

The effect of sampling effort on species richness estimates of
flower visitors

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ABSTRACT

Estimates of species richness, while useful and common to many subdisciplines of biology, are problematic in their reliance on adequate sampling effort. How much sampling is required for an accurate estimate of species richness, and what levels of sampling will render communities comparable? This study used estimates of species richness of flower visitors to four plant species in the presence and absence of an invasive plant as a case study to explore how much sampling effort is necessary to reach saturated species richness. Visitor species were recorded exhaustively in a single flowering season. Visitors were also tested for the pollen of the focal plant in order to ascertain whether visitor richness accurately measured pollinator richness. Empirical visitor accumulation curves for each plant species showed that even when sampling effort was maintained at a high level, saturated species richness estimates were elusive. Pollen data showed that not all visitor species carried pollen of the flowers that they were collected from, and that the frequency of visitation of a particular visitor species did not predict whether this species was likely to carry pollen. This study, in addition to documenting empirical and exhaustive pollinator taxon sampling curves for four plant species, uses those asymptotic richness counts to test whether visitor species numbers and diversity are affected by the presence of an attractive invasive. It also shows that species counts of flower visitors are not simply equivalent to species counts of pollinators.

KEYWORDS

Sampling effort; pollination; species richness; *Linaria vulgaris*; species accumulation curves.

INTRODUCTION

Quantitative descriptions of biodiversity provide a useful means of comparison between communities, and are often central to studies of change within communities (Memmott 1999). Methods for the measurement of species richness, whether applied directly to conservation studies or extended to the characterization of community interaction webs, have fallen prey to criticism based on sampling inconsistencies (Gotelli & Colwell 2001, Polis 1991). For example, differences in species counts, often attributed to biological phenomena, have been shown by Ollerton and Cranmer (2002) to be explained by differential levels of sampling effort between researchers or between study systems. These inconsistencies in sampling preclude honest comparisons between differentially sampled systems and mask true differences between communities.

How is it possible to be sure that species richness has been accurately measured, and that estimates between communities can be reliably compared? Equal sampling is not always an appropriate solution. Two communities may differ in the amount of effort required to quantify a given proportion of species present; some communities, such as those in tropical forests, may require vastly more sampling than others to be adequately described (Ollerton & Cranmer 2002). The method of rarefaction provides a means to compare species richness counts between communities that have been differentially sampled, using constructed predictions of subsample diversity from within the largest estimate available to compare diversity at the lowest common count of individuals or samples (Gotelli & Graves 1996). The estimates generated by rarefaction, while they cannot be used to extrapolate the asymptotic or saturated number of visitor species, can provide a means to compare the relative diversity of two visitor communities, given a

common number of individuals sampled (Herrera 2005). Rarefaction itself, however, requires a certain level of sampling to work and is unable to provide meaningful comparisons of species richness at low individual or sample counts (Gotelli & Colwell 2001).

It is possible to predict the theoretical number of species in a sampled community by estimating an asymptote that is approached as sampling is extended (Bunge & Fitzpatrick 1993). Given that sampling technique is consistent and able to account for the spatial distribution of individuals (Gotelli & Colwell 2001), species counts should rise in predictable curves and reach a fixed asymptote (representing true, or asymptotic richness) when sampling has been sufficient to capture present diversity. Knowing the shape of a given taxon sampling curve, however, is necessary to predict the asymptotic richness. The shape may vary based on a number of biological characteristics depending on the study system, such as species turnover rate or movement of individuals into or out of communities.

The aim of this study was to establish an empirical comparison between species richness counts in a study system of plant-pollinator interactions by building complete taxon sampling curves for eight related species assemblages. As a case study in species richness sampling, taxon sampling curves were restricted to measuring the flower visitor species to four selected plant species: *Geum triflorum* (Rosaceae), *Pseudocymopterus montanus* (Apiaceae), *Collomia linearis* (Polemoniaceae), and *Senecio bigelovii* (Asteraceae). Hereafter, I will refer to these species by their genus name. The richness of visitor species to each of these plant species was sampled exhaustively, using identical sampling methodology between species, in a single flowering season.

These four species are native to a community that is partially invaded by the introduced invasive *Linaria vulgaris* (Scrophulariaceae; hereafter *Linaria*). Two taxon sampling curves were constructed for each plant species, one in the presence and one in the absence of *Linaria*. By measuring asymptotic richness of pollinator visitors to these plants both with and without *Linaria* present, this study showed whether the number and diversity of pollinator visitors was affected – either facilitated or decreased – by the presence of the invasive, which offers substantial nectar and pollen rewards to pollinators and thus may attract them away from natives (Irwin, unpublished data). As a case study in the degree of sampling effort that is necessary to describe and compare communities, this work compared the related communities of flower visitors in areas with and without *Linaria* present.

