

Are native bees picky enough? The effect of an invasive plant, *Linaria vulgaris*, on the foraging of native bee pollinators

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Abstract

Invasive and native plant interactions are well studied, but the influence of invasive plants on native pollination systems is largely ignored. Native pollinators, specifically bees, feed their young with pollen and nectar they harvest from plants. An invasive plant could greatly augment the foraging patterns of the bees, especially if the plant has large nectar and pollen rewards. In 2004 and 2006, pollen balls were collected from *Bombus appositus* and analyzed to determine pollen composition. In 2004, 28.1% of the pollen found in *Bombus appositus* pollen balls was *Delphinium barbeyi*, and 24.9% of the pollen came from the invasive species, *Linaria vulgaris*. In 2006, 98.3% of the pollen in *Bombus appositus* pollen balls was from *Linaria vulgaris*, and only 1.5% was *Delphinium barbeyi*. Preliminarily, from the changes in proportions of pollen species found in pollen balls it is possible that *Linaria vulgaris*, the invasive species, is causing bees to change their foraging patterns between years.

Introduction

Biological invasions are widespread and are now considered one of the five leading causes of biodiversity loss worldwide (Sala et al. 2000). For example, plant invasions are known to decrease the abundance and diversity of native plant species, in addition to subsequent effects on ecosystem structure and function (Vila et al. 2006). The effect of invasive plants on the pollination of native plants has been examined (Brown et al. 2002, Chittka and Schürkens 2001). Brown et al. (2001) tested the effect of the pollen of an invasive plant, *Lythrum salicaria*, with a showy display of flowers on the seed set of the native plant, *Lythrum alatum*. They found that the presence of foreign pollen reduced the seed set of the native plant. Furthermore, Brown et al. (2002) explored the idea that invasive plants reduce the number pollinators visiting native plants, in turn lowering seed set of native plants.

Conversely, the effects of exotic flowering plants on native animals, in particular insect pollinators, are relatively unexplored. Pollinators rely on plants for more than just food for themselves; the females of native bees provision their young with pollen and nectar collected from plants in the local habitat (O'Toole & Raw 2004). Little is known about how much invasive pollen and nectar make up bee diets, the consequences for bees, and the performance of larvae fed invasive pollen and nectar. This lack of information is surprising considering the recent concern in global pollinator declines (Buchmann & Nabhan 1996, Kearns et al. 1998).

Invasive flowering plants may affect the performance of bee pollinators through a number of mechanisms, which are not mutually exclusive. One possibility is a change in the quantity or the timing of floral rewards in an area. Invasive species may flower

earlier or later in the season compared to native species, potentially extending the duration of high floral abundance. Therefore, the invasive flowers may provide rewards for pollinators, which could augment the amount of resources available. In addition, the quality of the resources available may also change when alien species are present, causing a response from the pollinator community, which may be different between pollinator species. On one hand, the invasive floral resources may be more nutritious to the insect pollinators, providing richer nectar or pollen rewards. Conversely, the resources may have less nutrition than provided by the non-alien plants, especially if the alien plant has secondary compounds in its nectar or pollen.

Linaria vulgaris (hereafter *L. vulgaris*) is a dominant flowering species in areas that are heavily invaded in the western Rocky Mountains. *L. vulgaris* may have positive or negative effects on the pollinator community that is present in the areas of heavy invasion, considering it produces secondary compounds (Saner et al. 1995). It may also have positive effects on the bee community by providing more resources for the bees to collect for their young.

My study addresses the effect of *L. vulgaris* on *Bombus appositus* (hereafter *B. appositus*) and solitary bee foraging and survivorship. Specifically, I addressed the following question: How do the foraging patterns of solitary bees and *B. appositus* relate to the amount of *L. vulgaris* in bloom? In other words, if there is a small amount or no *L. vulgaris* in bloom at the location where the bees are caught or nesting is there still *L. vulgaris* present in the pollen balls. If the bees preferentially visit *L. vulgaris*, in the plots where there is no *L. vulgaris* there will still be *L. vulgaris* pollen present in the provisions of the bees.

