Pollinator-mediated interactions between alien and native plants: alien status and spatial relationships

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ABSTRACT

The introduction of species outside of their native ranges has been extensively studied in ecology. Particular attention has been paid to examining interactions between alien and native plants, and a large proportion of this attention has focused on pollinator-mediated interactions. In order to interact through pollinators, plants must co-occur, coflower, and share pollinators; studies investigating pollinator-mediated interactions between alien and native plants frequently make fundamental assumptions about the definition of these prerequisites to pollinator-mediated interaction. The present analysis examines assumptions about plant co-occurrence and the effects that these assumptions have on study outcomes. In Chapter 2, I present the results of a meta-analysis of 76 studies which overturns previous findings that pollinator-mediated interactions between plants can be predicted on the basis of whether the neighbour is an alien, phylogenetic distance, or floral trait similarity. Moreover, I demonstrate that the spatial definition of the control group (i.e. the distance between the group of focal plants that ‘do not co-occur’ with the alien/alternate neighbour species and the nearest individuals of that neighbour species), and the spatial arrangements of plants within their treatment groups (i.e. the relative placement of the group of focal plants that ‘co-occurr’ with the neighbour species), both have a significant impact on the outcome of pollinator-mediated interactions between alien and native plants. I also emphasize evidence of bias in the selection of study systems and in the process of publication. In Chapter 3, I present the results of a field experiment testing the role of distance between interacting plants in determining patterns of visitation by insects. The results of this analysis are suggestive of visitor functional group-dependent effects but limited by low power. In both Chapter 2 and Chapter 3, I show that heretofore unexamined assumptions about definitions of co-occurrence of plants may be introducing bias into studies of pollinator-mediated interactions between plants, and that facilitation and competition between plants for visitation may be linked across different spatial scales.

Key words: pollination, pollinator-mediated interactions, methods, spatial scale, alien plants, alien-native interactions, meta-analysis

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STATEMENT OF CONTRIBUTIONS

Chapter 2

The meta-analysis presented in Chapter 2 is based in part on a data set made publicly available in a supplement to a meta-analysis published by Carolina Laura Morales and Anna Traveset (2009). This appendix consists of a spreadsheet containing the effect sizes, associated errors, and study systems for a large set of studies examining pollinator-mediated interactions between plants; the analysis presented in Chapter 2 makes unaltered use of the Morales and Traveset (2009) effect sizes and associated errors, as well as the publication information (i.e. reference of the article the data came from), the study system information (i.e. focal and neighbour species, and whether the interaction was native-native or alien-native), and the study approach (i.e. whether each data point came from observational or experimental research). The data set provided by Morales and Traveset (2009) included other information which was not used in the present analysis.

I conducted the literature search and expanded the data set to include results published since Morales and Traveset (2009) was published.

The data for all variables and grouping factors, for both the Morales and Traveset (2009) data set and for the subsequently retrieved data, were obtained in the course of this study. Risa Sargent contributed the phylogenetic distances, and helped to obtain floral symmetry and floral colour data. I personally retrieved all other variables and grouping factors following the methods specified in the methods section of Chapter 2.

Risa Sargent is co-author of the article presented in Chapter 2; in addition to the contributions outlined above, she advised on analyses and contributed to the writing.

I conceived and designed this study, conducted the analyses, and generated the figures.

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A version of this article has been submitted, and resubmitted with revisions, to Ecology Letters for publication. At the current time, there is no copyright impediment to its reproduction here.

Chapter 3

I conceived the idea for this study, conducted the planning and fieldwork, analysed the data, produced the figures, and wrote this article. Risa Sargent consulted on the design, analysis, and writing and will be co-author on the eventual manuscript prepared for publication.

CHAPTER 1: OVERVIEW

Relationship between spatial scale and ecological interactions

The association between landscape patterns and ecological patterns has been the object of considerable research effort (Turner 2005). In particular, the role of space and proximity in shaping biotic interactions is of increasing interest, particularly in landscape ecology. Indeed, biotic interactions are influenced by both local and regional factors (Turner 2005, Hambäck *et al.* 2014). Biotic interactions between plants, herbivores, and parasites, and the impact of community characteristics on these interactions, may both often be scale-dependent. For example, one study considered aphid abundance on wheat and the rate of parasitism on those aphids: aphid abundance decreased detectably with percent arable land at all of five spatial scales from 1 – 3 km, while the abundance of their parasites significantly increased with percent arable land only at spatial scales between 1 – 2 km (Roschewitz *et al.* 2005). Generally, it has been theorized that organisms at higher trophic levels should respond to community characteristics at a larger spatial scale than organisms at lower trophic levels, due to differences in the relevant spatial domains of organisms at each trophic level, but empirical evidence to support this hypothesis is lacking (Thies *et al.* 2003). In a review of biotic interactions, Underwood *et al.* (2014) proposed that the ecological and evolutionary role of biotic interactions in shaping communities cannot be clearly understood until research has better illuminated the relationship between local and landscape responses in biotic interactions.

It has been proposed that biotic interactions (including plant-pollinator interactions) can be affected at multiple scales simultaneously and non-unoformly by community context, e.g., the presence of heterospecific or heteromorphic neighbours, resulting in complex spatially-dependent biotic interactions (Underwood *et al.* 2014). For example, if a consumer is affected at a large/landscape scale by patch characteristics, then the choice of a consumer selecting a patch in which to forage may be altered by the presence of heterospecific or heteromorphic individuals in that patch. Meanwhile, within the patch, the heterospecific/heteromorphic individuals may have different effects on the consumer's use of a focal species (as proposed for herbivory by Hambäck *et al.* 2014). In effect, a consumer chooses first to forage in a patch, and subsequently chooses individual plants to forage on (Hambäck *et al.* 2014); consequently, it is possible for the presence of a neighbour to increase foraging on another species at one scale and simultaneously decrease it at another (Underwood *et al.* 2014). The first stage of consumer decision-making is likely influenced by community characteristics such as density, diversity, and species composition (Hambäck *et al.* 2014). In the case of pollinator-mediated interactions, this stage would also be impacted by foraging behaviour such as flight range and bout duration (Steffan-Dewenter *et al.* 2002). On the other hand, the latter stage of decision-making is more likely to be influenced by individual plant characteristics (Underwood *et al.* 2014), which in pollinator-mediated interactions includes individual traits such as floral display/arrangement, floral colour, reward, etc. (e.g., Grossenbacher and Stanton 2014, Beans and Roach 2015).

The multi-step consumer decision-making process proposed by Hambäck *et al.* (2014) for herbivores responding to plants likely extends to pollinator-mediated interactions as well (Underwood *et al.* 2014).

Known factors in pollinator-mediated interactions

The majority of flowering plant species (87.5%) rely on pollinators for reproduction (Ollerton *et al.* 2011), and plant reproductive success is commonly limited by insufficient pollination (Ashman *et al.* 2004). Many plants are generalists pollinated by a wide array of pollinator species, and many pollinators are likewise generalist plant visitors, visiting a wide assortment of plant species (Waser *et al.* 1996).

In order to interact through pollinators, plants must co-occur, co-flower, and share pollinators (Rathcke 1983). Despite being commonly treated as binary, all of these factors are, in fact, continuous: co-occurrence cannot be determined without defining the relevant spatial scale;

co-flowering may occur during only part of the flowering period; and although many species are linked through shared visitors (either species or individuals), there are usually some visitors that are not shared. Previous research has shown that all three of these factors can affect the outcome of pollinator-mediated interactions between plants (i.e., competition, facilitation, or neutral interactions): indeed, several researchers have shown that pollinator-mediated outcomes can vary depending on the scale of co-occurrence (Cariveau and Norton 2009, Albrecht *et al.* 2016, Bruckman and Campbell 2016).

Pollinator-mediated interactions among plants occur through two main mechanisms: alteration of pollinator visitation, and alteration of the type/quality of pollen transferred (i.e., con- *vs*. heterospecific) (Rathcke 1983). In the case of pollinator visitation, the presence of one plant species can affect the rate at which another receives pollinator visits (e.g., Kandori *et al.* 2009, Liao *et al.* 2011, Carvallo and Medel 2013); in terms of the type or quality of pollen transfer, the presence of one plant species can lead to post-pollination interference through heterospecific pollen deposition on another (e.g. Bell *et al.* 2005), or through conspecific pollen loss to heterospecific stigmas (e.g. Campbell and Motten 1985).

Although pollinator-mediated interactions between plants are often characterized as having a single outcome (i.e., facilitation, competition, neutral), it is possible for both competition and facilitation to arise in the same system, simply due to the movement of a relatively fixed number of pollinators across a landscape (Hegland *et al.* 2009). Indeed, several recent studies have demonstrated that the outcome of pollinator-mediated interactions depends on the spatial scale (e.g. local *vs*. landscape) at which the interaction is measured (Albrecht *et al.* 2016, Bruckman and Campbell 2016) (discussed below). It appears likely that, rather than being binary outcomes, as has been largely assumed by the literature to date, pollinator-mediated facilitation and competition may be linked across space.

The distance between interacting groups of plants is not the only aspect of co-occurrence that may alter interaction outcomes: in close proximity, the arrangement of interacting plants may also be relevant to the outcome of pollinator-mediated interactions (as discussed below). Whether individual plants are clumped or interspersed at small spatial scales has been shown to influence both pollinator visitation rates (Seifan *et al.* 2014), and heterospecific pollen transfer (de Waal *et al.* 2015). Moreover, the likelihood of post-pollination interference through heterospecific pollen deposition may depend on the distances between potentially interacting plants: in a field test of pollen transfer for the single species *Delphinium nelsonii*, Waser (1988) demonstrated that conspecific pollen transfer actually occurred over relatively short distances for bee (most pollen transfer occurred within 0.5 m, sparse from 1 – 10 m) and hummingbird (largely < 1 m, some from 1 – 10 m) pollinators. Consequently, the distances at which post-pollination interference between species can occur may depend on the distance over which the pollinator carries pollen.

Pollinator preference can also play a significant role in interactions among individuals and groups of plants. The outcome of pollinator-mediated interactions among different species of plants will depend in part on which plants are more attractive to relevant pollinators. Indeed, pollinator preferences have been established for particular colour morphs (e.g. Waser and Price 1981, Schemske and Bradshaw 1999, Grossenbacher and Stanton 2014), plant height (e.g. Andersson and Widén 1993, Gervasi and Schiestl 2017), floral shape (e.g. Andersson 1996, Campbell *et al.* 1997), floral size (e.g. Eckhart 1991, Conner and Rush 1996), and patch density (e.g. Mustajärvi *et al.* 2001, Dauber *et al.* 2010, Stein *et al.* 2013). Patch diversity also affects pollinator preference, but its effects vary: facilitation has been found to increase with increased patch diversity, due to resource complementarity (e.g., Ghazoul 2006); as has competition, in the latter case, possibly due to dilution of a fixed number of available visits across a larger population of plants (e.g., Montero-Castaño and Vilà 2015). Moreover, some pollinator preferences are affected by community context: for example, pollinator preference for floral colour morphs in *Mimulus bicolor* changed in the presence of congener *M. guttatus* (Grossenbacher and Stanton 2014); the presence of competitor *Impatiens glandulifera* has been shown to alter selection for floral shape in *I. capensis* (Beans and Roach 2015); and pollinator preference and constancy to three related species of *Castilleja* depended on community composition (Hersch and Roy 2007).

Alien-native interactions

There has been considerable research devoted to elucidating the potential impacts of species introduced outside of their native range on the native communities that they have been introduced to, and in understanding the mechanisms underlying those impacts (Levine *et al.* 2003); conservation is a commonly-cited reason to conduct studies of alien-native interactions (e.g., Aigner 2004, Kandori *et al.* 2009, Da Silva *et al.* 2013, Masters and Emery 2015), but the novel interactions brought about through these introductions also provide fruitful ground for the study of plant evolutionary ecology. There has been particular attention paid to the potential disruption of native plant-insect mutualisms by alien plants (Skurksi *et al.* 2014).

Pollinator-mediated interactions between alien and native plants are commonly reported to be competitive (Skurski 2014). Competition is expected because many aliens form dense monotypic stands (Bjerknes *et al.* 2007, Skurski *et al.* 2014, Goodell and Parker 2017), are escaped ornamentals selectively bred for large floral displays (e.g. Chittka and Schürkens 2001), and/or because of the potential to reallocate resources from defense against herbivory into floral display and fecundity following release from natural predators from the home range (Blossey and Notzold 1995). There is nevertheless no *a priori* reason to expect competition as opposed to facilitation, particularly given that density and large floral displays, used to justify the expectation of competition, are also traits associated with facilitation of neighbour plant pollination through the magnet species effect, wherein an attractive plant draws more visitors to the community in which it occurs and thereby improves visitation rates to co-occurring plants (e.g., Laverty 1992). Moreover, facilitation is well-known among plants (e.g., Johnson *et al.* 2003, Moeller 2004), was commonly found in a comprehensive survey of plant interactions in a grassland community (Hegland *et al.* 2009), and can be more common than competition in some ecosystems (e.g., Tur *et al.* 2016).

The most recent quantitative synthesis of studies examining pollinator-mediated interactions between alien and native plants concluded that alien plants generally impose competition on native neighbours for pollinator visitation and seed/fruit set (Morales and Traveset 2009). However, many new studies reporting facilitative or neutral effects have been published since 2009 (see Chapter 2). Harrison and Winfree (2015) speculated that there may be bias in terms of the study systems selected for study of alien-native pollinator-mediated interactions, while Levine *et al.* (2003) proposed the same for the study of alien-native interactions as a whole. If there is indeed bias in selection of study systems for evaluating the nature of pollinator-mediated interactions between alien and native plants, this could prevent researchers from drawing accurate conclusions about the overall relationships between alien and native plants.

Plant arrangements

Theoretically, there are three possible arrangements of multiple plant species relative to each other in space: interspersion, in which species alternate with one another in space; clumped, in which each species tends to aggregate in clusters or groups; and random. A diagram of these possibilities is provided in Chapter 2 (Figure 2.1). Thomson (1983) discussed the potential impact that different mechanisms of interaction between species, namely visitation and improper pollen transfer, could have on pollinator-mediated interactions between plant species in different arrangements. He proposed that visitation rates should increase as intermingling increases, due to the increased attractiveness of interspersed patches through density and/or diversity. However, he also noted that improper pollen transfer (i.e., heterospecific pollen deposition and/or pollen loss to heterospecific stigmas) should increase with greater interspersion of species, potentially leading to reduced seed set at high levels of interspersion. The theoretically ‘optimal’ arrangement, at least from the perspective of pollen transfer, is one in which plants exist in intermingled clumps, rather than in isolated clumps or in patches of intermingled flowers (Thomson 1983). These predictions have not been tested extensively. Some recent work found significant but species-specific effects of plant arrangement on visitation rates (Seifan *et al.* 2014), or unchanged visitation rates but improved reproductive success through reduced post-pollination interference when plants are clumped, but only at low densities (de Waal *et al.* 2015).

It is likely that the relationship between plant spatial arrangement and visitation is also affected by pollinator constancy (i.e., how consistently a pollinator visits a single species) (de Waal *et al.* 2015). Indeed, Seifan *et al.* (2014) found some evidence that the constancy of *Apis mellifera* may change with plant arrangement and density; constancy to the attractive neighbour plant *Centaurea cyanus*, and its effects (mainly competitive) on a variety of co-occuring focal species, increased with *C. cyanus* density and clumping. Similarly, Hersch and Roy (2007) showed that community context, with respect to the neighbourhood species composition, altered pollinator constancy in three hybridizing species of *Castilleja*, with the ‘preferred’ species in experimental arrays being the most frequent one in the floral neighbourhood. However, constancy declined in communities containing morphologically intermediate hybrids (Hersch and Roy 2007), suggesting that constancy may also be affected by morphological distinctiveness. Hersch and Roy (2007) only tested this effect with interspersed, not clumped, arrangements, but their results may also extend to other floral spatial arrangements.

Overall, the role of the spatial arrangement of plants has largely been ignored in experimental tests of pollinator-mediated interactions between plants. One of the goals of my thesis was to address this gap in the literature.