An introduced flowering plant such as *Linaria* may facilitate pollination of natives by attracting additional pollinator species to patches where they may service both native and invasive plants (Rathke 1983, Lopezaraiza-Mikel *et al* 2007). Alternatively, an introduced invasive may negatively affect pollination of natives by usurping pollinators or by causing non-native pollen to be deposited on native stigmas. Brown and Mitchell (2001) found that pollen from an introduced exotic plant was transferred to a native congener in the field and decreased seed set of the native plant, while Chittka and Schurkens (2001) attributed some of the invasive success of an introduced plant in Europe to its ability to usurp pollinators from native plants. Being able to accurately compare the number and diversity of pollinators to native plants in invaded versus non-invaded areas is important to elucidating the effect of *Linaria* on pollination. As

explained above, species richness estimates of flower visitors that are accurate and comparable based on sampling effort are needed to make these comparisons.

A pollination web is currently under construction that will quantify differences at the community level between plant-pollinator interactions in the presence and absence of *Linaria* (Irwin, unpublished), but three of the focal plant species under study here (*Geum*, *Collomia* and *Pseudocymopterus*) are relatively uncommon and small-flowered species that have received fewer recorded visitors in the Irwin pollination web study than plants that are common and showy. It is unclear whether these low estimates of pollinator diversity are accurate or whether they suffer from poor sampling effort due to the plants' rarity or inconspicuousness. This study afforded comparison of the asymptotic richness of visitors with richness measurements from the larger web, where sampling of visitor species to these focal plants has been minimal. These low-abundance measurements were tested with rarefaction to see whether sampling has been sufficient to reveal differences in visitor number and visitor diversity that are similar to those shown by this exhaustive study.

The fourth focal species, *Senecio bigelovii*, is a relatively common and apparent plant compared to the other three focal plant species. The asymptotic richness of its visitor species helps to show which differences in sampling effort required between the four focal species are caused by flower abundance. Because *Senecio* has many more recorded interactions in the Irwin pollination web, this study also shows whether those plants that have been relatively well sampled show accurate visitor species richness in the pollination web.

Since the sampling curves of this study measure only visitor species, which says nothing about the pollen transfer capability of each visitor, visitors were swabbed for pollen of the native focal plant which they were observed visiting. Checking for pollen of the focal plant established a relationship for each focal plant species between the number of visitors observed and the number of visitors that are likely pollinators. This comparison between flower visitors and likely pollinators also tested the hypothesis by Vasquez *et al* (2005) that observed interaction frequency can be used as a predictor of interaction strength between plants and pollinators.

METHODS

Visitors to the focal plant species were observed in small (~2m x 3m) plots both with and without *Linaria* present. Plots were chosen in paired replicates (*Linaria* present/absent) based on high density of the focal plant species. All plots were in Gunnison County, Colorado; they were in the Gothic townsite, on Slate River Road, and on the Lower Loop Trail. Observations were conducted on sunny days during the entire flowering period of the plant, as much as possible, for periods ranging from thirty minutes to three hours. Plot size, focal plant and floral density were recorded at each observation period. During each observation period, I identified visitors that probed the focal plant flowers. Those that could be identified to species in the field, such as most bumble bees and some Lepidopterans, were recorded without being collected; all other visitors were collected and retained in the lab for identification. I identified visitors to species when possible, and otherwise identified them to lowest taxonomic unit and then to morphospecies using discrete and repeatable characters.

Species accumulation curves were built for focal plant species with and without *Linaria*, using time of observation of each new recorded visitor species. I also built curves that were corrected for flower density and plot size by plotting total recorded visitor species against the total number of recorded visitors, divided by the number of focal flowers present at the time of each visitor observation.

To assess whether each collected visitor was carrying pollen of the focal plant that it was collected from and could therefore be considered a pollinator, I swabbed the insects systematically for pollen with a small cube (c. 2mm x 2mm x 1mm) of glycerine jelly containing basic fuchsin stain (Kearns & Inouye 1993). The cube was placed on a microscope slide, covered with a coverslip, and melted. I used a compound microscope to identify pollen of the focal plant species that the insect was collected from; pollen was identified with a reference collection of pollen grains collected from study sites.