Study System

L. vulgaris (common name: butter-and-eggs, Schrophulariaceae) is often a main flowering species in invaded habitats in the western Rocky Mountains. It was likely introduced as a garden plant, and quickly spread to other non-urban habitats (Saner et al. 1995). *L. vulgaris* is characterized by lanceolate shaped leaves distributed along a single stalk in an alternate pattern. The inflorescence is a raceme of yellow snapdragon-like flowers, and it produces seeds in ovate capsules, as well as producing ramets from underground rootstalks. *L. vulgaris* is self-incompatible and relies on insects for pollination. Its main pollinators are *Bombus* spp, *Psithyrus* sp, *Dialictus* spp, and *Halictus* sp. *L. vulgaris* contains secondary compounds, including alkaloids and flavonoids, possibly in the nectar and pollen in addition to foliage (Saner et al. 1995). These compounds may affect the bee species of the area, especially since they are feeding the pollen and nectar to their young (Saner et al. 1995).

Bombus species are abundant throughout most of North America. They are primitively eusocial, because each colony has a solitary phase and a subsocial phase. The queens mate in the autumn, over-winter as adults. In the spring they emerge, start foraging for pollen, and search for a nest site. As the summer progresses, the colony continues to forage for pollen, nectar, and becomes larger as workers are produced. Eventually, the colony has enough resources to start raising new queens (O'Toole & Raw 2004).

Solitary bees nest in tube-like cavities they find in dry wood or other materials after they have mated. At the end of the cavities, the female piles up mud and small

pebbles, and then begins to collect pollen and nectar. She forms the pollen and nectar into a plug in the tube. After the plug, called a provision is formed, she lays one egg on the provision, and then seals off the chamber with more mud and pebbles. She then starts another provision on the other side of the mud and pebble seal until the tube is full. After the larva eats its provision it spins itself a cocoon. If the bees develop late in the summer, they overwinter in a pupal state, and emerge the following spring (O'Toole & Raw 2004).

Field and Lab Methods

In order to determine how *L. vulgaris* affects the native bee communities in the western Rocky Mountains, several sets of observations were made. Three different sites were used around the Crested Butte and Gothic area in the Elk Mountains of Colorado: Lower Loop, Slate River, and the Gothic Townsite. Each site had three plots that were each approximately 50 meters by 50 meters. At each site, there was a plot from which *L. vulgaris* was absent, present, and present, but the buds were clipped creating and absence of flowers. Biomass was left in order to determine the affect of *L. vulgaris* on other species of plants, if it was not flowering.

In each plot two times per week, flower abundance was estimated using transects to compare the percent of *L. vulgaris* and other plants in bloom. Also, all bee visits to plants were recorded for up to 2 hours in both the afternoon and the morning each week. In addition to observing the presence of bee species in the plot, up to three bee boxes were put out at each plot either in trees or on the ground next to downed wood to collect solitary bees. A bee box consisted of a block of wood with approximately 10 holes drilled in it, and the holes were lined with removable paper tubes, so more than one

female could build a nest in a box. The boxes were checked once a week to see if any bees had nested in the tubes. If a female had provisioned the tube, it was removed, taken back to the lab, and the empty hole was filled with a new tube. Once back in the lab, I cut the tube open, and sampled a small amount of pollen from each provision with fuchin gel in order to stain the pollen. A small amount of pollen was removed from the provision in order to not alter subsequent larval development. Two pollen slides with two samples per slide were made from each provision. Under a compound microscope at 20x, I identified all the pollen to species on the slide using a reference pollen collection. The larvae were raised in the lab in individual plastic wells. Wells were checked three days per week to check for bee emergence. Once the bees emerged the date was recorded, they were weighed, and identified

Another bee group that was sampled was the *Bombus* species of the area to determine if, and how much *L. vulgaris* pollen they were using to provision offspring. Each week, starting the week of July 3rd 2006, any species of *Bombus* that was caught in the plots was identified, and one of their pollen balls was collected from their back femur. We did this in each plot until we had a maximum of three pollen balls per species, per plot. In addition to the pollen balls collected in 2006, I analyzed pollen balls collected from species of *Bombus* in 2004 around the Gothic townsite. The two years of *Bombus* data were compared to see if there have been any changes in the pollen foraged for in 2004 versus 2006. In the lab, the pollen balls were broken up and stained with fuchin gel to dye the pollen. To break up the pollen balls, they were placed in eppendorf tubes, in 100 microliters of water, and sonicated for 30 minutes. After they were sonicated, 400

microliters of fuchin dye was added, and then the tubes were sonicated for 10 more minutes. Then the eppendorf tube was heated in a water bath at 200° C, and a slide was spotted with 15 microliters of the pollen solution from the eppendorf tube. Two slides were made each with 2 dots of solution. The different types of pollen were then matched to a reference collection and quantified subsampling six squares under a compound microscope at 20x.

