (0.68±0.66 vs 0.53±0.49; paired t, df=52, t=0.77, P=0.44). The proportion of total fruits produced by hand-pollination of the experimental branch ranged from 0 to 1 for both species,
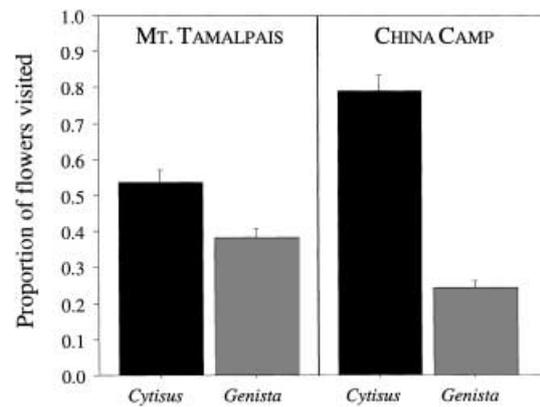

Fig. 3 Mean proportion of flowers visited over the entire season for *C. scoparius* and *G. monspessulana* at two sites. *Error bars* represent 1 SE. Results of analysis of variance shown in Table 1

Table 1 Analysis of variance of proportion of flowers visited over the entire season for *Cytisus* and *Genista* at two sites. Data were transformed using the arcsine of the square root of the proportion of flowers tripped

Source	df	SS	F	P
Species	1	8.40	150.7	<0.0001
Site	1	0.27	4.9	0.028
Species×Site	1	2.64	47.4	<0.0001
Residual	206	11.47		

with a mean of 0.21 for *Cytisus* and 0.18 for *Genista*. If reallocation were occurring, one would expect that as this proportion increased, the difference between the two control branches would increase; however, we did not find a significant relationship between these two variables (*Cytisus*, R^2=0.04, P=0.13, *Genista*, R^2=0.09, P=0.07; both slopes were negative). Because we did not find evidence of reallocation, all subsequent analyses on pollinator limitation compare the experimental branch to the control branch on the same plant.

Experimental pollination revealed significant pollen limitation in both species, but with differences between the two sites (Fig. 4). For *Cytisus*, the mean percent increase in fruit production was 176% and 49% at Mt. Tamalpais and China Camp, respectively. Fruit to flower ratios were significantly higher on the experimental branch than the control branch at Mount Tamalpais (paired t with Bonferroni adjustment, df=24, t=3.6, P<0.01), but not at China Camp (df=19, t=0.9, P=0.38). Hand-pollinated fruits also contained more seeds than controls at Mount Tamalpais (paired t, 7.8 vs 6.1, df=23, t=3.2, P=0.004), although no significant difference was found at China Camp (2.5 vs 2.2, df=7, t=0.68, P=0.52). For *Genista*, the mean percent increase in fruit number was 159% and 188% at Mt. Tamalpais and China Camp, respectively, and this pollen limitation was marginally significant at Mount Tamalpais (paired t with Bonferroni adjustment, df=26, t=2.2, P=0.07) and significant at China Camp (df=26, t=3.9, P<0.01). Unlike fruit produc-
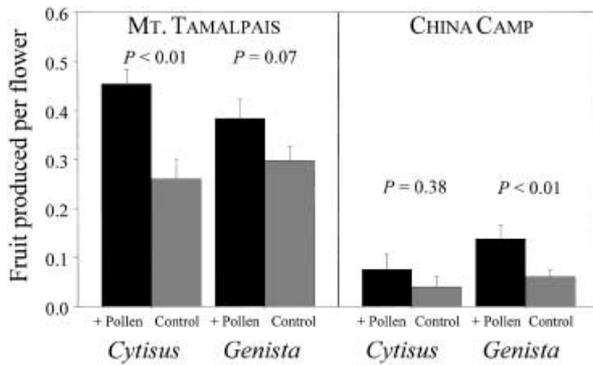
Fig. 4 Fruit production per flower on experimental (hand-pollinated) and control branches, for *C. scoparius* and *G. monspessulana* at two sites. *P*-values were generated from paired *t*-tests to assess pollen limitation (see text), and were adjusted across the four tests by the sequential Bonferroni method. *Error bars* represent 1 SE