Scale-dependent pollinator-mediated interactions between plants

Although this hypothesis has not received much research attention, pollinator choices may be influenced by information at multiple spatial scales simultaneously (Underwood *et al.* 2014). Moreover, a pollinator that makes a large number of visits and can recruit nestmates in the foraging effort (e.g., a honeybee) is likely to choose relevant foraging patches on a larger scale than a pollinator that makes a smaller number of visits and cannot recruit nestmates (e.g., a solitary bee) (Steffan-Dewenter *et al.* 2002). The small body of work investigating the effects of spatial scale on pollinator-mediated interactions between plants has uncovered evidence of scale-dependent effects. For example, Hegland (2014) showed that visitation to *Trifolium pratense* increased with both heterospecific and conspecific density at the plot (i.e., local) scale (facilitation), but that visitation decreased as neighbourhood (i.e., community) floral density increased (due to increased competition). In an experiment simulating different local and neighbourbood densities and plant compositions, Bruckman and Campbell (2016) showed that the presence of the showy alien plant *Brassica nigra* at the local scale increased visitation to the native *Phacelia parryi* at both low and high densities (facilitation), but that reproductive success increased only when the alien plant was present at the local scale in sufficiently low densities due to increased post-pollination interference through heterospecific pollen deposition with greater alien plant density. When the alien plant was present at the landscape scale but not the local scale, visitation and seed set were both reduced, suggesting pollinator-mediated competition imposed by the alien on the native species. Similarly, in an experiment examining the effect of the presence of the alien *Oxalis pes-caprae* on visitation and reproductive success to the native *Diplotaxis erucoides* at local and landscape scales, Albrecht *et al.* (2016) showed that when the alien was present at both scales, visitation was higher than when it was present at the landscape scale. This facilitation for visitation only resulted in higher seed set when the alien was present at both landscape and local scales. Changes in visitation rates were also visitor-specific, and so may have resulted in lower visit quality when the alien was present at the landscape scale but absent at the local scale (Albrecht *et al.* 2016). Such spatially-contingent visitor responses were detectable even at fine scales spanning 0-15m: Cariveau and Norton (2009) showed with experimental arrays of alien and native plants that visitation rates to native *Monarda fistulosa* were highest 1m and 15m from alien *Carduus nutans*, and lower at 5 and 10m.

Based on empirical data, Hamback *et al.* (2014) proposed a model of spatially-dependent herbivore-mediated interactions between plants (Hambäck *et al.* 2014). If extended to pollinator-mediated intractions (Underwood *et al.*, 2014), then spatially-dependent differences in the direction and effect size of pollinator-mediated interactions between plants could arise. Little is currently known about the movements of pollinators over landscapes and how floral signals at the community and individual levels might affect those movements. It is likely that the distances involved will change depending on the life histories of the interacting plants and their visitors. We know that large, highly social bees respond at greater distances to the presence of communities providing a wider diversity of floral resources than do smaller, less social bees (Steffan-Dewenter *et al.* 2002). Albrecht *et al.* (2007) found that the scale-dependent effects of the presence of an alien plant on pollinator visitation depended in part on the type of pollinator, with large bees responding over larger distances to community characteristics than small bees.

Due to Allee effects, plant mating success may be improved through the pollinator-mediated facilitative effects of a diverse flowering community (e.g., Moeller 2004). On the other hand, individuals in more dense/diverse communities may face increased competition for pollinators (Stein *et al.* 2013). Density-dependent visitation and/or reproductive success has been demonstrated in several systems (e.g., Mustajärvi *et al.* 2001, Moeller 2004, Stein *et al.* 2013), but little is currently known about whether visitor responses to changes in plant density/diversity are also scale-dependent. Determining the effect of spatial scale on how pollinators respond to floral community characteristics is crucial to understanding how trade-offs between community attractiveness and competition for pollinator services shape flowering communities. One consequence of insufficient knowledge in this area is that studies examining pollinator-mediated interactions among plants have no consistent, empirically determined definition of a control group (i.e., plants sufficiently removed from others that they can be reasonably expected not to interact through pollinators) and consequently such studies cannot be readily synthesized.

RESEARCH QUESTIONS

The research articles presented in Chapters 2 and 3 of my thesis examine different aspects of one larger question: how do spatial relationships between plants affect their pollinator-mediated interactions? Because pollinator-mediated interactions should depend on the scale of co-occurrence, I hypothesize that the outcome of pollinator-mediated interactions between plants will vary with scale of interaction, and that because different species of pollinators respond to community characteristics at different spatial scales, the relationship between spatial scale and interaction outcome will be affected by pollinator type.

In Chapter 2, I pursue this general question and hypothesis in a meta-analysis to examine several questions related to the above broader question:

1. Are alien-native interactions consistently competitive, even across multiple spatial scales and plant arrangements?
2. Does the spatial arrangement of study plants (interspersed, clumped) exert a predictable effect on outcomes of studies examining pollinator-mediated interactions between plants?
3. Does the spatial definition of the control group (i.e., the distance beyond which researchers implicitly hypothesize pollinator-mediated interactions cannot take place) in studies of pollinator-mediated interactions significantly affect study outcomes?

In Chapter 3, I pursue questions related specifically to the spatial scale of pollinator-mediated interactions and the potential interconnection of competition and facilitation across different spatial scales. With a field experiment, I examine the following questions:

4. How does visitation vary by proximity to an attractive floral resource in a natural system?
5. Does the distance over which an introduced plant influences pollinator foraging behaviour depend on the the particular floral visitor?

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CHAPTER 2: NO CONSISTENT POLLINATOR-MEDIATED IMPACTS OF ALIEN PLANTS ON NATIVES[1]

ABSTRACT

The introduction of an alien plant is widely assumed to have negative consequences for the pollinator-mediated fitness of nearby natives. Indeed, a number of studies, including a highly cited meta-analysis, have concluded that the trend for such interactions is competitive. Here we provide evidence that publication bias and study design have obscured our ability to assess the pollinator-mediated impacts of alien plants. In a meta-analysis of 76 studies, we demonstrate that alien/native status does not predict the outcome of pollinator-mediated interactions among plants. Moreover, we found no evidence that similarity in floral traits, or phylogenetic distance between species pairs influences the outcome of pollinator-mediated interactions. Instead, we report that aspects of study design, such as distance between the control and nearest neighbour, and/or the arrangement of study plants, better predict the impact of a neighbour than does alien/native status. Our study sheds new light on the role that publication bias and experimental design play in the evaluation of key patterns in ecology. We conclude that, due to the absence of clear, generalizable pollinator-mediated impacts of alien species, management schemes should base decisions on community-wide assessments of the impacts of individual alien plant species, and not solely on alien/native status itself.

INTRODUCTION

One of the most studied changes to plant communities is the introduction of an alien species. Generally, studies have focused on identifying the impacts of aliens, but not necessarily on the mechanisms that underlie them (Levine *et al.* 2003). A recent shift towards more mechanistic studies has revealed that indirect interactions between alien and native plants, such as those

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[1] A version of this chapter has been accepted for publication in *Ecology Letters* for publication. At the time of this writing, there is no copyright impediment to its reproduction here. The use of the word “we” instead of “I” in this chapter reflects R. D. Sargent’s co-authorship.

mediated by herbivores (Bhattarai *et al.* 2017), soil microbial communities (Hawkes *et al.* 2005, Mangla *et al.* 2008), and pollinators (Goodell & Parker 2017) can have significant ecological (Lau & Strauss 2005) and evolutionary (Lau 2006) consequences. Our ability to better understand the impacts of alien invasions requires more information about the mechanisms and processes involved (Levine *et al.* 2003).

The degree to which a plant receives adequate pollination to set seed is known to be influenced by the density, diversity, and identity of other plants residing nearby (Ashman *et al.* 2004, Vamosi *et al.* 2006). Pollinator-mediated interactions therefore offer a potential mechanism by which aliens can impact native plant communities. Indeed, over the past decade, the invasion literature has increasingly focused on the pollinator-mediated impacts of aliens – more so than any other mechanism (Skurski *et al.* 2014). Furthermore, evidence is mounting that the density and composition of species in a plant community have the potential to influence the size and diversity of the pollinator community (Moeller 2005, Westphal *et al.* 2003), and vice versa (Biesmeijer *et al.* 2006), suggesting that alien introductions could have widespread implications for pollination services (Traveset & Richardson 2014). Yet, whether any generalities can be drawn about how alien introductions influence pollination services to natives remains an open question. It is important that we better understand the pollinator-mediated impacts of aliens on native plant communities in order to: 1) gain a more complete picture of the overall impact of an alien introduction and 2) make better predictions about which aliens are expected to impose the most negative impacts, and where, enabling better management schemes.

Interspecific interactions, especially those involving alien species, have tended to be viewed through the lens of competition, and pollinator-mediated interactions are no exception. Alien plant species have been hypothesized to compete directly with native species for pollinators for a wide variety of reasons, including their propensity to grow in large, high-density populations (Bjerknes *et al.* 2007, Goodell & Parker 2017, Skurski *et al.* 2014), the potential for release from natural enemies to promote the evolution of greater investment in floral display (Blossey & Notzold 1995), and the fact that many are introduced as ornamental plants, which are typically specifically bred/selected for their floral display (e.g., Chittka & Schurkens 2001). Negative effects of alien plant species may also operate indirectly, through their influence on local pollinator populations, by, for example, outcompeting and driving to extinction the local plant species the pollinators rely on (Cox & Elmqvist 2000, Traveset & Richardson 2014). Finally, even if visitation is not altered, invasive species can negatively impact post-pollination processes in natives through the introduction of heterospecific pollen, which can clog native stigmas (e.g., Arceo-Gomez & Ashman 2016, Lopezaraiza-Mikel *et al.* 2007), or through pollen loss from native pollen deposited on non-native stigmas (e.g., (Campbell & Motten 1985).

On the other hand, as far as we can tell, there is no particular reason to expect that an alien plant might not facilitate pollination of nearby native species. Facilitation is well-known among plants (Johnson *et al.* 2003, Moeller 2004). Data from flowering plant communities in the Andes suggest that pollinator-mediated facilitation can be even more common than competition in some ecosystems (Tur *et al.* 2016). Similarly, Hegland *et al.* (2009) reported mainly facilitative interactions via pollinator attraction among six plant species in a temperate grassland community. Tellingly, for the same reasons described above to predict competition, alien species, growing in high-density populations, with conspicuous/showy floral displays, may facilitate pollination of natives through what has been termed the ‘magnet species effect’ (Da Silva *et al.* 2013, Thomson 1978). Furthermore, the introduction of an alien plant species may improve the near- and long-term prospects of pollination services to the local plant community by enhancing the population and/or year-to-year survival of its key pollinator populations – through processes such as complementarity (Waser & Real 1979). And yet, overall, the impact of alien species on the pollination of natives is frequently reported to be competitive (Bjerknes *et al.* 2007, Goodell & Parker 2017). In particular, a prominent meta-analysis, cited 137 times as of this writing, found evidence that these interactions tended to be competitive overall (Morales & Traveset 2009).

Notably, since Morales & Traveset (2009) was published, a number of new studies have reported neutral, or positive (facilitative) impacts of an alien neighbour on pollination of native species, leading to speculation that there may exist bias in the species selected for such studies (Harrison & Winfree 2015). There may also generally exist bias in which results tend to be published in ecology and evolution (Parker *et al.* 2016). Together, these factors cast doubt on the conclusion that alien species tend to impose competition on natives. Indeed, even in a single system, pollinator-mediated interactions have been shown to vary in direction. For example, Albrecht *et al.* (2016) found that invasive *Oxalis pes-caprae* reduced seed set of the native *Diplotaxis erucoides* when the invader was present only at the landscape scale, but improved seed set when it was present at both the local and landscape scales. Similarly, Bruckman & Campbell (2016) showed that either facilitation or competition can arise in interactions between the invasive *Brassica nigra* and the native *Phacelia parryi*, depending on whether the invasive was present at the landscape or local scale. These effects also depended on invasive plant density. In yet another study, McKinney & Goodell (2011), discovered that the direction of the impact on the invasive shrub *Lonicera maackii* on a native herb, *Hydrophyllum macrophyllum*, switched from competitive to facilitative depending on the degree of synchrony in flowering phenology between the two species. Overall, these studies indicate that multiple factors, many of which are not considered in any individual study, can impact the direction of pollinator-mediated interactions among aliens and natives.

In addition to alien/native status, species traits may influence the direction and intensity of pollinator-mediated impacts of a neighbour plant on a focal species. Theory predicts that plant species that share floral traits should compete more strongly for pollinators (Fenster *et al.* 2004, Fishman & Wyatt 1999, Grant 1972). On the other hand, trait similarity could promote facilitative interactions among plants (Moeller 2004, Sargent & Ackerly 2008). A number of studies have found that alien plants that share certain traits with a focal species (e.g., floral symmetry, floral colour) tend to impose more negative pollinator-mediated impacts than those that do not (Gibson *et al.* 2012, Goodell & Parker 2017, Morales & Traveset 2009), suggesting that a species' traits, rather than its alien/native status, may provide more power for predicting impact.

Here we address whether any generalizations can be made about the pollinator-mediated impacts of alien plant species on focal native plant species. Specifically, using a phylogenetic meta-regression approach, we test:

> whether alien plants have an overall competitive effect on pollinator visitation to and reproductive success of natives,
>
> whether there is evidence for publication bias in the set of existing studies, and
>
> whether trait overlap, the phylogenetic distance among species pairs, or particular elements of study design (e.g., distance between control plants and the nearest neighbour plants, arrangement of study plants, species choice) influences the outcome of studies of pollinator-mediated interactions among plant species.

METHODS

Data Collection

Our study expands on data collected for a meta-analysis published by Morales & Traveset (2009), which is composed of experimental and observational studies measuring the effect of the presence of a neighbouring species (alien or native) on the pollinator-mediated aspects of fitness (i.e., pollinator visitation and/or plant reproductive success) of a particular focal species. To expand the set of studies included, we searched the electronic databases Scopus, Web of Science, and Google Scholar, using their same search terms: [pollinat* OR visit* OR reproduc*] AND [compet* OR facilita* OR "interspecific interaction" OR "plant-plant interaction"]. Once studies were assessed for inclusion criteria (see below), we were able to add a total of 60 new outcomes for both native-

native and alien-native pollinator interactions to the original set, 40 reporting visitation outcomes and 20 reporting reproductive success outcomes; we did not add any unpublished records.

For each new study, we recorded the mean, standard deviation, and sample size for visitation and/or fruit or seed set, measured in the presence (treatment) or absence (control) of the neighbour species, and used this information to calculate effect size. When data were not available from tables or in the text, they were obtained from published figures using DATATHIEF III software (Tummers 2006), which uses axis measurements to determine the precise values presented in figures. We also recorded data for several independent variables (see below). In cases where the necessary data could not be obtained, the study was excluded.

Criteria for study inclusion

Independent observations were selected based on the following criteria:

Studies of the same neighbour species, focal species, or neighbour-focal pair were treated as independent observations if they were reported in different articles.

11 articles reported results for more than one species pair (e.g., Gibson *et al.* 2013); each species pair was treated as an independent observation.

For articles where results were reported using both observational and experimental approaches (n = 4 for visitation data; n = 2 for reproductive success data) for a single species pair, each approach was treated as a separate study (e.g., Baskett *et al.* 2011).

When a single article reported results for multiple sites (e.g., Gibson *et al.* 2013), years (e.g., Liao *et al.* 2011) treatment densities (e.g., (Sun *et al.* 2013), or other factors (e.g., spatial scale, Albrecht *et al.* (2016)) for a single species pair, one observation was randomly selected for inclusion.

In three studies, pollinator visitation to both the neighbour and the focal species were reported (i.e., with a control and treatment group for both); these were considered as different species pairs. Consequently, these studies supplied one observation for each species pair.

In Morales & Traveset (2009), summary analysis was used to obtain an overall effect size and mean study variance across data reported for multiple days for two studies (Bell *et al.* 2005, Jennersten & Kwak 1991). These observations were retained as reported by Morales & Traveset (2009).

Studies that separated control and treatment groups temporally rather than spatially were excluded from the data (e.g., Takakura *et al.* 2009), as we consider this type of design fundamentally different from one in which the control group is defined on the basis of distance from the nearest neighbour plants.

Observations from unpublished sources reported by Morales & Traveset (2009) were excluded from the present analysis because relevant information about the factors under investigation could not be obtained.

In the Morales & Traveset (2009) dataset, conspecific pollen load was used as a proxy for visitation frequency for two studies (Caruso 2000, Larson *et al.* 2006). These data points were retained as reported by Morales & Traveset (2009).