Data Analysis

Data was analyzed by average percent of a species of pollen per pollen ball across two years of pollen balls collected from *B. appositus*. The samples of pollen from solitary bees were analyzed by the average percent of a species of pollen found in the provisions for 2006.

Results

In 2004, I found that *B. appositus* on average comprised 28.1% of their pollen balls from *Delphinium barbeyi*, 24.9 % from *L. vulgaris vulgaris*, 21.8 % from *Geum trifolium*, and 11.8 % from *Aconitum columbianum* (n=29, Figure 1). The other species that were found in the pollen balls in small percents (less than 5.0) were *Potentilla gracilis*, *Trifolium rapens*, *Lupinus argenteus*, *Erigeron elator*, *Senecio sp*, *Hydrophyllum sp*, *Helinthella sp*, *Androsace sp*, *Teraxacum officinale*, *Polemonium sp*, *Pseudopsymopterous sp*, *Arabis sp*, and *Heracleum sp*. The pollen balls collected in 2006 on average, were comprised of 98.6 % *L. vulgaris* pollen, 1.5 % *Delphinium barbeyi* pollen, 0.33 % *Heliomeris multiflora* pollen, and 0.27 % *Penstemon sp* pollen (n=9, Figure 2). The other species of pollen that appeared in small amounts in the *B. appositus* pollen balls from 2006 were

Latherous leucanthus, *Matricaria perforata*, *Cirsium sp*, *Pyrrocoma sp*, and *Trifolium repens*.

Porportion of Pollen Species in *Bombus appositus* pollen balls
2004

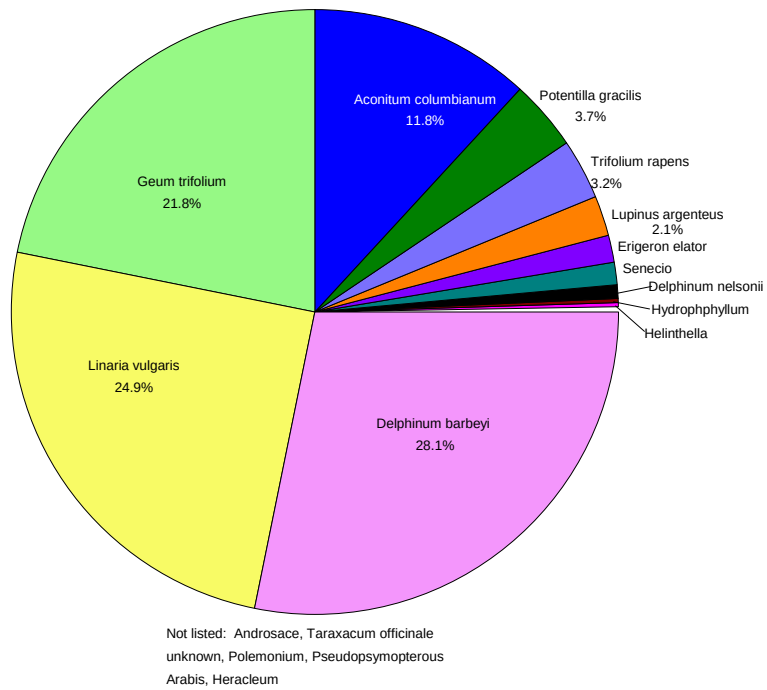
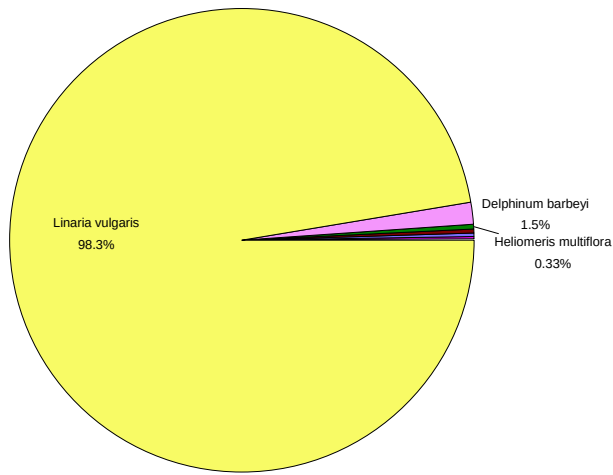


Figure 1

Porportion of Pollen Species in *Bombus appositus* pollen balls
2006



Not Listed: *Lathyrus leucanthus*, *Matricaria perforata*, *Cirsium*,
Pyrocoma, *Trifolium repens*, *Penstemon*.