RESULTS

According to the flowering duration of each plant species and my time constraints, I observed each plant species for a total of between 13 and 45 hours (table 1), and observed a total of 1,699 flower-visitor interactions. While *Geum triflorum* received more visitors in the absence of *Linaria*, more visitor species were recorded in *Linaria*-present plots; *Collomia linearis* received more visitors and more visitor species in the absence of *Linaria*; and both *Pseudocymopterus montanus* and *Senecio bigelovii* received more visitors and more visitor species in the presence of *Linaria* (table 1). The effect of *Linaria* on visitor number and species was therefore mixed between the four focal native species. (These data have not yet been tested for significance.)

Figure 1 shows the species accumulation curves for each plant species, with separate curves for plots in the presence and absence of *Linaria*. When standardized for flower density and plot size, the shape of the curves is quite different (Figure 2). Figure 3 shows separate species accumulation curves for each site where observations were conducted, exposing site-by-site differences that may be due to microclimate. Figure 4 shows the relative number of each visitor species that was observed (all plots were combined for a single plant species). *Thricops species2* (Diptera: Muscidae) was the most common visitor to *Geum*, *Pseudocymopterus*, and *Senecio*, and was the second most common visitor to *Collomia* (after *Anastoechus species1* (Diptera: Bombyliidae)).

The pollen swab test showed that not all of the visitors that were collected at focal plants were carrying the pollen of the focal plant. For the ten most common visitor species for each focal plant, table 2 shows the percent of individuals of that species that were found to be carrying pollen of the focal plant that they were collected from. Sixty-six percent of recorded visitor species to *Senecio* carried pollen of *Senecio*; 54% of visitor species to *Pseudocymopterus* carried *Pseudocymopterus* pollen; 41% of visitor species to *Geum* carried pollen of *Geum*; and 39% of visitor species to *Collomia* carried pollen of *Collomia*.

DISCUSSION

It appears that the asymptotic richness of flower visitors was reached in *Pseudocymopterus* and *Geum*, but not in *Senecio* and *Collomia*. The shape of the sampling curves, and the proximity of the empirical curves to an apparent asymptote, is also greatly affected by the number of flowers present, as shown by the curves that are

standardized for floral density and plot size. This calls into question whether, indeed, the saturated or asymptotic richness of visitor species was reached in these four plant species. The plant that most closely shows an asymptote in the sampling curve, that for *Geum*, is also the plant that received the most hours of observation. It seems possible that although observation was done for the maximum possible number of hours for this study, sampling time was still inadequate to measure the full richness of flower visitor species.

However, there is not a clear pattern between number of hours of observation and proximity to an asymptote in the sampling curve. The curve that most nearly approaches an asymptote, after *Geum*, is *Pseudocymopterus*; this plant received by far the least amount of observation time.

The species that visit and pollinate flowering plants do not represent a static, unchanging community. Instead, the complement of species that visit a plant varies depending on temperature suitability for emergence and on resource availability, especially as other flowering plants emerge and become available. A visitor species may visit one flower species until its preferred species becomes available, at which point it may cease visiting the first species. This factor of turnover in visitor species composition, combined with the limited time for observation that ephemerally blooming plants provide, may mean that “new” visitor species are constantly arriving at plants throughout the entire blooming period. This alone would mean that an observational sampling curve, even if constant observation were maintained over the blooming period, would never “flatten out” or reach an asymptote.

If this is indeed true and a saturated species richness of visitors cannot be reached in a single flowering season, it may be more useful to build species accumulation curves

over many flowering seasons, or to restrict species counts further to a short “optimum sampling” period, such as that proposed to measure the biodiversity of arthropods in Mediterranean communities (Cardoso *et al* in press).

Observations of *Geum*, *Collomia* and *Pseudocymopterus* were carried out during times that *Linaria* was not blooming. Differences in visitor number and visitor species between plots with and without *Linaria*, if significant, are probably therefore due to indirect effects of *Linaria*, such as competition with native plants for nutrients and water, that may affect floral characters. Because observations were carried out on *Senecio* while *Linaria* was blooming, differences in visitor number and richness are likely to be combined effects of direct and indirect interactions with *Linaria*.

There was no clear pattern between the number of visits that a given visitor species paid to a focal plant, and that species’ likelihood of carrying pollen of the focal flower. These data suggest that visitation frequency can not be used as an indicator of the strength of a plant-pollinator interaction. *Collomia* most strongly exemplified this conclusion, where the most abundant visitor by far (*Anastoechus species1*) was not found to carry *Collomia* pollen. Tests of *Senecio* visitors, at the opposite extreme, showed a high incidence of *Senecio* pollen. The relationship between visitor number and pollinator number appears to vary widely between plants, with some plants receiving comparably fewer pollinator services per visitor than others.