Effect size

A single effect size metric and its associated variance were calculated for each observation included in the analysis; because data compared responses in control vs treatment conditions, we selected Hedge's *d* as our effect size metric (Hedge & Olkin 1985). Hedge's *d* is a popular estimate of the standardized mean difference between treatments that adjusts for differences in variance in the two groups and incorporates a correction term to remove bias from small sample sizes (Rosenberg *et al.* 2013).

$$d = \frac{\acute{Y}_1 - \acute{Y}_2}{\sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}} J \quad \text{where} \quad J = 1 - \frac{3}{4(n_1 + n_2 - 2) - 1}$$

Positive values of *d* indicate that the outcome (visitation or reproductive success) is higher in the treatment group (i.e., facilitation), while negative values indicate that the outcome is lower in the treatment group (i.e., competition). The larger the absolute value of *d*, the greater the difference. The estimate of variance on this metric was used to weight studies in the model:

$$w_i = \frac{1}{v_i + \hat{\tau}^2}$$

Consequently, studies with higher sample sizes and/or greater precision were more highly weighted than those with low sample sizes and/or precision.

Independent variables

Using phylogenetic meta-regression (described below), we explored whether the following independent variables impacted overall study outcome:

(1) *Alien-native status of the neighbour species*

We distinguished whether the neighbour species studied is native or alien to the community studied.

(2) *Treatment arrangement*

The arrangement of the neighbour species in the treatment plots was categorized into one of the following: natural, wherein plant arrangements were not manipulated or controlled by the researchers; clumped, wherein neighbour plants were present in a clump or cluster close together within or adjacent to the treatment; interspersed, where neighbour plants alternated with focal plants or were regularly spaced through the treatment; or random, where the spatial arrangement of neighbour plants was determined randomly (Figure 2.1A).

(3) *Distance between the control group and the neighbour species*

For the purposes of this investigation, the minimum distance of the control to the nearest ‘neighbour’ plant was determined, as per the description in the methods section of the study, as the distance between treatments in experimental studies using blocked designs, or as the edge of the largest extent the researchers specifically verified or treated as the exclusion zone for the neighbour (e.g., the size of a clipping treatment that removed the competitor neighbour (Figure 2.1B). Some studies provided maps of their sites, or GPS coordinates, which were used to determine the distance of the control groups to the nearest neighbour plant when this information could not be gleaned from the text; when maps with scale bars were provided, measurements were obtained using calipers and printed maps; when GPS coordinates were provided, measurements were obtained using online Google Maps.

(4) *Study approach*

We distinguished whether the study used an observational or experimental approach.

(5) *Phylogenetic distance*

A tree of study species was creating by pruning a recent tree of angiosperms (Zanne *et al.* 2014). A phylogenetic distance matrix among species pairs, based on the branch lengths from the Zanne tree, was then created in R 3.2.3 (R Core Team 2015) using tools from the package Ape (Paradis *et al.* 2004).

(6) *Floral trait overlap*

For each pair of species, we assessed similarity of (1) floral colour, and (2) floral symmetry. Data on flower colour was obtained, by preference, directly from the descriptions in the article, or by searching for the species of interest in the USDA plants database (USDA 2017), and, if the floral colour was not listed, by searching for photographs of the species and manually coding their colour. Similarity in floral colour was determined as follows: like colours were treated as similar (e.g. red and red), and different colours were treated as dissimilar (e.g. red and white), following the conventions of previous work (e.g., Morales and Traveset 2009). In all cases, floral colour was coded based on human perception and so does not constitute a complete representation of the spectral properties of the species in question (i.e., UV reflectance data were not available for the vast majority of species, and were therefore not included). Data on floral symmetry was obtained using genus or family descriptions from Mabberley (1997) or Judd *et al.* (2002), or, less commonly, through searching the internet for a reliable species description.

Publication bias

Finally, we explored the potential for systematic bias in the choice of alien-native species pairs and/or publication bias:

(1) *Publication bias*

As described in Supplement 1, we examined funnel plots to look for possible asymmetry in the distribution of effect sizes and their variances around the population means according to the following: (a) alien-native neighbour distinction, (b) time of publication (pre-2009, post-2009), and, in the Morales & Traveset (2009) data set, (c) published and unpublished records.

(2) *Bias in selection of species pairs*

There are several ways by which the particular species chosen for study could bias our ability to meaningfully assess the overall impact of a neighbour species on a focal species through pollinators. For example, if researchers tend to select pairs of species that are close relatives, the overall outcome might differ compared to a sample of pairs selected randomly with respect to relatedness. In order to test for bias in the diversity or phylogenetic distance among species selected for study, we examined whether there was a relationship between the phylogenetic distance between species pairs and the reporting period (i.e., whether or not it was included in Morales & Traveset (2009), see S1).

Statistical Analyses

To test the effects of the independent variables on *d*, we fitted a restricted maximum-likelihood (REML) (Viechtbauer 2005) meta-regression model to a) the visitation data and b) the reproductive success data. Meta-regression tests the simultaneous effects of multiple explanatory variables against inter- and intra-study variability (Mengersen *et al.* 2013).

Normality of the data was tested graphically and with Shapiro-Wilk tests (Shapiro & Wilk 1965). We examined plots of residuals against fitted values to test for heteroscedasticity and identify outliers in the fitted models (Viechtbauer 2010). We checked for publication bias in our dataset using funnel plots (Sterne *et al.* 2005) (S1.1A & S1.1B).

Analyses were run in R 3.2.3 (R Core Team 2015) using the Metafor 1.9-9 package (Viechtbauer 2010).

A restricted maximum-likelihood meta-regression model was fitted to test for the effects of neighbour status (alien or native), control plot distance to the nearest neighbour, study approach, treatment arrangement, phylogenetic distance, and floral trait similarity between species pairs, as well as all first-order interactions among these explanatory variables, on the effect size, *d*. Because we fitted mixed-effects models, we used a Knapp and Hartung adjustment (Knapp & Hartung 2003), which accounts for uncertainty in the between-study variance estimate in random- and mixed-effects models; with this adjustment, residual variability is tested against a *Q*-distribution, overall model significance against an *F*-distribution, and individual effects against *t*-distributions (Viechtbauer 2010); we report each of these statistics in the results section. Variables which had no significant effect and no significant interactions were dropped from the model and the model was fitted again. The results of the full and reduced models were compared to confirm that the removal of the non-significant factors did not alter the predictions of the model.

Subsequent to the abovementioned analysis, we fitted a follow-up maximum-likelihood meta-regression model to test whether a change in visitation (*d*, visitation) could predict a change in reproductive success (*d*, reproductive success).

The articles from which our data were obtained are listed in Supplement 2.

RESULTS

Visitation

The results of a meta-regression testing the effect of our independent variables (alien or native status of the neighbour, study approach, phylogenetic distance, floral trait overlap, plant arrangement in the treatment group, and distance from the control group to the nearest neighbour) on the direction of the impact (i.e., facilitation, competition, or neutral) of a neighbour species on visitation to a focal species (hereafter, 'visitation outcome') indicate that neighbour status (i.e., alien/native) does not explain a significant amount of the variation in visitation outcome (Figure 2.2A; $t = 0.894$, $n = 76$, $P = 0.658$). This result does not change if we test the impact of neighbour status alone, without controlling for other effects ($F = 0.997$, $n = 76$, $P = 0.321$).

Similarly, we found no significant effect of study approach (i.e., observational vs. experimental ($t = -0.444$, $P = 0.658$, $n = 76$), or phylogenetic distance between species pairs ($t = 1.30$, $n = 76$, $P = 0.197$), or floral trait overlap (colour similarity: $t = 0.054$, $n = 76$, $P = 0.967$; symmetry similarity: $t = 0.481$, $n = 76$, $P = 0.632$), on visitation outcome. Plant arrangement in treatment plots (Figure 2.1A) was found to significantly impact visitation outcome ($F = 2.81$, $n = 76$, $P = 0.056$), however, this result was not robust, as it disappeared when the single study that randomized plant arrangement in the treatment group (Brown *et al.*, 2002) was excluded (Figure 2.3: $F = 0.663$, $n = 75$, $P = 0.578$).

The distance between the control group and the nearest neighbour plant had a significant influence on visitation outcome, with competitive outcomes much more likely for greater distances among control and neighbour plants (d_{slope}: -0.0001 (-0.0001 to -0.0000), $t = -2.39$, $n = 76$, $P = 0.019$). When this relationship was modelled while pooling across other factors, the intercept was positive, indicating facilitation is more commonly found at the closest distances, although we note high variability in study outcomes at the shortest distance (Figure 2.4: $t = 2.01$, n = 76, P = 0.049, $d = 0.1890$ (0.001 to 0.377)).

Reproductive Success

As with the visitation data, we found no evidence that alien or native status of a neighbour had a significant effect on the reproductive success of a focal plant (Figure 2.2B: $t = 1.28$, $n = 50$, $P = 0.208$). This result did not change if we tested the impact of status alone, without controlling for other effects ($F = 0.551$, $n = 50$, $P = 0.462$).

We found no significant effect of study approach ($t = -0.560$, $n = 50$, $P = 0.552$), distance from the control group to the nearest neighbour plant ($t = -0.113$, $n = 50$, $P = 0.911$), floral trait overlap (colour similarity: $t = -1.250$, $n = 50$, $P = 0.218$; symmetry similarity: $t = 0.248$, $n = 50$, $P = 0.806$) or phylogenetic distance between species pairs ($t = 0.0238$, n = 50, $P = 0.981$) on reproductive success outcome.

However, the arrangement of neighbour and focal plants within the treatment group significantly affected reproductive success outcomes, with competition more likely to be reported for studies that interspersed neighbour and focal plants ($n = 7$), as well as in the single study that arranged plants randomly in treatment plots (Brown *et al.* 2002) (Figure 2.3: $F = 8.05$, $n = 50$, $P = 0.0002$). The effect of plant arrangement remained significant when the single study with a random plant arrangement was excluded ($F = 8.313$, $n = 49$, $P = 0.0008$).

Publication bias

The current dataset shows no evidence of publication bias either for visitation or reproductive success outcomes (Figure S1.1A & S1.1B). However, results published prior to 2009 tended to report more competitive outcomes, while results published since 2009 reported more facilitative outcomes; overall effect size for visitation outcomes in alien-native studies in the pre-2009 and post-2009 data are significantly different (Supplement 1). Funnel plots describing the distribution of study effect sizes according to their variance, when divided according to their origin (i.e., the data collected by Morales & Traveset *vs.* the results we added) reveal differences in their degree of asymmetry. In particular, there is asymmetry with greater representation of competitive outcomes in the older data set, and a symmetrical representation of outcomes in the more recently published results (Figure S1.2). In addition, for the pre-2009 data, the published effect sizes are significantly more extreme than unpublished ones (S1).

Relationship between visitation and reproductive success

For studies that looked at both factors, there was an overall significant effect of visitation outcome on reproductive success outcome (i.e., seed or fruit set: $F = 10.4$, $n = 41$, $P = 0.0025$). Although the intercept of the relationship between visitation and seed/fruit set was not significantly different from zero (Figure 2.5: $d = 0.0007$ (-0.237, 0.239), $t = 0.006$, $n = 41$, $P = 0.996$), the slope was (Figure 2.5: $d = 0.553$ (0.206, 0.899), $t = 3.23$, $n = 41$, $P = 0.003$).

Outliers

All outcomes reported in the Results section above are robust to outlier effects unless otherwise specified; conclusions remain statistically and qualitatively unchanged when models are fitted without influential data points identified using the suite of diagnostic statistics available in Metafor (Viechtbauer 2010). Consequently, we report model estimates and outcomes with influential data points included.

DISCUSSION

Understanding the mechanisms that underlie the impacts of alien plant species on native communities is considered essential for successful invasive species management (Levine *et al.* 2003). In the case of pollinator-mediated impacts, a common conclusion is that aliens tend to depress the fitness of their neighbours through competition for shared pollinators (Bjerknes *et al.* 2007, Morales & Traveset 2009, Skurski *et al.* 2014). Our results, a meta-analysis of 76 studies, directly contradict this assertion. Specifically, we found that neither the rate of pollinator visitation, nor the seed/fruit set of a focal plant species, could be predicted on the basis of the alien or native status of a neighbouring plant. In fact, we found that alien neighbours appear to facilitate the pollination of natives as often as they reduce it. Further, we discovered that experimental design appears to have an outsized impact on the results of this type of study. For example, facilitation for visitation was more likely to be found when control plants were located closer to the nearest alien/native neighbour species, while competition was more likely to be reported as control plants were located farther from the nearest neighbour (Figure 2.5). Moreover, a reduction in focal plant seed/fruit set (competition) was more likely to be found when treatment plants were arranged in an ‘interspersed’ pattern with a neighbour, relative to natural or clumped arrangements (Figures 2.1 and 2.3). Overall, factors relating to experimental design were better able to predict study outcome (i.e., existence and/or direction of the impact) than were neighbour status (i.e., alien or native), or similarity in species traits, raising serious concerns about the ability of certain experimental designs to meaningfully assess the impact of an alien or native neighbour on pollinator-mediated interactions with a native.

Visitation was the most commonly collected response variable for studies included in our analyses: of the comparisons we considered, 58 reported visitation alone, 19 reported fruit or set alone, and 41 reported both visitation and seed/fruit set. Visitation has been criticised as a misleading proxy for pollination because visitation rates do not distinguish between mutualist and antagonist visitors (King *et al.* 2013), because visitation is not consistently associated with pollen transport (King *et al.* 2013, Popic *et al.* 2013), and because visitor effectiveness can change from year to year (Fishbein & Venable 1996). Despite these criticisms, we found that a change in visitation rate was significantly associated with a change in seed/fruit set (Figure 2.5). Nevertheless, considerable variability in reproductive success outcomes remains unexplained by variation in visitation outcomes, indicating that visitation is a useful but imperfect proxy for reproductive success.

Although less commonly reported, lifetime reproductive success is arguably the most defensible way to measure the ecological or evolutionary impacts of neighbour interactions on a focal plant. Several prior studies, including a meta-analysis, have reported an overall negative impact of an alien neighbour on the seed/fruit set of a focal species (Morales & Traveset 2009). Once again, our results contradict: we found that, similar to visitation, there was no overall impact of a neighbouring plant on a focal plant's seed/fruit set, with no difference between alien and native neighbours. Our results strongly suggest that the impact of a neighbour on native plant visitation and reproductive success cannot be predicted on the basis of the alien or native status of the neighbour plant.

Trait overlap

The high variability in interaction outcomes suggests that other factors (e.g., trait overlap, density effects (e.g., Bjerknes *et al.* 2007, Muñoz & Cavieres 2008), or interference competition through heterospecific pollen deposition (e.g., Brown & Mitchell 2001, Da Silva & Sargent 2011, Matsumoto *et al.* 2010) could explain some of the remaining variation in study outcome. However, contrary to our expectation, neither phylogenetic distance among species pairs (as a proxy for trait overlap Harvey & Pagel 1991)), nor similarity in specific floral traits (i.e., colour and symmetry), were significant factors in our meta-regression, directly contradicting the findings of earlier studies.

Although floral trait overlap has been described as a predictor of the direction and severity of the impacts of alien plants on pollinator-mediated interactions with natives, the actual findings are mixed. In their meta-analysis, Morales & Traveset (2009) reported that similarity in floral traits (measured, as we report here, as the phylogenetic distance, similarity in floral symmetry and similarity in flower colour among species pairs) was associated with more negative outcomes of pollinator-mediated interactions among aliens and natives. On the other hand, in a study of pollinator-mediated interactions between the showy invasive *Lythrum salicaria* and 36 native co-flowering plants, species with more dissimilar floral symmetry experienced a *more detrimental* impact of the invasive, while overlap in colour and inflorescence traits had no significant effect on impact (Goodell & Parker 2017). Meanwhile, Gibson *et al.* (2012) reported that, while trait overlap did predict the sharing of pollinator species among invasives and natives, there was no subsequent impact on visitation. These findings are consistent with our analysis: it would appear that the association between trait similarity among species pairs and study outcome is highly variable, with no differences associated with alien or native status of the neighbour.