Figure 2

Linaria Pollen Depending on Site Treatment

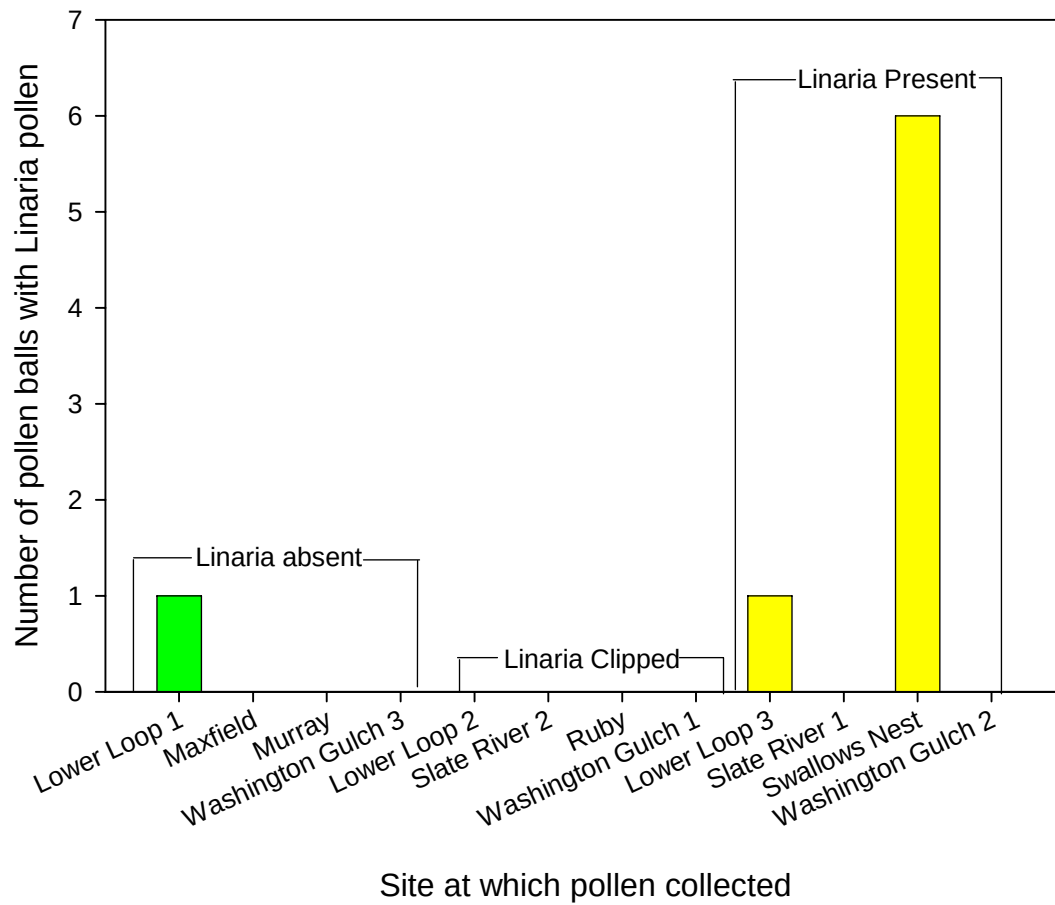


Figure 3

The pollen collected from solitary bees was quantified by determining what percentage of provisions in which a certain species of pollen appeared. *Senecio sp.* appeared in 18 % of the provisions, *Potentilla gracilis* appeared in 13.1 % of provisions, *Teraxicum officinale* appeared in 11.5 % of provisions, and *Helianthella quinquenervis* appeared in 11.5 % of provisions (n=16, Figure 4). The species *Delphinium nelsonii*, *Pentaphylloides floribunda*, *Erigeron sp.* *Lathyrus leucanthus*, *L. vulgaris vulgaris*, *Hymenoxys hoopesii*, *Pseudopsympterosus sp.* and *Geranum richardsonii* appeared in provisions less then 10% of the time.

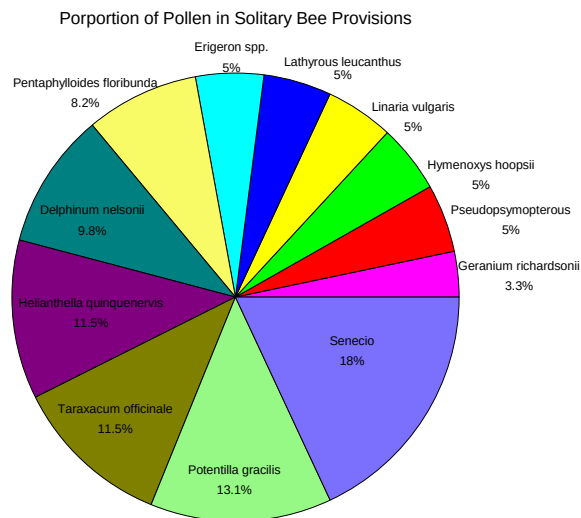


Figure 4

Discussion

The effect of invasive species on pollinator assemblages is largely unexplored, and upon first investigation it appears that an invasive species, specifically the presence of *L. vulgaris* augments the foraging preference of *B. appositus*. In the year between sampling, the foraging preference of *B. appositus* changes drastically. In 2004, the