In the future, apparent differences in visitor number and diversity between sites with and without *Linaria* will be tested for significance with ANOVA; visitor accumulation curves will be tested for similarity with the Kolmogorov-Smirnov test. Species richness counts from this study will be rarefied to the abundance of species

counts within the larger pollination web using EcoSim® software (Gotelli & Entsminger 2001), and these rarefied counts will be compared to the larger dataset in order to show the effect that sampling effort has had on species richness counts. Pollen carrying data will be further tested by quantifying the amount of pollen that swabbed visitors are carrying.

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FIGURES AND TABLES

Table 1. Visitors recorded, visitor species recorded, and number of hours of observation (hh:mm) for four focal plant species in the presence of the invasive *Linaria vulgaris* (Linaria+) and in the absence of *Linaria* (Linaria-).

	Visitors	Species	Time in observation
<i>Geum triflorum</i>			45:27
Linaria+	67	19	
Linaria-	162	16	
<i>Pseudocymopterus montanus</i>			13:11
Linaria+	127	21	
Linaria-	69	10	
<i>Collomia linearis</i>			30:10
Linaria+	42	11	
Linaria-	58	16	
<i>Senecio bigelovii</i>			36:08
Linaria+	713	56	
Linaria-	461	46	

Table 2. The ten most common flower visitor species to each of four focal plant species, in descending order, and the percentage of individuals of each visitor species that were found to be carrying pollen of that focal plant species.

<i>Geum</i>		<i>Collomia</i>	
Visitor species	% carrying pollen	Visitor species	% carrying pollen
Muscidae: <i>Thricops</i> sp2	17	Bombyliidae: <i>Anastoechus</i> sp1	0
Syrphidae: <i>Platycheirus</i> sp3	25	Muscidae: <i>Thricops</i> sp2	30
Muscidae: <i>Thricops</i> sp1	25	Syrphidae: <i>Sphaerophoria</i> sp1	40
Anthomyiidae: <i>Crinurina</i> sp1	0	Bombyliidae: <i>Conophorus</i> sp1	0
Syrphidae: <i>Platycheirus</i> sp2	50	Muscidae: <i>Thricops</i> sp1	0
Andrenidae: <i>Andrena crataegi</i>	100	Syrphidae: <i>Platycheirus</i> sp4	100
		Anthomyiidae: <i>Paregle</i>	
Halictidae: <i>Sphecodes sedi</i>	33	<i>aestiva</i>	50
Megachilidae: <i>Osmia</i> sp1	100	Syrphidae: <i>Sphaerophoria</i> sp3	50
Megachilidae: <i>Osmia</i>			
<i>bucephala</i>	100	Asilidae	100
Megachilidae: <i>Osmia</i> sp2	33	Bombyliidae: <i>Villa darkwing</i>	0
<i>Pseudocymopterus</i>		<i>Senecio</i>	
Visitor species	% carrying pollen	Visitor species	% carrying pollen
Muscidae: <i>Thricops</i> sp2	38	Muscidae: <i>Thricops</i> sp2	86
Muscidae: <i>Thricops</i> sp1	29	Muscidae: <i>Thricops</i> sp1	100
Syrphidae: <i>Platycheirus</i> sp5	100	Apidae: <i>Bombus bifarius</i>	100
Syrphidae: <i>Hiatomyia</i> sp1	100	Halictidae: <i>Sphecodes tenax</i>	100
		Anthomyiidae: <i>Botanophila</i>	
Syrphidae: <i>Cheilosia</i> sp1	100	<i>sp1</i>	86
Syrphidae: <i>Platycheirus</i> sp4	100	Apidae: <i>Bombus sylvicola</i>	100
Muscidae: <i>Phaoria</i> sp1	50	Apidae: <i>Bombus flavifrons</i>	100
Syrphidae: <i>Melangyna</i> sp1	0	Calliphoridae: <i>Pollenia</i> sp2	100
		Megachilidae: <i>Megachile</i>	
Colletidae: <i>Hylaeus</i> sp1	67	<i>frigida</i>	71
Andrenidae: <i>Andrena birtwelli</i>	0	Anthomyiidae: <i>Adia</i> sp2	67