Publication Bias

There are several ways by which the literature on the ecological impacts of invasive species could suffer from publication bias. First, studies reporting significant results may be more likely to get published (Levine *et al.* 2003). Second, alien/invasive species are widely considered harmful, and thus, studies that suggest positive impacts (even if they are not really positive, see below) could face additional scrutiny by reviewers, making them less likely to be published. Finally, data that refute the commonly held idea that alien species impose ecological harm may face stronger scrutiny by authors themselves – if authors of such studies anticipate a difficult reviewer process, they may delay submitting the data for publication for longer, leading to a ‘file drawer effect’. We believe there is good evidence to support one or more of these mechanisms as an explanation for the differences in our findings in comparison to those reported in an earlier meta-analysis (Morales & Traveset 2009). Especially for visitation, there is an unexplained gap in studies showing mild facilitative effects and also low sample size in the earlier data (Figure S1.2A), while no such gap exists for studies published since 2009 (Figure S1.2B); we also found a significant difference in study outcomes depending on whether the data were published before or after 2009 (Supplement 1), and that the competitive effect of alien neighbours on native plant visitation and reproductive success disappears with the addition of the more recent data (Figure 2.2). Together, these findings suggest that the expectation that alien plants impose pollinator-mediated competition on native plants is subject to the decline effect (Schooler 2011), in which support for a given hypothesis diminishes over time.

The decline effect been previously demonstrated in ecology and evolution: Jeschke *et al.* (2012) showed that decline effects are the norm for six major hypotheses in invasion biology, and

Jennions & Moller (2002) found that support for many hypotheses across various fields in ecology and evolution declined with time. The decline effect is usually attributed to publication bias, wherein positive effects with high statistical significance are most likely to be published first, while subsequent work shows moderate or no effects (Jeschke *et al.* 2012). The results presented here, with respect to the change in conclusion since the publication of Morales and Traveset (2009), appear to be consistent with such a pattern.

Study design

Spatial Arrangement of Plants

Only one factor in our model was significantly associated with a directional impact of a neighbour species on a focal plant's reproductive success: the spatial arrangement of the plants in the treatment group. Specifically, when focal and 'neighbour' plants are arranged in a clumped or natural design (Figure 2.1A), the impact of the neighbour on the seed/fruit set of the focal species is mixed (i.e., neutral, competitive or facilitative). In contrast, when focal and neighbour plants are arranged in an 'interspersed' design (Figure 2.1A), the impact on seed/fruit set of the neighbour tends to be competitive, although there is no overall impact on visitation.

Theory suggests that the spatial arrangement of plants in a community influences pollinator behaviour in a manner that can impact the direction of the effect on a focal plant's visitation and reproductive success (Hanoteaux *et al.* 2013). In an experimental test, Seifan *et al.* (2014) demonstrated that the density and spatial arrangement of an introduced, conspicuous neighbour plant species influences whether it tends to facilitate or compete for pollinators with other species in a community. Although Seifan *et al.* (2014) were unable to identify a consistent effect of spatial arrangement on reproductive success outcomes for the focal species, their study was performed by introducing a conspicuous species to plants co-flowering in a single meadow community. Conversely, de Waal *et al.* (2015) varied the spatial arrangements of their focal species and found no consistent effects of plant arrangement on visitation rates, but plants that were clumped together had higher reproductive success at low densities.

It is notable that while some spatial arrangements influenced seed/fruit set, none had a detectable effect on visitation, suggesting that spatial arrangement might impact visit quality rather than visit quantity, through changes in heterospecific pollen transport. It has previously been found that the spatial arrangement of plants can influence pollinator behaviour in a manner that could impact heterospecific pollen deposition (Lopezaraiza-Mikel *et al.* 2007, Thomson 1983).

Distance to nearest neighbour

There was little agreement about the appropriate distance for the placement of the control group in the studies included in our analysis. Indeed, the distance between the control and the nearest neighbour varied widely across studies, from 1 – 30 000 m, with the majority of studies placing their control group within the first 50m of the nearest neighbour. The preponderance of neutral and facilitative effects in these latter studies raises the concern that there may be interactions between the control group and the neighbour species when they are not sufficiently distant from each other (i.e., < 50m). It is possible that it is better to be very close to an attractive neighbour (i.e., treatment condition, *c.* 1m) than only somewhat removed from it (i.e., control condition, *c.* 20-50m). Such scale-dependent effects have recently been identified in a variety of systems (Albrecht *et al.* 2016, Bruckman & Campbell 2016, Cariveau & Norton 2009). Consequently, control groups may be inappropriately defined in many studies.

Species selection

Although there has been no formal analysis, there is speculation that studies of pollinator-mediated interactions among alien and native species have tended to focus on showy alien species (e.g., Bjerknes *et al.* 2007, Chrobock *et al.* 2013, Harrison & Winfree 2015). In a broad review of the invasion literature, Levine *et al.* (2003) reported that nearly all studies were performed in systems where the alien exhibited larger than average potential to impose impacts. Recently, more studies that compare the impacts of one or more alien species on a multitude of natives have emerged (e.g., Chrobock *et al.* 2013, Garbuzov & Ratnieks 2014, Goodell & Parker 2017, Williams *et al.* 2011). We found that although phylogenetic distance between species pairs studied has not changed over time, there is significantly greater variability in phylogenetic distance between pairs in more recently published results, suggesting that a greater variety of systems are being considered in recent studies than previously (Supplement 1). By considering an entire community of interacting aliens and natives, such studies are less likely to focus on pairs that may simply reinforce the widely-held assumption that aliens tend to impose pollinator-mediated competition on natives.

Implications

Several major reviews have reached the conclusion that, in terms of the description of hypotheses and study conclusions, the scientific literature on invasion biology is imbued with the language of competition (Levine *et al.* 2003, Skurski *et al.* 2014, Vila *et al.* 2011). This is as true of studies focused on community structure as those concerned with ecosystem processes (Levine *et al.* 2003). The impact of plant invasions on mutualisms, in particular, has received the lion’s share of attention; with most studies reporting that alien plants tend to negatively interfere with interactions between native plants and their mutualists, including seed dispersers, beneficial microbes, and pollinators (Traveset & Richardson 2006, Traveset & Richardson 2014). Our results suggest that facilitation, at least in the context of pollinator-mediated interactions, may be much more common than previously thought. However, we put forth that there is no *a priori* reason to suspect that facilitation is any less deleterious than competition. For example, in the case of pollinator-mediated facilitation, increased visitation to a newly introduced species implies the movement of pollinators *away* from one set of plants to another (Bjerknes *et al.* 2007). In other words, as denoted by the few studies that have looked at pollinator-mediated interactions across multiple spatial scales, facilitation at one scale may well be connected to competition at another scale, either within or across a regional pool of species (Hegland 2014). Finally, as discussed earlier, what appears to be facilitation at the level of visitation may belie competition at another if increased visitation is accompanied by increased heterospecific pollen transfer (Lopezaraiza-Mikel *et al.* 2007).

Less well-studied are the impacts of alien introductions on interactions with plant antagonists, such as herbivores and seed predators. One underappreciated, but potentially widespread interaction is the phenomenon of 'apparent competition', in which competition between species is mediated through shared enemies (Bonsall & Hassell 1997). For example, in a classic case, the presence of the alien plant species *Medicago polymorpha* can lead to increased herbivory on a native Californian species, *Lotus wrangelianus*, by an exotic alfalfa weevil (*Hypera brunneipennis*). In this system, Lau & Strauss (2005) have demonstrated not only that the presence of the alien reduces *Lotus* fitness directly through competition for space and resources, but also indirectly through an increase in the abundance of their shared herbivore. Recently, authors have speculated that apparent competition could lead to enemy release of an alien, thereby promoting its invasion (Bhattarai *et al.* 2017). Unfortunately, studies of apparent competition in the context of invasions are rare, and overall, the degree to which alien plants impact natives via herbivory is poorly understood. In general, given our findings, it seems likely that publication bias, combined with an overall lack of studies, is influencing our ability to draw reliable conclusions about the impacts of invasion on all types of species interactions.

In addition to its implications for invasion biology, our study has implications for the study of ecological patterns in general. Our results underscore the concern that publication bias is hampering our ability to draw accurate conclusions in the field of ecology, and indeed, in science as a whole (Parker *et al.* 2016, Mueck 2013). Across scientific disciplines, the proportion of published studies reporting negative or neutral results is on the order of 10-15%, suggesting the literature is strongly biased towards positive results (Mueck 2013). This obviously puts disconcerting limits on our ability to reach general conclusions, even using quantitative methods such as meta-analysis. It seems likely that in particularly controversial fields, such as invasion biology (Davis *et al.* 2011), it would be even more difficult to make accurate generalizations, because the probability of missing data is higher. We echo the recent concerns and proposed solutions raised by our colleagues about this real, and worrying issue (Parker *et al.* 2016).

CONCLUSIONS

We conclude that, through publication bias, and perhaps because of an early, undue focus on unusually ‘showy’ alien species as study subjects, the literature has conflated the pollinator-mediated impacts of (certain) neighbouring alien species with the impact of alien/native status itself. We provide compelling evidence that publication bias is likely part of the explanation for the difference between our findings and those of earlier reviews of this topic. We demonstrate that, when developing studies to predict the outcome of pollinator-mediated interactions among neighbouring plant species, one needs to pay careful attention to the potential effects of experimental design and species choice on outcome. Our results highlight the inherent limitations of trying to draw general conclusions from studies that examine interactions between a single pair of plant species: flowering plant communities rarely consist of just two interacting species, and yet this is where the vast majority of studies focus. Finally, our results suggest that our ability to draw general conclusions about ecological patterns could often be hampered by the availability of data, especially in fields where certain types of results are likely to face additional scrutiny in the publication process.

FUTURE DIRECTIONS

Several key questions about the pollinator-mediated impacts of alien neighbours remain unanswered: (1) is the impact of an alien plant species on native visitation/reproductive success *disproportionate to its attractiveness/density*?; (2) is competition for pollination one of the avenues by which alien plant species become invasive?; and (3) what long-term effects do we expect from the disruption of plant-pollinator interactions (either facilitation or competition) through invasion? To address the first question, more studies are required in which the effect of an alien plant on native plant visitation and reproduction is compared to the effect of a similarly attractive native plant (e.g., Cariveau & Norton 2009). To address the second question, investigations would be better directed at comparing the competitive abilities (for attraction of pollinators) of invasive and naturalized alien plants. More work focusing on the direct impacts of alien introductions on pollinator communities (e.g., Stout & Tiedeken 2017), and the effects of targeted ecosystem restoration (e.g., Kaiser-Bunbury *et al.* 2017), would help address the third question. Overall, more studies that focus on the impact of an alien on multiple native species across a variety of community contexts (e.g., Goodell & Parker 2017), are badly needed.

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FIGURES

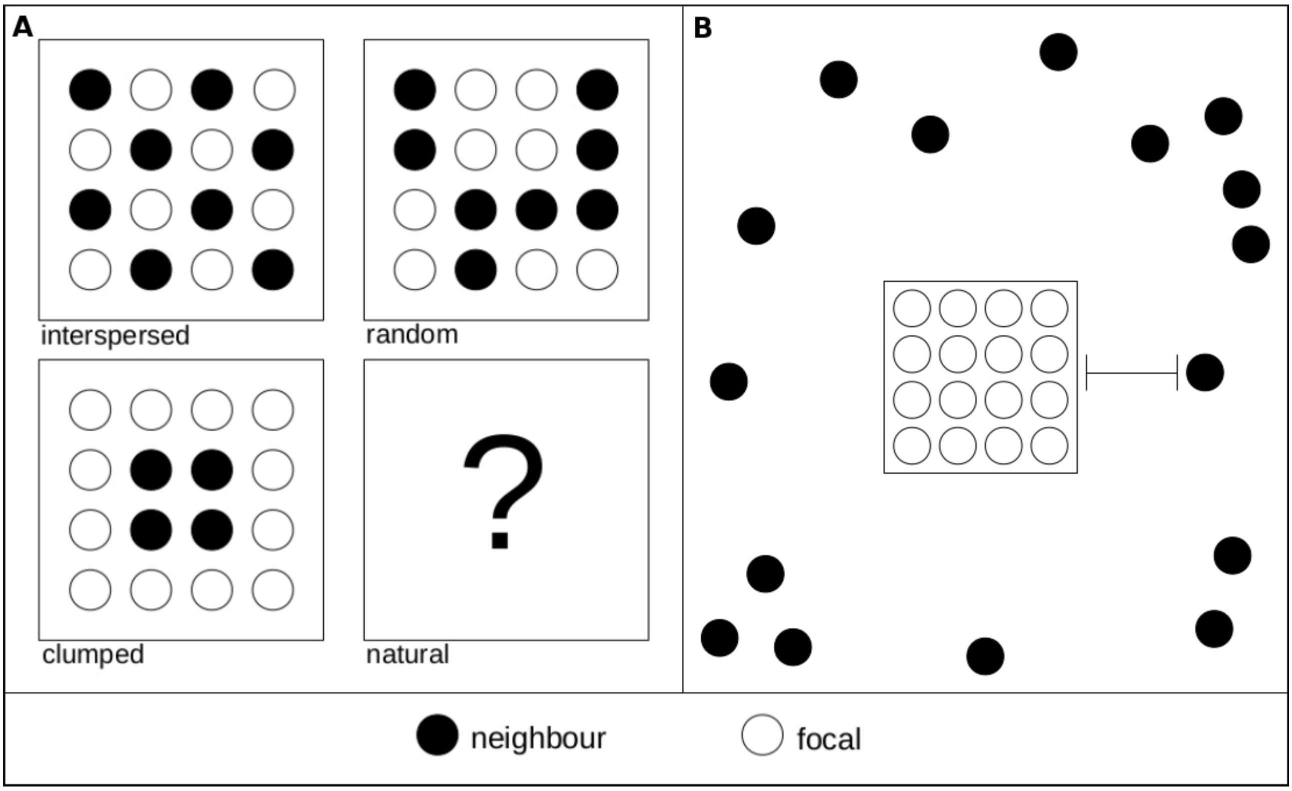

Figure 2.1. Graphical representation of different arrangements of neighbour and focal plants in treatment groups (A), and of the definition of the factor “distance of the control group to the nearest neighbour plant” (B).

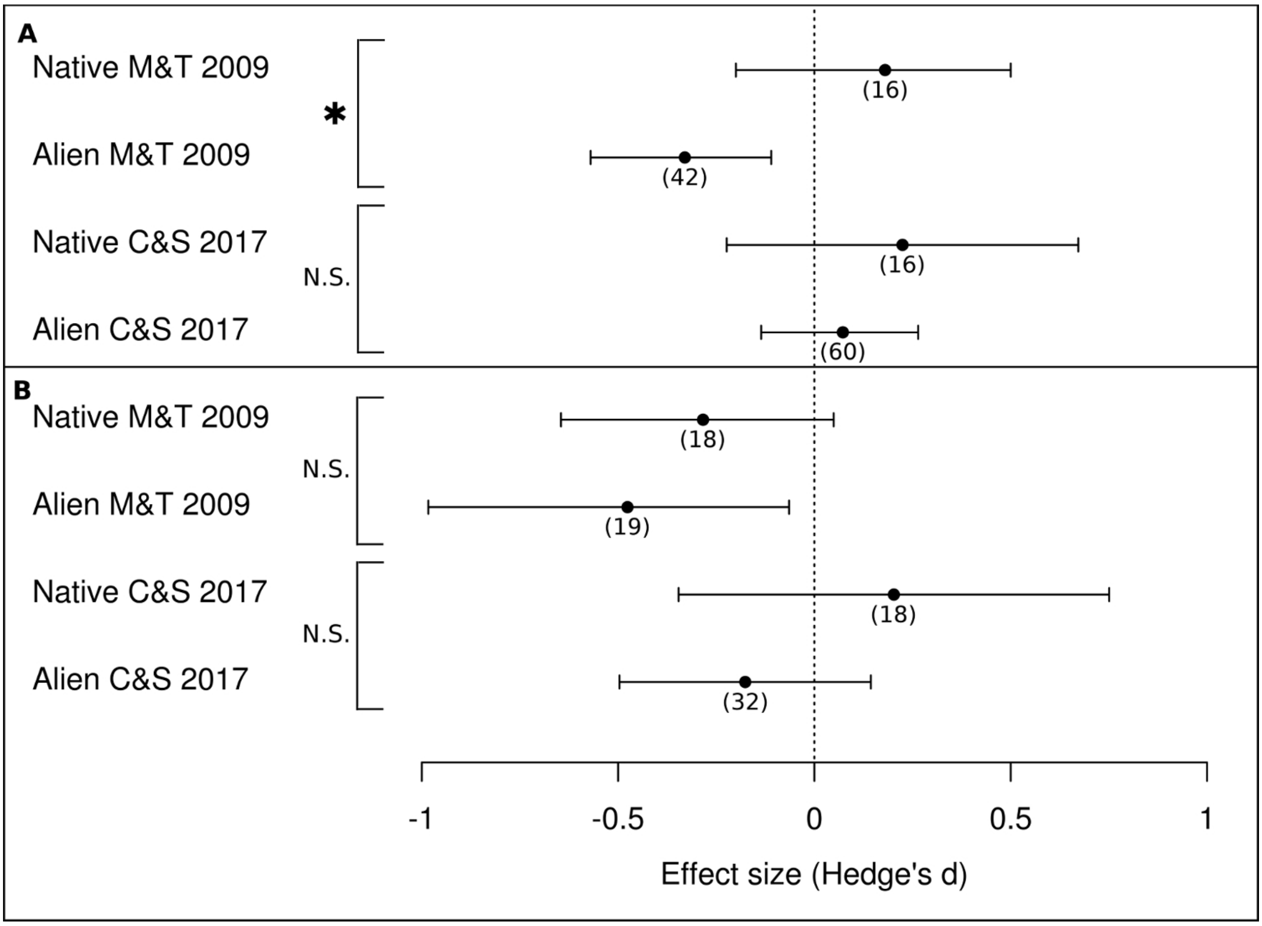

Figure 2.2. Forest plot of effect size and bootstrap-corrected 95%CIs in a prior meta-analysis MT (Morales and Traveset (2009)), and Knapp and Hartung-adjusted estimates and 95%CIs for the current data set (CS), examining the impact of alien or native neighbors on visitation (A) and reproductive success (B). Sample size for each estimate indicated in parentheses. Statistical results of pairwise comparisons between group estimates presented on the left, significant comparisons indicated with *, non-significant differences denoted N.S.