Table 2 Analysis of variance of pollen limitation, measured as the fruit per flower of the experimental branch minus that of the control branch, for *Cytisus* and *Genista* at two sites. Data were arcsine square-root transformed

Source	df	SS	F	P
Species	1	0.03	0.96	0.33
Site	1	0.18	5.44	0.022
Species×Site	1	0.11	3.31	0.072
Residual	95	3.20		

tion, seed number per fruit in *Genista* did not increase with pollen addition at either site (paired *t*, experimental vs control: 4.6 vs 4.8 at Mount Tamalpais, df=18, t=1.6, P=0.13; 3.8 vs 4.1 at China Camp, df=16, t=1.9, P=0.08).

Our metric of pollen limitation (fruit/flower for experiment – control) showed a significant effect of site and marginally significant interaction between site and species, but no significant main effect of species (Table 2). Planned contrasts showed a significant difference between the sites for *Cytisus* (F=7.8, P=0.006) but not for *Genista* (F=0.15, P=0.70). There was a significant difference between the two species at Mt. Tamalpais (F=4.2, P=0.04) but not at China Camp (F=0.3, P=0.57).

Linking pollinators to fecundity:
patterns in fruit production at different scales

Patterns of natural variation in fruit set among individuals were consistent with our experimental results on pollen limitation (Fig. 5). In *Cytisus*, we found a highly significant relationship between tripping rate and fruit set at Mt. Tamalpais (P<0.001, R^2=0.31), but no significant relationship at China Camp, the site where *Cytisus* was not significantly pollen limited (P=0.66, R^2=0.01). In *Genista*, we found a significant relationship between tripping rate and fruit production at both sites, although R^2 values were fairly low (Mt. Tamalpais, P<0.002, R^2=0.17, China Camp, P=0.02, R^2=0.09).
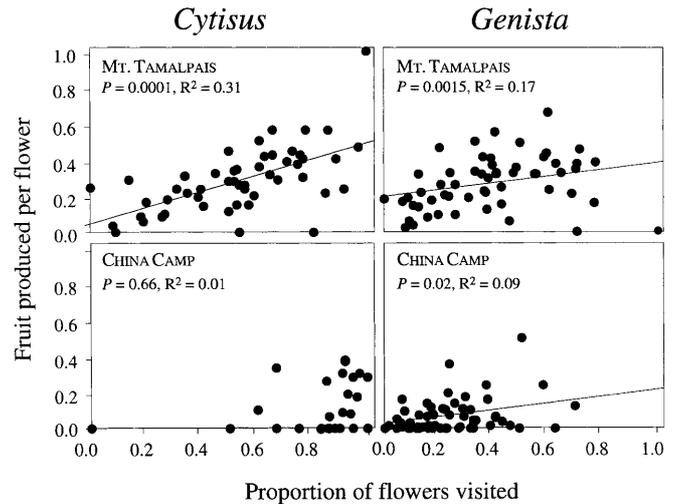

Fig. 5 Fruit production per flower on naturally pollinated branches as a function of the proportion of flowers visited by pollinators, for *C. scoparius* and *G. monspessulana* at two sites. Regression equations are as follows: *Genista* at Mt. Tamalpais, Y=0.27+0.35×X (n=55); *Genista* at China Camp, Y=0.09+0.26×X (n=57); *Cytisus* at Mt. Tamalpais, Y=0.14+0.48×X (n=51); *Cytisus* at China Camp Y=0.26+0.06×X (n=37). Regression equations, *P*-values and R^2 based on arcsine square root transformed data

Table 3 Analysis of variance of fruit per flower for naturally pollinated branches, for *Cytisus* and *Genista* at two sites. Data were arcsine square-root transformed

Source	df	SS	F	P
Species	1	0.0001	0.00	0.96
Site	1	1.90	51.2	<0.0001
Species×Site	1	0.07	1.87	0.17
Residual	97	5.67		

ANOVA on natural fruit set showed a highly significant effect of site (Table 3), and orthogonal contrasts revealed that the two sites differed significantly both in *Cytisus* (F=40.0, P=0.0001) and in *Genista* (F=15.3, P=0.0002). We found no significant effect of species and no significant interaction between species and site (Table 3). Thus patterns in natural fruit production (Fig. 4) did not directly parallel patterns in pollinator visitation (Fig. 3). These data suggest that there were differences between the sites in the level of resources available, or other factors affecting fruit production besides pollinators, and that both species responded in a similar way.