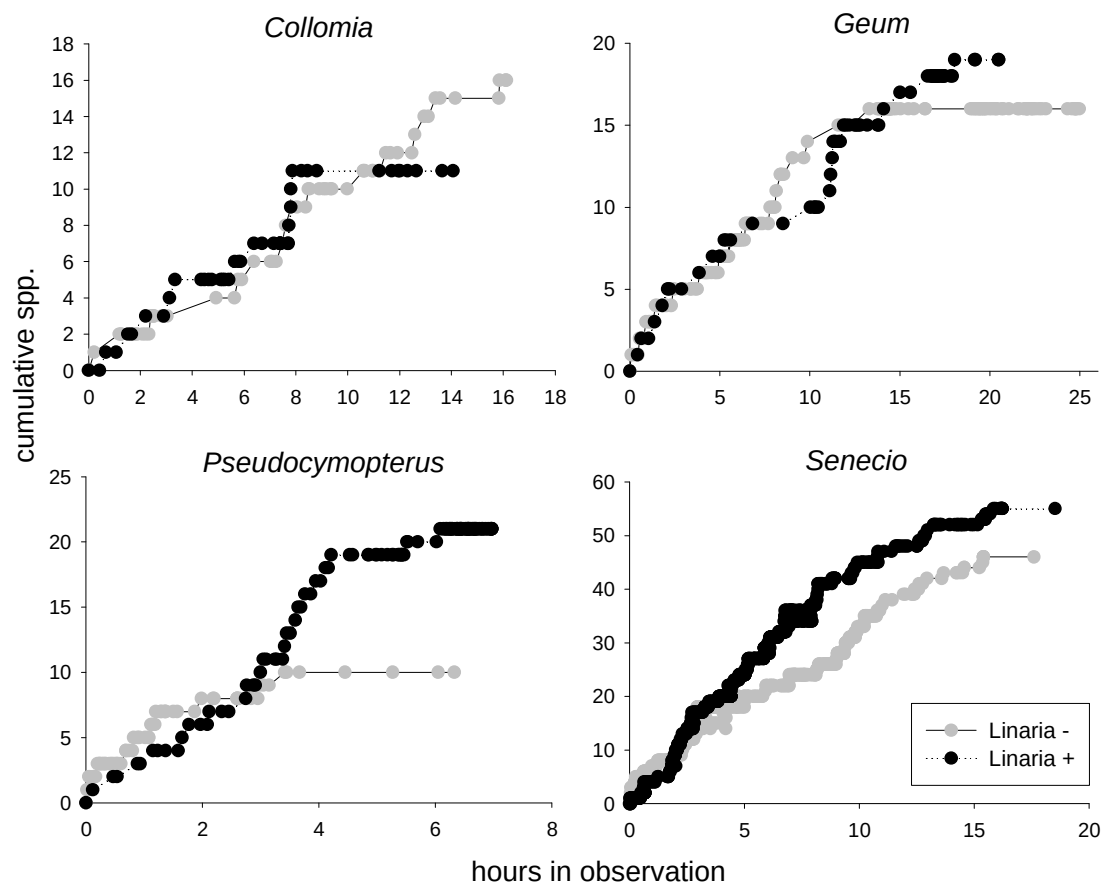


Fig. 1. Species accumulation curves for flower visitor species to four focal plant species in the absence (grey line, Linaria -) and presence (black line, Linaria +) of the invasive *Linaria vulgaris*. Data points correspond to individual visitor observations.