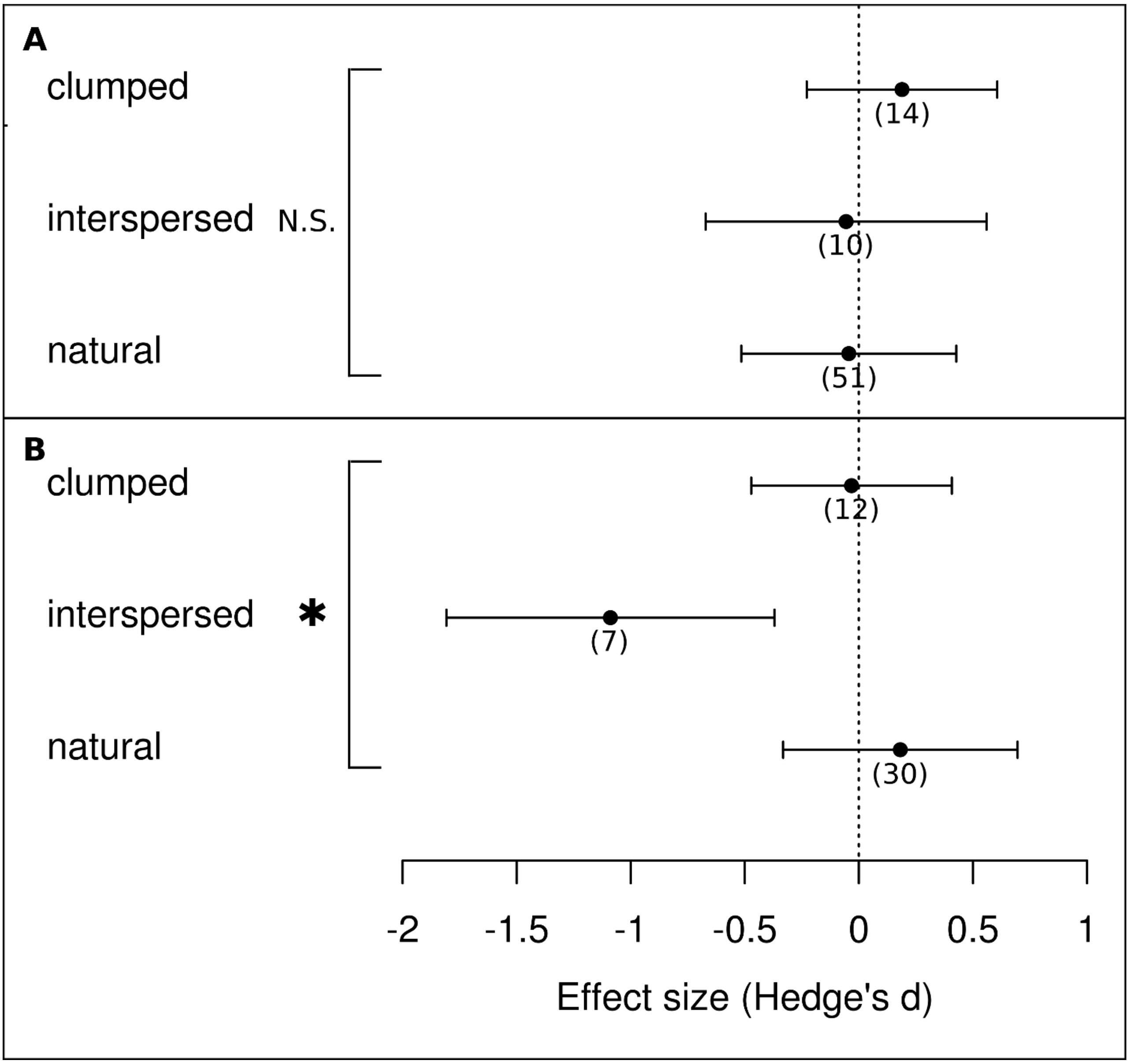

Figure 2.3. Forest plot of Knapp and Hartung-adjusted 95%CIs of the effect of arrangement of neighbour and focal plants in the treament group on (A) visitation and (B) reproductive success; sample sizes contributing to each group estimate indicated in parentheses. Brackets indicate differences in estimates depending on category membership (N.S. = not signficiant, * = significant)

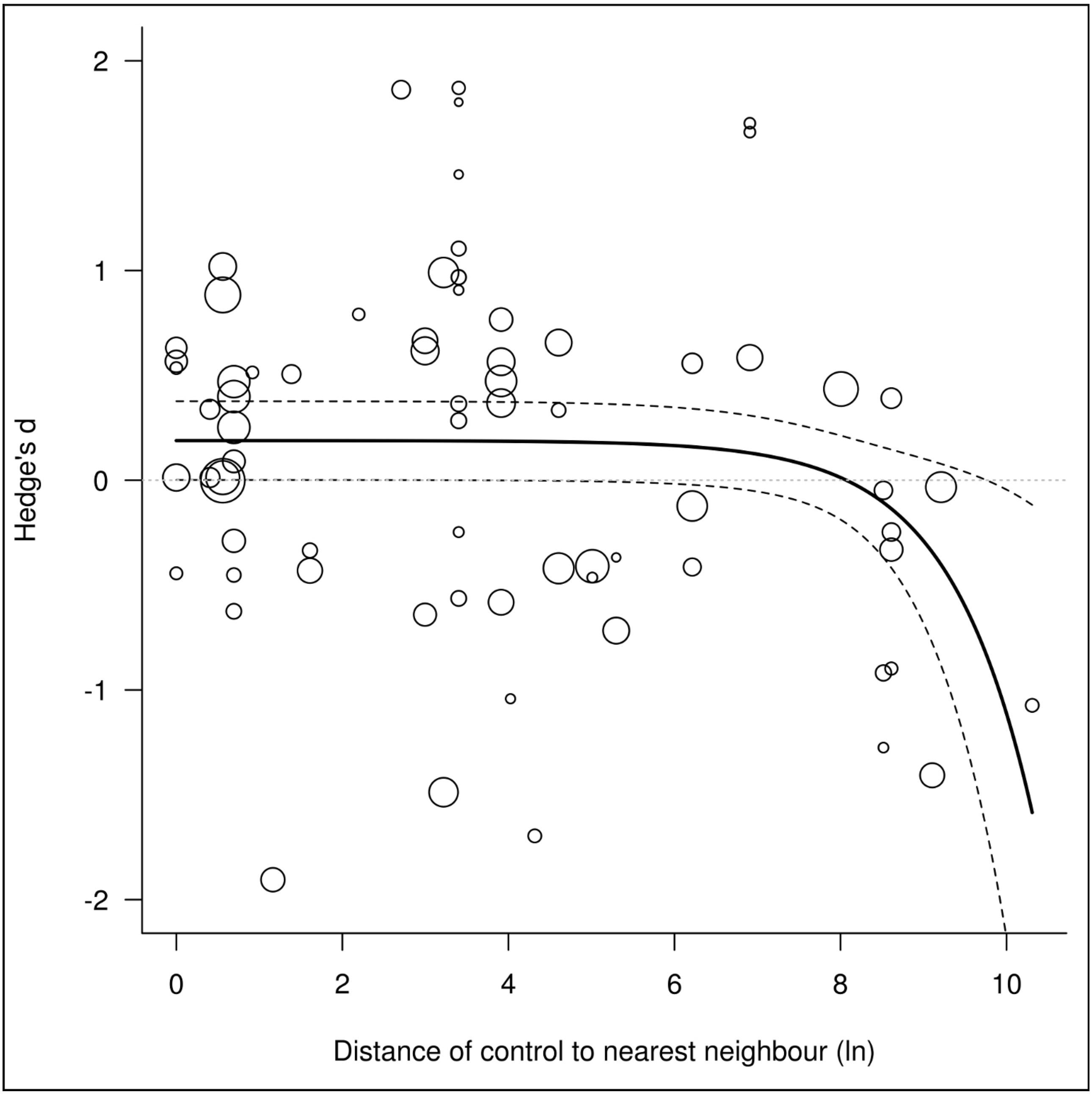

Figure 2.4. Standardized mean difference in visitation to native plants in [presence - absence] of a potential competitor by distance from that competitor in the control group. Point size reflects study weighting in the model, by inverse of variance. Solid line displays model regression (y = -0.0001x + 0.198) (d_{slope}: -0.001 (-0.0001 to -0.0000); $d_{intercept}$: 0.189 (0.001 to 0.377)), converted to a natural log scale to encompass the entire range of x values (1m:30,000m); dotted lines show 95%CI.

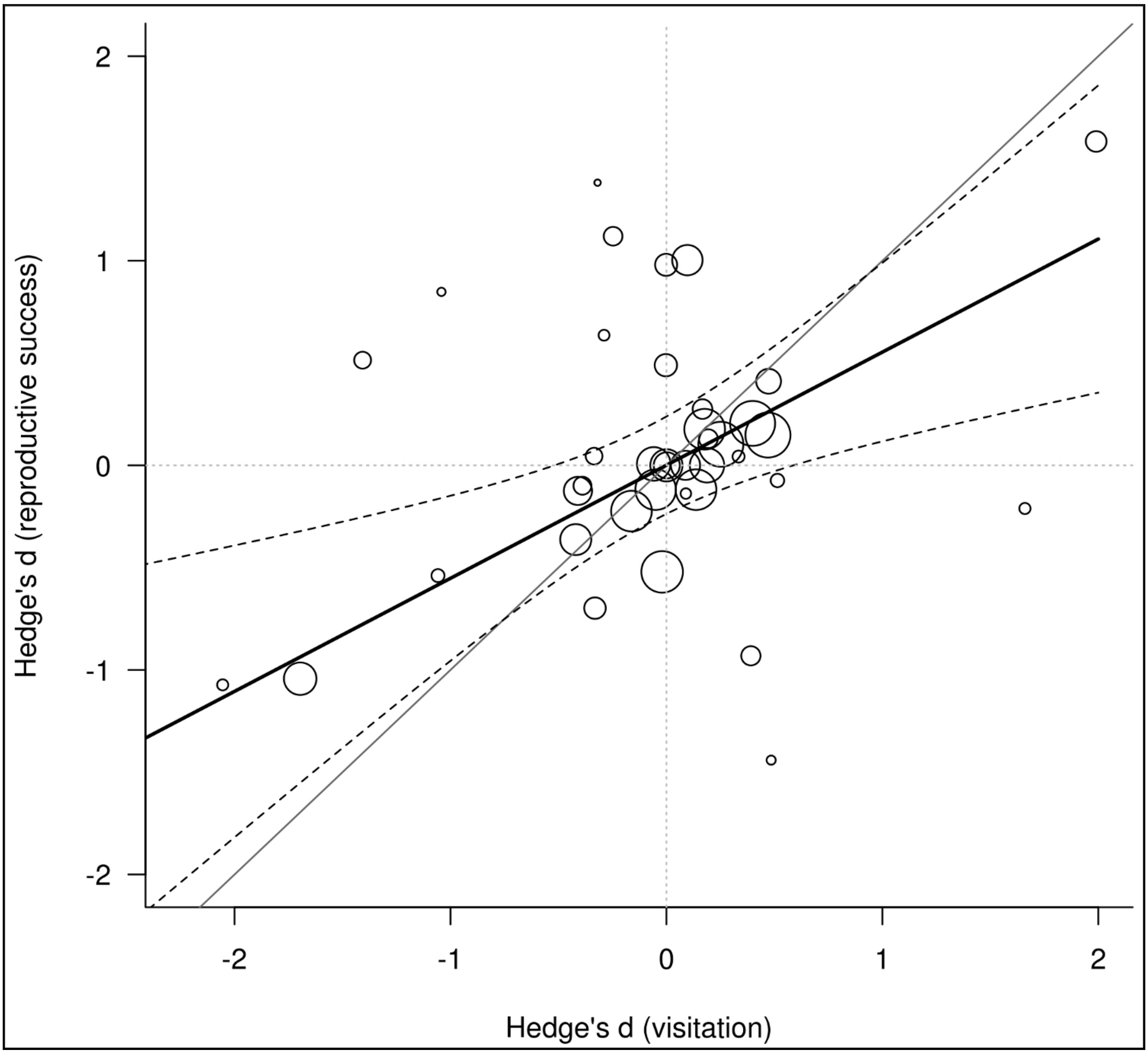

Figure 2.5. Relationship between change in visitation and change in reproductive success. The solid black line shows the predicted relationship between change in visitation and change in reproductive success (y = 0.5528x + 0.0007; d_{slope} = 0.552 (0.026, 0.899); $d_{intercept}$ = 0.0007 (-0.237 to 0.239)); the black dotted lines show the 95%CI on the estimated relationship; the solid grey line shows the expected slope of a perfect relationship between change in visitation and change in seed set; the grey dotted lines show the zero line of each axis.

CHAPTER 3: HOW FAR DOES AN ALIEN REACH? A FIELD TEST OF THE ROLE OF SPATIAL PROXIMITY IN POLLINATOR-MEDIATED INTERACTIONS BETWEEN ALIEN AND NATIVE PLANTS

Charlebois, Julia

ABSTRACT

Pollinator-mediated interactions between alien and native plants are one of the most extensively studied areas of invasion biology. However, the majority of studies on the topic consider interactions at only a single spatial scale. Here I evaluate the influence of spatial scale on visitation rates by pollinators from six different functional groups to naturally existing plant communities that varied in their distance from an introduced array of the alien plant species, *Cirsium vulgare*. Visitor frequency depended significantly on visitor functional group. Ants, commonly-disregarded in studies of plant-pollinator interactions, along with Diptera and honeybees, were the most frequent visitors to the experimental plants. I found non-significant patterns of visitation by distance from the alien plant arrays. Due to low sample size and high variance, however, this null result does not conclusively refute the hypothesis that pollinator-mediated interactions between plants may depend on spatial proximity and visitor functional group.

INTRODUCTION

Interactions between alien and native plants have received extensive research attention (Levine 2003). Recent research strongly suggests that community context matters in biotic interactions (reviewed by Strauss and Lau 2006). Indeed, Chamberlain *et al.* (2014), in a meta-analysis that compiled studies of species interactions, found that species interactions are context-dependent, and that studies of mutualisms in particular were highly variable in their outcomes (i.e., competition, facilitation, or neutral) depending on the location of the study (i.e., different geographic sites).

Similarly, a large-scale experiment examining biomass, growth, and reproduction of 115 plant species in 11 mountain ranges found that interactions between plants were increasingly facilitative with increasing elevation and that there was a greater frequency of positive interactions at low temperatures, regardless of elevation (Callaway *et al.* 2002). Similarly, the effect of heterospecific neighbouring plants on plant-herbivore interactions has been proposed to vary across scales: at the landscape scale, the presence of a heterospecific neighbour may affect the response of the consumer to a patch of focal plants; while at the local scale (i.e., within a patch), a heterospecific neighbour may directly impact the attractiveness of a focal plant and thereby modify consumer response to individual focal plants (Hambäck *et al.* 2014); the effects of the presence of the neighbour on consumer choice may differ at these two scales, resulting in complex spatially-dependent biotic interactions (Underwood *et al.* 2014). Underwood *et al.* (2014) suggested that these complexities may extend to plant-pollinator interactions.