Discussion

Pollen limitation of *Cytisus* and *Genista*:
can invasions be limited by mutualists?

We found significant pollinator limitation of reproduction in both *Cytisus* and *Genista* in central California. This finding was consistent with earlier work showing

strong pollinator limitation of *Cytisus* in the Pacific Northwest (Parker 1997), and suggests that pollinator control over reproduction may be a common feature of non-clonal, invasive shrubs. As more than half of all successful woody plant invaders in North America show no clonal reproduction (S. Reichard, personal communication), pollinators may turn out to be a key factor in determining rates of spread in many weedy invaders. In order for pollinator limitation to contribute to variability in invasion, of course, there must be a demographic link between seed production and population growth and spread. Stage structured models constructed for *Cytisus* in Washington State projected a dramatic increase in population growth under increased pollinator visitation (Parker 1997). It is also true that at the start of any local invasion, or at the edge of any invading population, a non-clonal invader will always be seed limited and dispersal limited. Therefore, to understand what limits spread of these species we must understand controls over reproduction.

The reproductive ecology of invaders

Despite generalizations about successful invaders being either clonal, apomictic, or autogamous (Allard 1965; Baker 1965; Brown and Marshall 1981), *Cytisus* and *Genista* do not show a substantial level of any of these forms of reproductive assurance. Because small flower size often is often associated with a high degree of selfing (Charlesworth and Charlesworth 1987; Lloyd 1987; Brunet 1992), we predicted that *Genista* would show higher levels of autogamy than *Cytisus*, but this was not the case. However, *Genista* did experience less depression of fecundity in self-fertilized flowers than did *Cytisus*. Fruit production for selfed flowers in *Cytisus* was reduced by nearly an order of magnitude, and seed number per fruit was also reduced. In contrast, in *Genista* fruit production for selfed flowers was reduced by less than 50%, and seed number per fruit was indistinguishable from flowers pollinated with outcrossed pollen. The reduction of seed set could be caused either by partial self-incompatibility or by early acting inbreeding depression. Observations of pollen tube growth in selfed versus outcrossed flowers suggested the possibility of weak stylar or stigmatic self-incompatibility in both species, but *Cytisus* did not show stronger effects than *Genista* (I.M. Parker and K.A. Haubensak, unpublished data). Therefore, inbreeding depression probably played a role in determining variability between the species, with *Cytisus* exhibiting more inbreeding depression than *Genista*.

In colonizing species, we expect breeding systems to evolve away from self-incompatibility and toward strategies more tolerant of selfing. Colonizing plants should commonly experience founder effects that will favor the ability to mate successfully with close relatives (Barrett et al. 1989). Given its history as an invader, it is surprising that *Cytisus* expresses as strong a reduction in fitness at seed set under selfing as it does. We conclude that beyond simply visiting flowers, pollinator behavior (with resultant effects on pollen quality) should play a larger role in determining seed set in *Cytisus* than in *Genista*.

Patterns of visitation also refuted the idea that the smaller and more accessible flowers of *Genista* resulted in less specialization and therefore more pollinators. Pollinator observations revealed the same suite of insect species visiting both invaders (Parker et al. in press), and *Genista* did not receive more visits. Rather, *Cytisus* was pollinated more frequently than *Genista* at both sites. This suggests that large flowers and copious pollen made *Cytisus* a better competitor for pollinators than *Genista*.