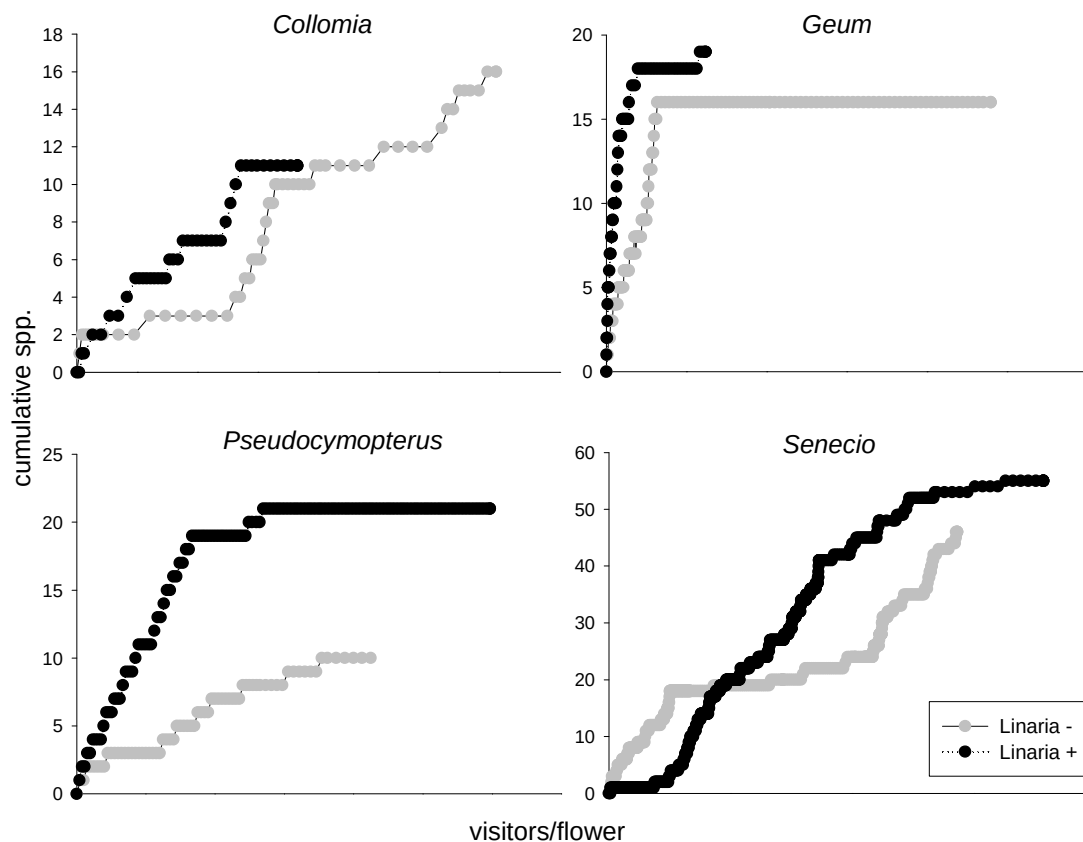
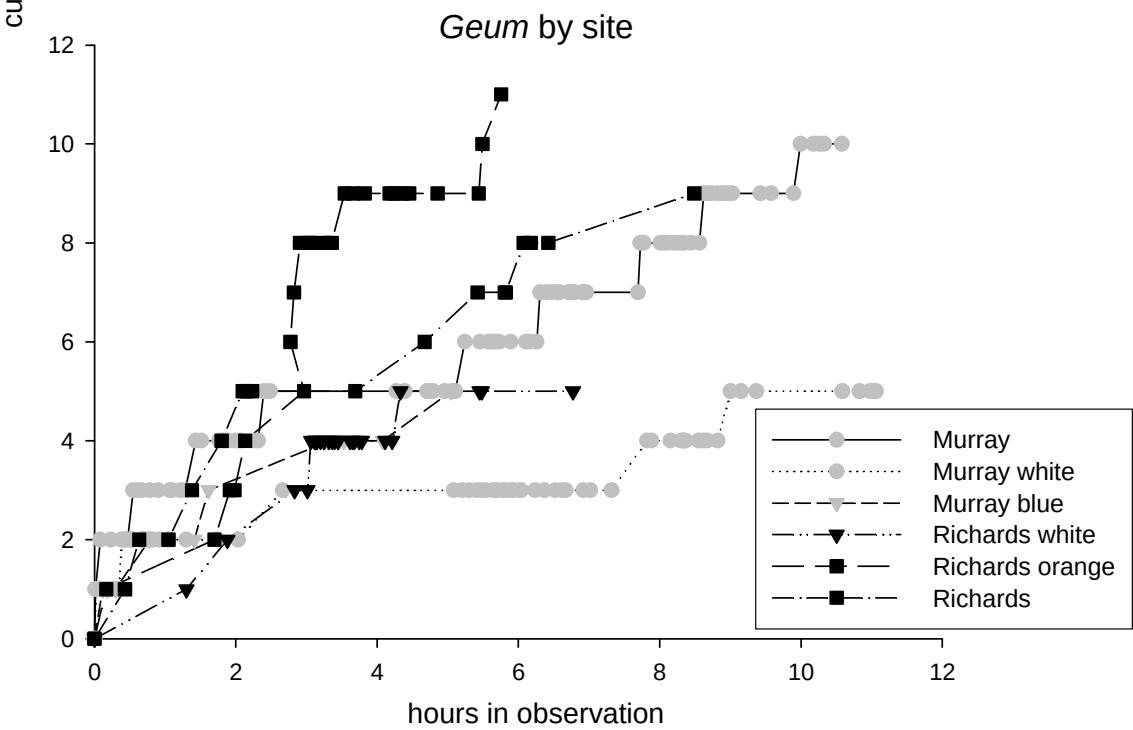