Although the potential for alien plants to disrupt native plant-pollinator mutualisms has been studied extensively (Skurski *et al.* 2014), almost all previous analyses on this topic have tested the possible interaction between plants at only a single spatial scale (Chapter 2). And yet, several studies have demonstrated that facilitation for pollinator services at one scale may be linked to competition at another (Albrecht *et al.* 2007, Bruckman and Campbell 2016), particularly if facilitation and competition are linked across a landscape due to spatially-dependent pollinator responses to plants (Hegland 2014). It has also been proposed that plant-pollinator interactions may be location- and/or site-dependent (Burkle *et al.* 2016). Understanding how variability in space and time alters biotic interactions is critical for determining the ecological and evolutionary impacts of these interactions (Underwood *et al.* 2014). Understanding how context alters species interactions is a critical missing link in our understanding of how pollination shapes plant populations (Maron *et al.* 2014).

Competitive (e.g., Chittka and Schürkens 2001, Baskett *et al.* 2011), neutral (e.g., Kaiser-Bunbury and Müller 2009), and facilitative (e.g., Ferrero *et al.* 2013, Masters and Emery 2015) pollinator-mediated impacts of alien plants on natives have been demonstrated across a variety of different systems, and in studies that examine the pairwise interactions between one alien and multiple native plants in the same community, outcomes are mixed (e.g. Thijs *et al.* 2012, Woods *et al.* 2012, Gibson *et al.* 2013, Goodell and Parker 2017). It has been speculated that the diversity of outcomes of pollinator-mediated interactions among alien and native plant species may, at least partially, reflect differences in the spatial scale at which different studies are conducted (Jakobsson *et al.* 2009). Moreover, it seems likely that scale-dependent responses of pollinators to community context will differ by pollinator functional group: larger pollinators such as honeybees and bumble bees are likely to be affected by landscape characteristics at greater distances than solitary bees, because they have longer flight distances, have individuals specialized for foraging, can recruit other foragers, and are more social (Steffan-Dewenter *et al.* 2002, Albrecht *et al.* 2007). Indeed, Albrecht *et al.* (2016) demonstrated that honeybees and bumblebees showed a greater response to landscape-scale presence of an alien plant than did non-corbiculate wild bees, which responded primarily to a difference in alien presence at a smaller spatial scale. Pollinators differ in their foraging behaviour; they may therefore differ in the scale of their response to landscape characteristics.

One aspect of foraging behaviour that changes with pollinator type is foraging range, i.e., the distance a pollinator travels from its nest in order to obtain resources. Thies *et al.* (2003) proposed that the size of the relevant spatial domains of organisms – at different trophic levels, at least – may alter the scale of their response to landscape characteristics. If this extends to pollinators, then those with larger foraging ranges may have larger relevant spatial domains and consequently respond to landscape characteristics at a larger scale. Pollinator foraging ranges vary considerably across functional groups: maximum foraging distances for *Bombus* spp. (bumblebees) are estimated to range from 450 – 800 m (Osborne *et al.* 1999, Darvill *et al.* 2004, Knight *et al.* 2005, Wolf and Moritz 2008), while *Apis mellifera* (honeybees) has been observed to forage as far as 9 km (mean 5.5 km) from the hive in one year, with a mean of 1 km from the hive in a subsequent year (Beekman and Ratnieks 1998). Maximum solitary bee foraging distances range from 150 m – 1.4 km (Gathmann and Tscharntke 2002, Zurbuchen *et al.* 2010), and larger solitary bees have the greater maximum foraging distances (Gathmann and Tscharntke 2002). One seed-eating North American ant species, *Pogonomyrmex barbatus*, forages within 10 – 20 m of the nest (Gordon 1995). These differences in foraging range may alter the size of relevant landscape for each forager type, and consequently their response to spatial relationships between plants.

Pollinator responses to spatial relationships among plants may also be affected by foraging bout duration, i.e., the length of time that a pollinator spends visiting plants in a patch. For example, ants typically spend several hours on foraging trips outside the colony (Gordon 1995), while honeybee foraging bouts can last from 10 – 16 minutes (Mattu and Bhagat 2016), and solitary bee foraging bouts increase with bee size and range from 6 – 28 minutes (Gathmann and Tscharntke 2002). Foragers that engage in longer foraging bouts may travel farther and visit more plants, therefore have a larger relevant patch size, and thus respond to patch characteristics at greater scales.

Some recent research has examined the effect of spatial scale and pollinator identity on the outcome of pollinator-mediated interactions. Albrecht *et al.* (2016), in a study of the effect of local- and landscape-scale presence of the invasive alien *Oxalis pes-caprae* on visitation and reproductive success of the native *Diplotaxis erucoides*, found that the alien facilitated visitation to the native plant when present at both landscape and local scales, relative to visitation when it was present at only one or neither of these scales, but that this only led to improved seed set of the native plant when the alien was present at both scales. They further concluded that the larger-scale response of larger pollinators such as the honeybee, *Apis mellifera,* as compared with smaller solitary bees, which responded only to local-scale community characteristics, might partially explain these findings. Conversely, a similar study examining the effect of the showy alien *O. pes-caprae* on visitation to the native *D. erucoides* at local, landscape, or both scales, showed that there was no difference in visitation to the native plant when the alien was present or absent at both scales, but that when the alien *O.s pes-caprae* was present at the landscape scale and absent at the local scale, visitation to the *D. erucoides* increased (Jakobsson *et al.* 2009). Similarly, Bruckman and Campbell (2016) showed that the showy alien plant *Brassica nigra*, when present at the local scale at both high and low densities, increased visitation to the native *Phacelia parryi*. However, it reduced visitation to the native plant when present only at the landscape scale, suggesting movement of pollinators away from the native. Increased visitation to the native *P. parryi* only resulted in improved seed set when the alien was present at low densities, as there was greater post-pollination interference through heterospecific pollen densities when the alien *Brassica nigra* was present at higher densities.

Such scale-dependent effects may operate even at much smaller scales than those studied using landscape-ecological approaches: Cariveau and Norton (2009) established experimental arrays, with the attractive alien *Carduus nutans* in the centre, then observed visitation to native *Monarda fistulosa* placed at 1, 5, 10, and 15 m from the alien plants. They found that visitation rates were highest closest (1 m) and farthest (15 m) from the attractive alien, while visitation rates were significantly lower between (5 m, 10 m). Indeed, in Chapter 2, I showed that the definition of the control group (i.e., the distance between ‘control’ plants and the nearest neighbour) in studies of pollinator-mediated interactions between plant species affects the outcome, and that it may be better (in terms of visitation) for a native plant to be very close to an alien than moderately distant from it. In this chapter, I follow up on this using the range of distances commonly used to define a control group in studies of pollinator-mediated interactions between plants (i.e., < 50 m), and further examine the role of visitor functional group in shaping the relationship between distance from an attractive generalist plant and visitation to native plants.

Because pollinator-mediated responses to community and individual floral characteristics may be distance-dependent even at relatively small scales (i.e., < 50 m), and because relevant scales may vary by foraging behaviour, I hypothesized that the effect of an attractive alien plant on visitation rates to a native plant community will vary by distance and by the visitor functional group.

Here I describe a field experiment that involved the introduction of arrays of the attractive generalist invasive alien species *Cirsium vulgare* (Savi) Ten. to an old field. I then observed pollinator visitation to plants in the community along a gradient of distances from the introduced array. My study addresses two major questions about pollinator visitation to a plant community in response to the introduction of an alien plant:

(1) How does distance from an introduced array of plants influence its impact (i.e. magnitude, direction) on pollinator visitation to local plants?

(2) Does the influence of distance from the array of alien plants on the impact/direction of plant-pollinator interactions depend on the pollinator functional group?

I predicted that visitation rates should differ significantly according to proximity to the alien plant array; I did not predict any particular shape of response, given the scarcity of experimental evidence in this area, the results of previous work indicate that visitation rates should change by distance from the attractive array (e.g., Cariveau and Norton 2009). I further predicted that, because some visitors have different foraging bout range and duration, and because foraging bout range and duration should alter the relevant scale of response (Hambäck *et al.* 2014), that different functional groups would respond to floral resources at different spacial scales (e.g., Jakobsson *et al.* 2009, Albrecht *et al.* 2016, Bruckman and Campbell 2016). Because experimental data in this area is currently sparse, I had no specific *a priori* predictions about the shape or direction of this relationship.

METHODS

Study species

Cirsium vulgare (Savi) Ten. (Asteraceae) is an introduced species found in all 10 Canadian provinces; it is listed as a noxious weed in Ontario, Saskatchewan, and Manitoba, as an invasive plant in British Columbia, and as an agricultural weed in Quebec (Plants of Canada Database 2015). It is widespread across Canada, particularly in agricultural areas (Klinkhamer and de Jong 1993), and produces local populations varying widely in size (a few individuals to several hundred) and density (from 0.01/m^2 – 8/m^2) (Klinkhamer and de Jong 1993).

Cirsium vulgare is a primarily outcrossing biennial: in the first year, it germinates and develops a large taproot up to 70cm long (Klinkhamer and de Jong 1993); in the second year, it produces nectar-rich light purple flowers presented in terminal groups of 1-3 capitulae on central or branching stalks (Michaux 1989). Each capitulum produces over 200 seeds; large individual plants can produce over 50,000 seeds, the majority of which are dispersed within 1.5 times the height of the plant (Michaux 1989). *Cirsium vulgare* was selected for this study because it is a highly attractive generalist-pollinated plant and thus likely to draw a wide range of visitors away from local plants; known floral visitors to *C. vulgare* include *Apis mellifera* (honeybees), *Bombus* spp. (bumble bees), Syrphidae, adult Lepidoptera, Thysanoptera, and Hymenoptera (*Leioproctus* spp.) (Michaux 1989), Bombyliidae, and Syrphidae (Klinkhamer and de Jong 1993).

The focal plants in this study were not restricted to one species; whatever flowering species occurred in the randomly located community plots (described below) were included. Although there were a large number of flowering plants at the field site, the flowering community was reasonably uniform: in most places the site was dominated by a single species, with occasional individuals of many other species occurring throughout. At the time of the study, the local flowering community was dominated primarily by *Solidago* spp. (Asteraceae), particularly *S. canadensis* L. Other species occurred at low frequency but with reasonable consistency, including *Centaurea jacea* L. (Asteraceae), *Spiraea alba* Du Roi (Rosaceae), *Aster* spp. (Asteraceae), *Eutrochium purpureum* (L.) E. E. Lamont (Asteraceae), and *Pastinaca sativa* L. (Apiaceae). A list of flowering species at the field site, their estimated prevalence, distribution across the field, occurrence in the plots, and proportion of visits received, are provided in Supplement 3. Note that some of these species are considered aliens in the region of this field site (specified in Supplement 3).

The dominant flowering species in the field site were species in the genus *Solidago*. *Solidago* are native to Canada and characteristic of abandoned farmland and occasionally grazed pastures, waste areas, and tall-grass prairies (Werner *et al.* 1980). They are rhizomatous perennials producing stems 25-200cm tall topped by a terminal paniculum with recurved-secund branches and small yellow capitulae composed of a few florets (Werner *et al.* 1980). Ray florets are female and fertile, disc florets bisexual and fertile; the fruit is an achene with pappus, dispersed by wind primarily within 0.3-0.6m of the parent plant (Werner *et al.* 1980). *Solidago canadensis* spreads aggressively and is considered a noxious weed in Europe where it has been introduced (Werner *et al.* 1980). *Solidago* are an important source of nectar for honeybees; they are frequently visited by bumble bees, and are associated with diverse insects whose interactions with the plant are unknown, including beetles, flies, lepidopteran larvae, moths, etc. (Werner *et al.* 1980).

Experimental plants

More than 400 *Cirsium vulgare* individuals in their second (flowering) year were collected June - July from pastures and recently logged clearings < 9 km from the field site (2nd year plants only) and potted in three- or five-gallon pots depending on the size of the taproot, and maintained at a location 10 km from the field site. The field site was searched on foot for *C. vulgare*, and these plants were removed (< 15 individuals) and added to the experimental population. The plants were tied to stakes to support the stems and watered daily.

Field site

The experiment was carried out in July and August 2016 at a 42 ha abandoned field in Bouchette, QC (46.188, -75.949). The site was formerly maintained for forage haying of *Phleum pratense* L. (Timothy grass) but this activity ceased prior to 2012. The site was subsequently left fallow and has now been colonized by a variety of introduced and native species of flowering plants and small shrubs. A map of the site is provided in Figure 3.1A. The site is 250 m wide and 1.5 km long, with the short ends bordered along the west by the Gatineau River and along the east by mixed deciduous forest. Along the north side is an additional lot, 250 m wide and 1.5km long, which is also abandoned, and beyond this abandoned strip is an active farm including corn fields and pasture for cattle. Finally, along the south side an area 500 m wide and 1.5 km long is likewise abandoned, with active pastureland beyond. The site is bisected by a narrow dirt road running east-west. There are two irrigation ditches approx. 2m deep diverting a stream into the site and running north-south, both densely overgrown with trees and shrubs rendering their immediate environs unusable for the field research. The western half of the site has drainage ditches (approx. 1.5 m deep) along the road, also overgrown and unsuitable for research use. A relatively small portion of the site in the northwest was low and damp, dominated by wetland grasses and reeds, with extremely rare occurrences of *Iris versicolor* L. (Iridaceae); this area was treated as unsuitable for the experiment, as it contained almost no animal-pollinated flowering plants of any species. The canopy at the site is entirely open, and the majority of the plants at the site were forbs and grasses.

Because the sap of *P. sativa* can cause phytophotodermatitis (Cain *et al.* 2010), a portion of the site dominated by this species was excluded from the research area. Research was conducted in places where *P. sativa* density was low enough that a person could navigate through the plants without significant risk of harm.

To facilitate navigation throughout the field, a grid of guiding poles was placed throughout the site at 50 m intervals along the north-south line and at 100m intervals east-west.

Experimental Design

Each replicate consisted of an array of 15 flowering potted *C. vulgare* individuals, selected haphazardly from the source population and placed at a random location within the field. Plants were chosen to ensure a reasonably balanced number of open capitulae at each replicate; however, because the plants were subsequently left for several days in the field, the actual number of open capitulae at the time of the observations varied from approx. 25-40. I used 15 individuals because this was the approximate size of medium-sized populations of this species locally. Random locations in the field were generated with a random number table and applied to a coordinate system that treated the southwest corner of the site as (0,0). Each randomly determined location for a replicate was designated the centre, and around this centre the 15 potted plants were placed in a circle at the locally normal density of 1-2 m distance between individuals. If the random location fell within one of the excluded zones described above, or was within 200 m of another replicate, a new random location was selected.

Once plants were placed in the array they were maintained for a minimum of 3 days before observations were begun, as is common practice in order to ensure that the local pollinator populations had sufficient time to find and respond to the new resource. At any given time, there were a maximum of 3 arrays of *C. vulgare* at the field site, at a minimum distance of 200 m apart.

Once the array had been in the field for minimum 3 days, pollinator observations were conducted over the course of a single day at the following set of distances from the *C. vulgare* array: 1 m, 5 m, 10 m, 15 m, 20 m, 30 m, 40 m, and 50 m. These distances were selected based on the frequently-chosen distances between control and nearest neighbour plants as described in previous studies (see Chapter 2). Each observation plot, consisting of a circle with 0.5 m radius, was placed at the assigned distance along a random compass bearing away from the array of *C. vulgare* (Figure 3.1), within a 1 m allowance to ensure that the plot contained flowering plants. If a location did not contain flowering plants within a metre, or if it was located in one of the excluded areas described above, a new random compass bearing was selected and the plot was placed instead along this new random compass bearing. The plants within each observation plot were then surveyed and all entomophilous flowering species were identified and the number of flowering and vegetative stems of each species was recorded. The total number of flowering stems in the plot was used to estimate floral density – as most plants were *Solidago* spp., this was a fairly uniform method of estimating floral density.

Plots were observed for 20 minute intervals; each plot was observed a minimum of 2 times during the period of the experiment. The order of the observations was randomly determined but stratified by morning and afternoon, so that each plot at each distance (per array) had at least one morning and one afternoon observation. Generally, plants were observed between 9:30am and 5:30pm. Observations were delayed if conditions that might inhibit pollinator activity occurred (e.g. cold, wind) (Kearns and Inouye 1993).