Geographic variation in plant-pollinator mutualisms: *Cytisus scoparius*

When a species' range covers a large geographic scale, interactions with other species are expected to vary considerably over that range. Changes in the composition of interacting species, as well as abiotic factors such as climate or topography that may influence the interactions, should all contribute to a mosaic of species interactions (Thompson 1994). For *Cytisus*, visitation levels at our sites (54–84%) were considerably higher than those found in Washington prairies, which varied between 3–29% over 2 years in 2 sites (Parker 1997). In Washington, *Cytisus* produces flowers early in the season relative to most native plants and relative to the peak activity of pollinators (I.M. Parker, unpublished data). While data from our Mt. Tamalpais site suggest that insufficient visitation may be a common characteristic of *Cytisus* ecology, pollinators seem to play a much larger role in limiting reproduction in the northern part of its introduced range. One possible explanation for this geographic pattern is that greater force is required to trip *Cytisus* flowers at cooler ambient temperatures (B. Burley, R. Martin, and K. Karoly, unpublished data). The interaction of flower biophysics with abiotic conditions influences both the composition of insects capable of tripping the flowers, as well as the energetically based foraging decisions made by individual pollinators (Herrera 1995).

Consequences of increased visitation: the coupling (and decoupling) of visitation and reproduction

Because of the difficulty of obtaining data on pollinator visitation to individual plants, few studies have addressed the relationship between natural pollination and pollen limitation, or seed set, at the level of the individual. For three cases (those sites/species that did show pollen limitation), we found a direct relationship between visitation and natural variation in the fruit production of individuals. The R^2 values for these regressions, however, were generally low, 0.09 and 0.17 in *Genista* and 0.31 in *Cytisus*. Many factors can influence reproduction,

adding variability to the relationship between visitation and fruit set. Resource limitation can vary between individuals and throughout a season (Pellmyr and Thompson 1996). Pollen quality, that is, the proportion of self versus outcross pollen delivered by a pollinator, could also influence the link between visitation and successful reproduction. Observations of pollinators at the two sites showed that bees on average visited only 6.9 flowers per plant for *Cytisus* and 3.9 flowers per plant for *Genista* (Parker et al, unpublished data). Therefore these plants do not seem to experience high levels of self-pollination, despite their large floral displays. In addition, because *Genista* had fewer within-plant bee visits and also showed more reliable fruit production under selfing than did *Cytisus*, we would expect a tighter relationship between the number of visits and number of fruits in that species. However, the R^2 values were not uniformly higher for *Genista* than *Cytisus*. We are led to conclude that differences in inbreeding and in the response to inbreeding were not the primary explanation for variability we saw in the relationship between visitation and fruit set in these two species.

Overall patterns of reproduction between the species and between the two sites also showed variability that was not always consistent with patterns of pollination. Pollinator visitation to *Cytisus* was consistently higher than visitation to *Genista*, but fruit production was similar in the two species. Comparing the sites for *Genista*, Mt. Tamalpais had higher rates of pollinator visitation as well as higher fruit production. However, in *Cytisus*, the Mt. Tamalpais population had fewer pollinator visits yet produced more fruits. Fruit production was quite low at China Camp for both *Genista* and *Cytisus*, suggesting that resources available to the plants could have been more limited at China Camp. Herbivory was also a factor curtailing reproduction at that site. Large numbers of buds were eaten by florivores and were unavailable to pollinators, and deer pruned back many entire plants at China Camp (personal observation). Others have noted that the pollen versus resources dichotomy is an incomplete description of reproductive success, that patterns in reproductive output can be driven by species interactions with non-pollinators as well as pollen and resources (Cunningham 1995).

In the Pacific Northwest, very strong pollinator limitation, along with the absence of other factors such as catastrophic herbivory, created a tight link between pollination and reproductive success among individuals, as well as among populations and habitats (Parker 1997). Where visitation was greater, fruit production was greater, and this contributed to increased population growth rates (Parker 2000). In contrast, in Marin County, a more complex and variable abiotic and biotic environment apparently de-coupled the relationship between pollinators and fruit production. Our results suggest that the role of mutualisms in promoting or constraining invasions is likely to vary considerably among invaded communities. Such variability will increase uncertainty and hinders our ability to predict invasiveness and invasibility.

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