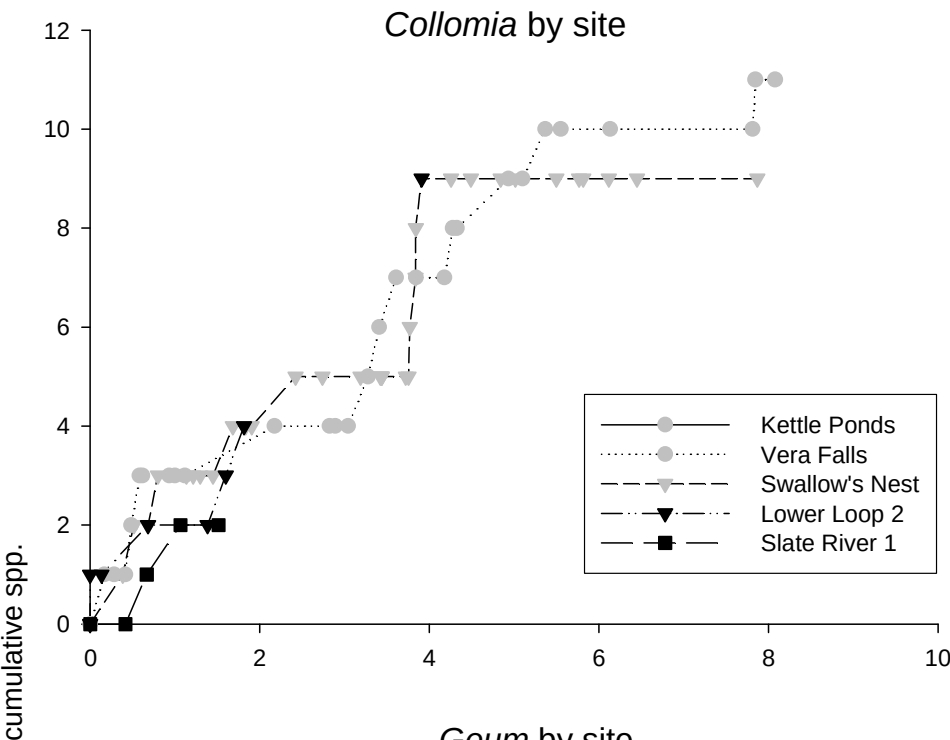