During each 20 min observation period, every visit by any insect (no other visitor types were observed visiting flowers during the course of the experiment) was recorded and the insect identified 'on the wing' to functional group (i.e., honeybee (*A. mellifera*), bumble bee (*Bombus* spp.), solitary bee/wasp, syrphid fly, fly (non-syrphid), Coleoptera, moth, skipper, butterfly, ant, other). An insect was considered a visitor when it made contact with the reproductive organs of the flower (Kearns and Inouye 1993). In the event that an insect made contact with the reproductive organs, ended contact (i.e., flew away or returned to the stem if non-flying), and then returned, the return was counted as a new visit.

Visitation rate for each visitor in every functional group category was calculated as the number of observed visits per hour. I divided visitation rates by the number of flowering stems in the visitation plots in order to control for density effects; hereafter this visitation rate will be referred to as visits/flower/hour. I did not control for diversity as almost all plots were dominated by one species to the exclusion or near-exclusion of all other species; plots were primarily dominated by *Solidago*, and rarely dominated by *C. jacea*.

Once the observations for each replicate were completed, the *C. vulgare* array was moved to a new random location in the field and any plants no longer flowering were replaced with flowering individuals from the source population.

This procedure was repeated 16 times from July 29 to August 31, 2016, resulting in a total of 16 independent replicates for analysis.

To ensure that no *C. vulgare* seeds were scattered at the site, the plants were checked regularly and senescing capitulae were cut, bagged, and subsequently autoclaved and composted off-site. At the end of the study, all flowers were bagged and disposed of, and the plants were uprooted from their pots and burned. The pots were emptied and sterilized.

Statistical analysis

In order to meet the assumption of normality, the response variable was (natural) log-transformed prior to modelling. I fitted a log-normal Linear Mixed Model (LMM) ([1]) with visitation rate (visits/flower/hour) as the response variable. Explanatory variables (fixed, categorical) were: visitor functional group (i.e., honeybee, bumblebee, solitary bee/wasp, Diptera, ant, or ‘other’), distance from the *C. vulgare* population (close = 1m, 5m, 10m; mid-distance = 15m, 20m, 30m; far = 40m, 50m), and the interaction between visitor functional group and distance (see reasons for pooling across explanatory variables below). Replicate, and therefore also day, as each replicate was observed on a single day, was also included as a random factor. Statistical analyses were run in R 3.2.3 (R Core Team 2015) using the lme4 package (Bates *et al.* 2015).

[1] visits/flower/hour ~ functional group * distance + (1 | replicate)

The initial model with all individual functional groups and distances was heteroscedastic, so the data were pooled across like factors, as indicated above, until it was possible to produce a final model that conformed to statistical assumptions (see Supplement 4).

Model least squared means and standard errors were estimated using the lsmeans package in R (Lenth 2016); because the model was fitted in log space, the standard errors converted to natural numbers are asymmetrical around the means. A table of the original logleast squared means and log standard errors, as well as the conversion into natural numbers, is provided in Supplement 4.

RESULTS

A total of 12 264 visits were observed in 128 observation plots placed at eight distances for each of the 16 replicates. Although we noted the presence of a total of 40 flowering plant species (Supplement 3), only 20 occurred in at least one of the observation plots; of these 20, 19 received at least one visit, but only 7 received more than 1% of the total observed visits throughout the experiment. The vast majority of visits were made to the most dominant species: *Solidago* spp. received 65% of observed visits; *C. jacea* received 25% of the visits; *S. alba* received 4% of the visits; *P. sativa* received 2% of the visits; *E. purpureum* received 2% of the visits; and *Aster umbellatus* received 2% of the visits.

The full LMM results are presented in Table 3.1. The final model adheres to the assumption of homoscedasticity of the variance, and that the response variable is normally distributed (Supplement 4).

There was a highly significant effect of visitor functional group on visits/flower/hour (Figure 2: $F = 12.85$, $df = 5$, $P < 0.0001$). The most frequent visitors were ants (1.53 visits/flower/hour, +SE/-SE 2.10/1.16), Diptera (1.21 visits/flower/hour, +SE/-SE 1.60/0.91), and honeybees (0.94 visits/flower/hour, +SE/-SE 1.26/0.70). Solitary bees and wasps were less frequent (0.61 visits/flower/hour, +SE/-SE 0.84/0.45), as were ‘other’ visitors (0.35 visits/flower/hour, +SE/-SE

0.51,0.25) . The least frequent visitors were bumblebees (0.18 visits/flower/hour, +SE/-SE 0.27/0.12).

Differences in visitation rates across distances (*F* = 1.090, *df* = 2, *P* = 0.338), and by distance and functional group (*F* = 0.576, *df* = 10, P = 0.833) were not significant (Figures 3.3, 3.4). Because the total sample size was low (*N* = 16), and the effect sizes fairly large (see below), it is not possible either to conclusively reject the hypothesis that visitation rates vary by distance or by visitor functional group, nor to reject the null hypothesis.

Across all visitor groups, visitation rates were lowest closest to the array of *C. vulgare* (0.57 visits/flower/hour, +SE/-SE 0.77/0.43), and at middle distances (0.61 visits/flower/hour, +SE/-SE 0.82/0.46), and were highest in the farthest plots from the arrays (0.75 visits/flower/hour, +SE/-SE 1.00/0.56).

The mean visitation rates and their associated standard errors for each functional group at each distance from the *C. vulgare* array are reported in Table 3.1. Honeybees and ants both visited the plots farthest from the *C. vulgare* array most frequently (40 – 50 m). These functional groups visited approximately 21-25% less when closest (1 – 10 m) to the *C. vulgare* array, and visited 55% and 34% less at middle distances (15 – 30 m) from the *C. vulgare* array. Diptera visited most frequently at 15 – 30 m and visited 17% less frequently at 1 – 10 m and 23% less frequently 40 – 50 m from the *C. vulgare* array; solitary bees and wasps visited very little within 10 m of the *C. vulgare* array, but increased their visitation rates by approximately 80% from 15 m onward; bumble bees visited too rarely to show any discernable pattern. Other insects visited the plots 46% less frequently within 1 – 30 m of the *C. vulgare* array than when 40 – 50 m away.

The statistical conclusions shown above remain unchanged when tested in a fixed model without accounting for the effect of replicate (effect of distance: *F* = 1.39, *P* = 0.25; distance * functional group: *F* = 0.58, *P* = 0.83), when the interaction term is dropped from the mixed model (effect of distance: *F* = 1.39, *P* = 0.25), and when the effect of distance is the only fixed effect included in the mixed-effects model (effect of distance: *F* = 1.16, *P* = 0.32).

DISCUSSION

I found only limited, non-significant support for the general claim that the outcomes of biotic interactions with mutualists are particularly variable across geographic space (Chamberlain *et al.* 2014), and failed to find support for the the extension of models of consumer choice at multiple spatial scales (Hambäck *et al.* 2014) into pollinator-mediated interactions. Finally, I could not confirm the existence of scale-dependence in the responses of floral visitor to community characteristics, nor could I confirm that these varied by visitor functional group (Steffan-Dewenter *et al.* 2002, Hegland 2014). Though neither the effect of distance from the alien array nor the interaction between functional group and distance are statistically significant, the effect sizes are large (up to 80% change in visitation rates by distance for some visitors); low sample size and high variance may be reducing power such that a potentially biologically significant effect cannot be detected; high variability in the data could be the result of environmental variability across date, or from variability by visitor functional group; this high variability might also be the result of genuine stochasticity in visitation. Consequently, although the model fails to reject the null hypothesis that there is no effect of distance, nor any interaction between distance and functional group, on visitation rates, the results are not sufficiently strong to contradict the findings of previous work suggesting that these factors should be important in affecting visitation rates (e.g., Cariveau and Norton 2009, Jakobsson *et al.* 2009, Albrecht *et al.* 2016, Bruckman and Campbell 2016). Visitation rates in this community differed significantly by pollinator functional group, suggesting that some floral visitors may have greater relative importance to plant reproductive success than others. Moreover, some common visitors are known to be at least occasional floral antagonists (e.g., ants, Coleoptera (Lindsey 1984)) and therefore interactions between the attractive alien neighbour and the local plant population through these visitors may be antagonistic visitor-mediated interactions, rather than pollinator-mediated interactions, further complicating interpretation.

Though pollinator-mediated interactions between plants have been extensively studied, ants are often omitted from discussions of the topic, either explicitly excluded (e.g., Aigner 2004, Baskett *et al.* 2011), or dismissed as a separate case (e.g. Moragues and Traveset 2005). The broad tendency to ignore ant visitors may stem from uncertainty about the role of ants in pollination systems: they may be nectar-thieving antagonists (Galen and Butchart 2003, Galen and Geib 2007), pollen-sterilizing antagonists (Galen and Butchart 2003), mutualists offering defense against herbivory in exchange for floral nectar (Yano 1994), or mutualist pollinators (Ashman and King 2005). However, the numerical dominance of ants as floral visitors in this system highlights the need for improved understanding of the impacts of ant floral visitation on plant fitness. Moreover, the second most frequent visitors are Diptera, which may impose different selection on plant traits than bee pollinators (Gervasi and Shiestl 2017).

The non-significant effect of scale- and visitor-dependent effects of the *C. vulgare* array on visitation to native plants is not consistent with previous findings suggesting that pollinators respond to community context (e.g., Albrecht *et al.* 2016, Bruckman and Campbell 2016), and that those responses differ by pollinator functional group (e.g. Albrecht *et al.* 2016). As some previous work has found effects even at smaller spatial scales (i.e., <15 m; Cariveau and Norton 2009), the lack of significance here may not be due to small spatial scale.

For visitors with complex responses including peaks and valleys of visitation (e.g., ants, honeybees, Diptera), the non-significant results resemble the possible outcomes suggested by Hamback *et al.* (2014)’s multi-step consumer decision-making process, wherein a consumer first chooses a patch in which to forage based on community characteristics and then chooses individuals on which to forage based on individual characteristics, can be extended to pollinator-mediated interactions between plants (Underwood *et al.* 2014). Other visitors (e.g., solitary bees and wasps, ‘other’ visitors) may respond only to information at a single spatial scale. Unfortunately, though the effect of functional group was significant, the effects of distance and its interaction with functional group were not significant.

CONCLUSIONS

I do not find evidence to support the claim that floral visitor-mediated interactions between plants are scale- and visitor-dependent, as has been suggested for various biotic interactions (Chamberlain *et al.* 2014, Burkle *et al.* 2016). Previous work has established that facilitation and competition for pollination can coexist across landscape and local scales in a single system (Jakobsson *et al.* 2009, Albrecht *et al.* 2016, Bruckman and Campbell 2016), and that differences are detectable even at very small scales (e.g. Cariveau and Norton 2009). The results presented here cannot support the hypothesis that these spatial patterns of competition and facilitation may also occur over relatively small scales and depend on the visitor functional group, which has been previously shown at landscape scales (Steffan-Dewenter *et al.* 2002, Albrecht *et al.* 2007). The proposed mechanisms should apply equally to attractive native generalists as to attractive aliens, and ought to reflect a broader set of principles with respect to the role of spatial scale in pollinator-mediated interactions between plants.

I did, however, find evidence for significant differences in visitation rates by different pollinator functional groups, which may affect the outcomes of pollinator-mediated interactions between plants and their impacts on plant fitness. Ants may play a larger role in floral visitor-mediated interactions than generally assumed, as potential floral mutualists or antagonists; consequently, their importance in pollination may be understated in some systems.

FUTURE DIRECTIONS

Further work needs to be conducted in order to establish whether the non-significant patterns of functional group-dependent spatial effects of an alien plant on native plant visitation are non-significant because there is no effect, or if low sample size and high variance are preventing the detection of a true effect.

Further investigation in this direction should make some minor modifications to design which might help to reduce variance: 1) restriction of observations to only one plant type, e.g., *Solidago* spp.; and 2) focus on replication by julian date rather than across space, i.e., observe visitation rates at multiple replicates simultaneously.

If the effect can be confirmed, this will raise a several other questions as well: (1) How does the size of the alien plant array influence the relationship between visitation rate, distance, and functional group?; (2) Is this effect limited to alien neighbour species, or does it represent a more general pattern?; (3) Does the effect change depending on the focal plant being visited?; and (4) Do visitor foraging range and foraging bout lengths explain the differences in the spatial patterns observed?

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TABLES

Table 3.1. Results of log-normal general linear mixed model, with F test of factor significance.

Factor	**Sum Sq**	**Mean Sq**	**Num Df**	**F**	**P > (F)**
Distance from *C. vulgare array*	3.369	1.6847	2	1.09	0.34
Visitor functional group	99.326	19.8653	5	12.85	>0.0001
Distance from *C. vulgare* array * visitor functional group	8.905	0.8905	10	0.58	0.83

Table 3.2. Least squares mean visitation rate and standard error by visitor functional group and distance from the *C. vulgare* array.

Distance from *C. vulgare*	**LS mean visits/flower/hour**	**-SE**	**+SE**
Ants			
1-10m	1.44	1.04	1.99
15-30m	1.28	0.92	1.79
40-50m	1.93	1.42	2.62
Dipterans			
1-10m	1.16	0.83	1.63
15-30m	1.40	1.01	1.93
40-50m	1.08	0.77	1.53
Honeybees			
1-10m	1.05	0.74	1.48
15-30m	0.60	0.40	0.90
40-50m	1.34	0.96	1.85
Solitary bees & wasps			
1-10m	0.42	0.27	0.66
15-30m	0.75	0.51	1.09
40-50m	0.74	0.50	1.08
Other visitors			
1-10m	0.29	0.17	0.48
15-30m	0.29	0.18	0.49
40-50m	0.54	0.36	0.83
Bumble bees			
1-10m	0.17	0.09	0.32
15-30m	0.22	0.12	0.39
40-50m	0.16	0.08	0.31

FIGURES

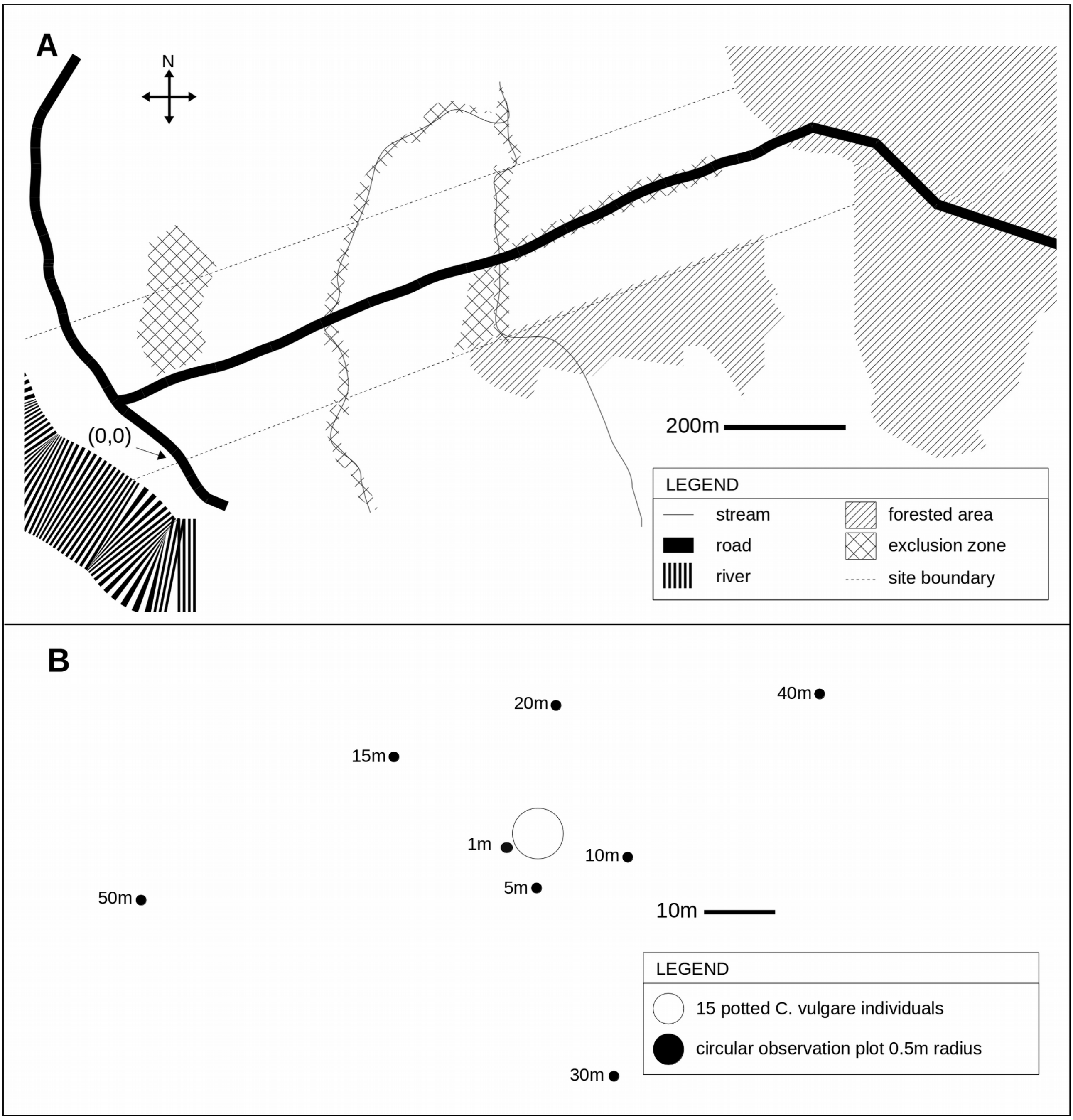

Figure 1. (A) Map of the field site showing site boundaries, general topography, point selected as origin (0,0) for the coordinate system of replicate placement, and areas which were excluded from the study, and (B) Schema of a hypothetical replicate showing the central *C. vulgare* array placed following a random coordinate system, and the 0.5m-radius observation plots at fixed distances and random compass bearings from the *C. vulgare* array.