composition of *B. appositus* pollen balls was 24.9 %(Figure 1). The 2004 pollen composition is starkly contrasted by 2006 pollen balls, which were comprised of 98.6 % *L. vulgaris* pollen (Figure 2). Between the two years, the presence of *L. vulgaris* has cause the bee species to augment its diet, represented by the large difference in the composition of the pollen balls. It has been previously demonstrated that invasive species affect the behavior of native species. Mayfly larvae, native to streams in Australia, are shown to change the time that they move in the water column based on the presence of the invasive brown trout (McIntosh and Townsend 2004). The presence of *L. vulgaris* greatly alters the habitat of *B. appositus*, and therefore the bee has shifted its foraging preference in response to the alteration. It is possible *L. vulgaris* offers a greater amount of pollen based on anther pollen loads and plant abundance, than many native species. As a result, it is advantageous for *B. appositus* to spend the most time foraging on the invasive plant. Investigation of alternate hypotheses is necessary to fully understand the drastic change in *B. appositus* foraging preference between 2004 and 2006.

The sample size for 2006 is not as large as in 2004, but in 2006 pollen balls were collected in a larger number of locations, allowing for more variation in floral communities. The increase in variation of floral communities could result in the bees foraging on a larger number of species, but this outcome is not what is found. Instead, *B. appositus* was only found with pollen balls in plots that contained *L. vulgaris* (Figure 3). The exception to this observation is one pollen ball collected from Lower Loop 1, which is a site where *L. vulgaris* is absent. The lack of *B. appositus* presence in non-*L. vulgaris* sites indicates that the bee species spends a large amount of time foraging in areas where

L. vulgaris is present. Native species response to invasive species is poorly documented, but Rudgers et al. demonstrate the presence of the invader in response to the native species in 2005. The study showed that the invasive species, *Lolium arundinaceum*, was only able to persist in highly diverse habitats when a mutualistic fungus, *Neotyphodium coenophialum*, was present. It is possible that *L. vulgaris* is only successful in habitats with high populations of *B. appositus*, and therefore the bee is facilitating the invasion of the plant, which is known to happen in pollination systems (Richardson et al. 2000).

While *B. appositus* reacts to the presence of *L. vulgaris* by foraging for pollen on the plant, the solitary bees react to the presence of *L. vulgaris* in a different way. The invasive pollen was not found in solitary bee provisions. Instead, solitary bees use the native plant community as a resource for pollen provisions (Figure 4). Leonie E. Valentine has showed this same result in habitat preference of several lizard species in riparian communities of Australia in 2006. The habitat was similar in both native, and habitats invaded by rubber vine. The lizards still preferred the native habitat opposed to the invaded rubber vine habitat, much like the solitary bees prefer the native flowering species to *L. vulgaris*. Although, the apparent lack of preference may be a result of physiological constraints. The construction of *L. vulgaris* flowers is such that a pollinator needs to press down the lip of the flower in order to enter the calyx. Solitary bees are, on average smaller than *B. appositus*, and may not be strong enough to push down the lips of the flowers. Regardless of the mechanisms that control the preference of solitary bees, it is obvious that they do not use *L. vulgaris* to provision their young.

In conclusion, I found pollen from the invasive plant *L. vulgaris vulgaris* is in the pollen balls of *B. appositus*, but not in the provisions of solitary bees. Therefore, *B.*

appositus has a preference for foraging on the invasive plant, while solitary bees largely ignore it. Future interests may include comparing pollen volume to see if *L. vulgaris* comprises a significant volume of the pollen balls. Though *L. vulgaris* does not have an effect on solitary bees, further investigation into the effect of the plant on other *Bombus* species is needed to fully understand the changes in bumble bee foraging patterns. Upon initial investigation it appears that invasive plants do affect pollen foraging of at least one species of native pollinator, and perhaps more. Further differences in foraging behavior will become clear with investigation of the entire bee assemblage.

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