Fig. 2. Species accumulation curves, standardized for floral density and plot size, for visitor species to four focal plants in the absence (grey line, Linaria -) and presence (black line, Linaria +) and of the invasive *Linaria vulgaris*. These curves, instead of showing the accumulation of species over time, show differences both in total number of visitors received (measured on x-axis) and in number of visitor species (shown on y-axis).



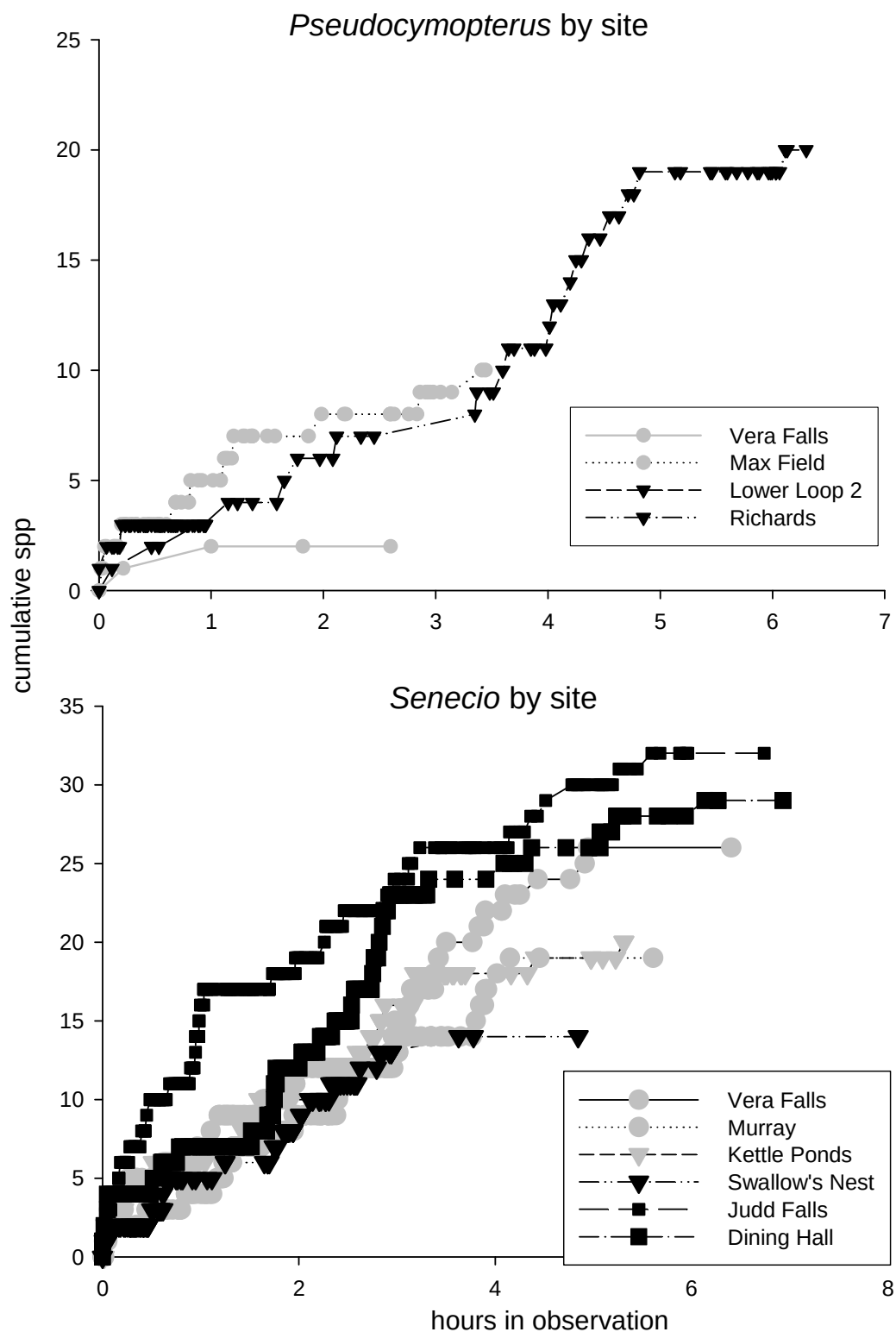


Fig.

3. Species accumulation curves for four plant species in the presence (black lines) and absence (grey lines) of the invasive *Linaria vulgaris*. Separate curves are shown for each site where observations were conducted; site names are indicated in the legend for each graph.

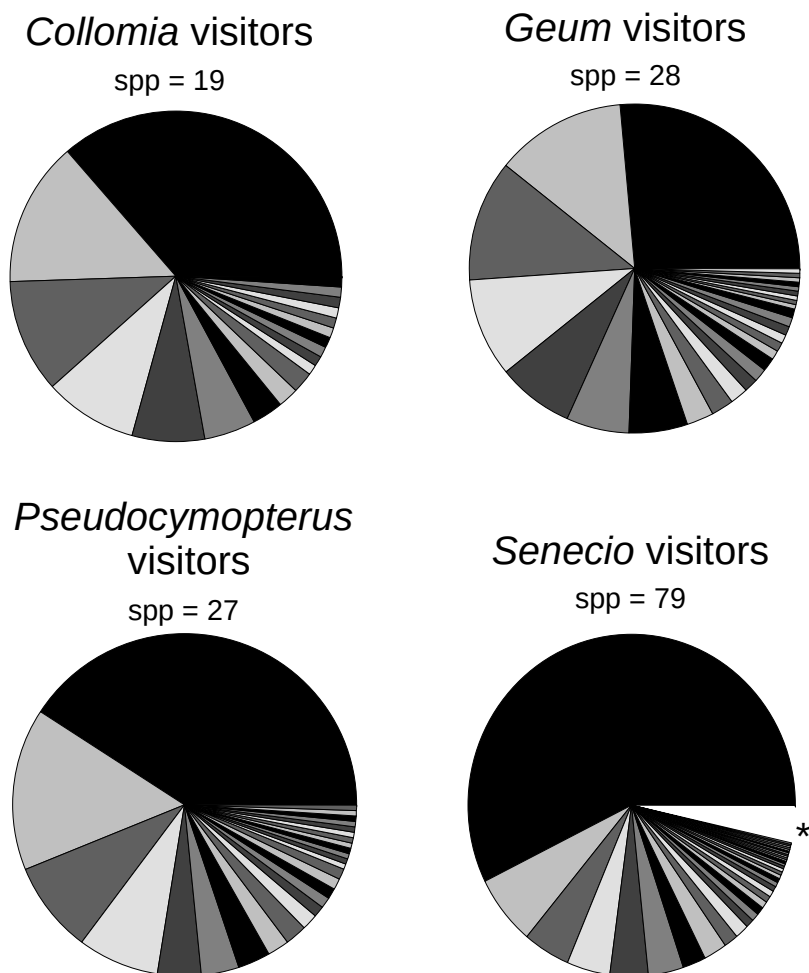


Fig. 4. Floral visitor species to four focal plant species, showing the number of visitor species recorded to each plant species and the relative number of individuals recorded from each visitor species. All observation plots for each plant species are combined.

* Due to graphic error, the number of species that fall in this blank space cannot be shown; 43 additional species should fill this space.