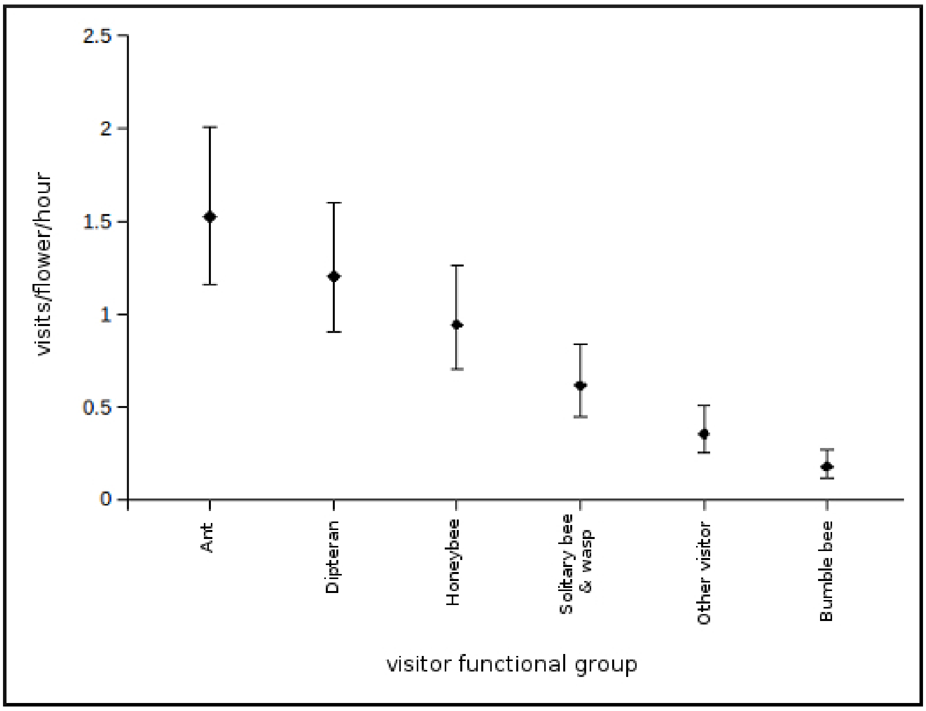

Figure 2. LMM least squares mean (±SE) visitation rate for each visitor functional group, pooled across distances.

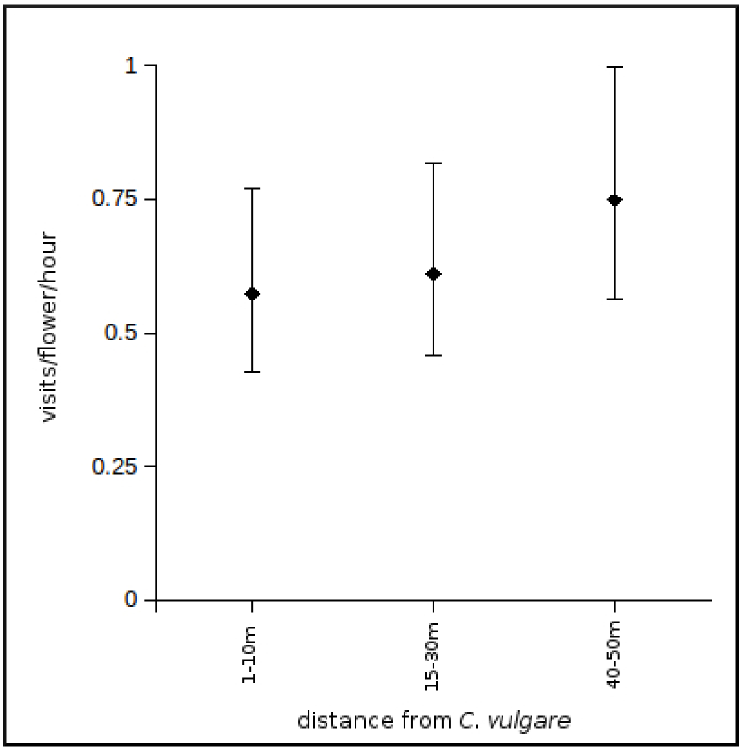

Figure 3. LMM least squares mean (±SE) visitation rate for each distance from the *C. vulgare* array, pooled across visitor functional groups.

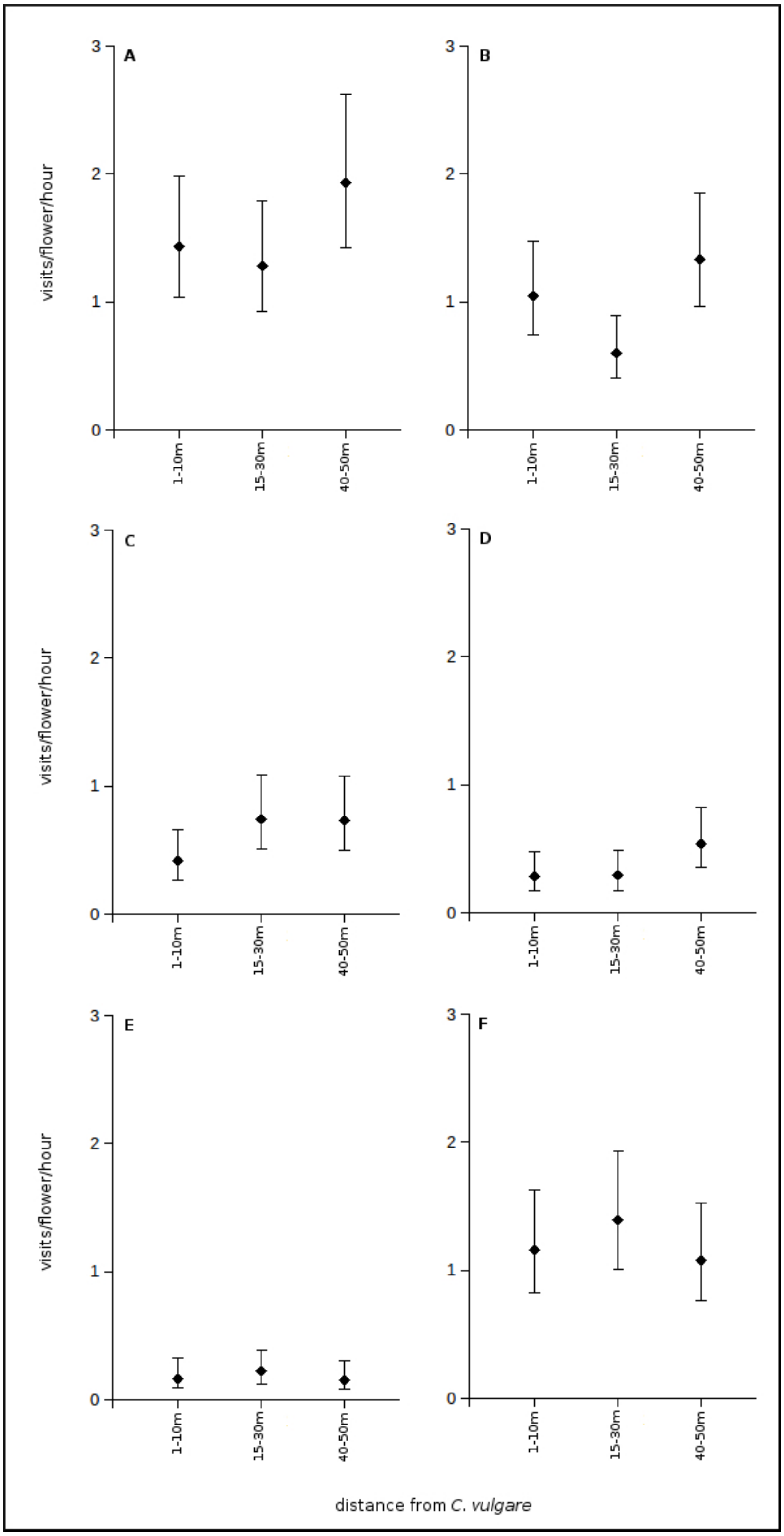

Figure 4. Boxplot of mean and interquartile ranges of model fitted values for visitation rate by distance from the *C. vulgare* array for (A) ants, (B) *A. mellifera*, (C) solitary bees and wasps, (D) other visitors, (E) *Bombus* spp., and (F) Diptera.

CHAPTER 4: DISCUSSION & FUTURE DIRECTIONS

DISCUSSION

Rather than repeating discussion of the specific findings of the analyses (see Chapter 2 discussion, page 42; and Chapter 3 discussion, page 80), I will focus here on the broader implications of the results of the articles presented in this thesis.

I have demonstrated that methodology, from the choice of study system to the definition of a control group and the management of spatial relationships between study plants, has a significant impact on the detection of outcomes of pollinator-mediated interactions between plants.

Implications for previous work

It is unlikely that previous studies examining pollinator-mediated interactions at a single spatial scale or spatial arrangement reflect the entirety of the pollinator-mediated interactions taking place in any given system; the attempt to simplify pollinator-mediated interactions between pairs of species to a single outcome limits the potential of each study to draw truly generalizable conclusions. Consequently, the conclusions of these previous analyses (see list in Supplement 2, composed largely of studies interpreting a single pollinator-mediated outcome between species pairs) may be overextended relative the scope of inference arising from the design.

Implications for future work

The foundational criteria for potential pollinator-mediated interactions between plants may have been oversimplified in being converted from continuous to binary factors: if co-occurrence is treated as binary, we may fail to capture the connection between facilitation and competition at the landscape scale; if co-flowering is treated as binary, we risk failing to capture the effect of sequential flowering and resource complementarity on interactions; and if we assume that floral visitors are either shared or not shared, rather than shared to varying extents, we may not be able to accurately predict the outcome of pollinator-mediated interactions between plants through different pollinators. It will be crucial to consider the impact on inference of design-based decisions with respect to the definition of co-occurrence, co-flowering, and pollinator sharing.

FUTURE DIRECTIONS

Alien-native interactions

The current set of studies examining pollinator-mediated interactions between alien and native plants is likely biased and consequently cannot be used to synthesize the possible effects of alien plants on native plant pollination. The crucial next step is the development of a suitably representative sample of possible interactions between alien and native plants. Further, a commitment on the part of researchers, reviewers, editors, and publishers to encourage the dissemination of null or controversial results is necessary for the development of a body of literature suitable for valid study of alien-native interactions.

Study design & methodology

The analyses in this thesis only examine the choice of study systems and the varying definitions of co-occurrence; I have not directly examined the impact of (temporal) flowering overlap on pollinator-mediated impacts, nor did I look at the effects of different degrees of pollinator sharing on study outcomes. A thorough investigation of these factors is required for the development of proper study designs to examine pollinator-mediated interactions between plant species. Until our assumptions about how plants interact through pollinators are thoroughly assessed, it will be impossible to determine the scope of inference for studies of pollinator-mediated interactions between plants.

SUPPLEMENT 1: COMPARISON OF STUDY OUTCOMES IN MORALES & TRAVESET 2009 AND DATA PUBLISHED SUBSEQUENTLY

Funnel plots, which plot the distribution of variances according to effect sizes for data reported in a meta-analysis, are commonly used to search for possible bias in the data (Sterne *et al.*, 2005). Specifically, in the absence of publication bias, funnel plots should show that effect sizes that deviate considerably from the group mean should also have greater associated error, i.e., there should be greater uncertainty associated with estimates that are particularly different from the population-level effect. We note that funnel plot asymmetry is not necessarily evidence of any particular type of bias (Sterne *et al.*, 2008), and must be interpreted cautiously. In an effort to explain differences between our findings and an earlier meta-analysis (Morales & Traveset, 2009), we compare funnel plots of the two datasets. Our investigation is based on the subset of studies with alien neighbour species, as this is where our findings deviate from previous work.

There is no evidence of asymmetry or bias in the (alien-neighbour) visitation data used in the meta-regressions reported in the body of our paper (Figure S1.1A). The funnel is symmetrical, with no evident gaps. The reproductive success data used for the meta-regression is comparatively sparse (Figure S1.1B), but displays no obvious asymmetry.

Both our analysis, and the one presented by Morales & Traveset, are based on a random subset of the available studies (see methods of the main paper); consequently, the data used below are representative of the set of outcomes in the Morales & Traveset (2009) analyses, but cannot replicate it exactly. For this same reason, the results presented here are not identical to those reported by Morales & Traveset (2009). They state that their statistical and qualitative conclusions are robust to selection of data points; because we are able replicate their statistical and qualitative outcomes with our sample of their original data, we believe that the data set we have generated from their publically available data set is representative.

We found that whether the data was available to Morales & Traveset (2009), or published after their paper was published, significantly affected the prediction of the outcome of studies of visitation (*F* = 12.7897, *n* = 82, *P* = 0.001). Unsurprisingly, the Morales & Traveset (2009) data overall shows a strong tendency toward competitive outcomes (*d* = -0.6163 (-0.9593 to -0.2734)) while results published since tend to show neutral or facilitative effects (*d* = 0.2942 (0.0367 to 0.5516)). This can be seen in the funnel plots (Figure S1.2A & S1.2B). Morales & Traveset did not find that alien or native status impacted the outcome of studies that looked at reproductive success (*F* = 3.2036, *n* = 38, *P* = 0.082).

Although we excluded some data from the Morales & Traveset (2009) data from our own analysis (see study inclusion criteria in the methods of the paper), the exclusion of these data points is not driving the difference between Morales & Traveset's conclusions and our own; when we test for an effect of neighbour status on study outcome on the full visitation data including the data excluded from Morales & Traveset (2009), we still find no significant effect of neighbour status on study outcome (*F* = 2.695, *n* = 99, *P* = 0.104). This did not change when we controlled for phylogenetic distance between species pairs (*t* = 1.0937, *n* = 99, *P* = 0.277). Likewise, adding the excluded data points to the model of reproductive success outcomes does not change the conclusions of the model (*F* = 0.5054, *n* = 59, *P* = 0.480). Again, there was no change when we also controlled for phylogenetic distance between species pairs (*t* = 0.1702, *n* = 59, *P* = 0.8655).

Given our finding that the impact of alien/native status on visitation to a focal species depends on the date of publication, we have conducted a follow-up analysis to identify possible sources of bias in results.

When the results were examined based on when they were collected (i.e., prior to 2009, or published since[2]), we found that the distribution of outcomes of visitation from studies included in

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[2] The results of one study (Carvallo & Medel 2013), were first incorporated in Morales and Traveset (2009) as unpublished results; as we wished to identify differences between the findings of the previous meta-analysis and our own, these results have exceptionally been analyzed as belonging to the 'pre-2009' data despite having been published subsequently.

Morales & Traveset (2009) was distinctly asymmetrical when compared with studies published since 2009 (Figure S1.2A & S1.2B). There is a notable gap in the bottom right portion of Figure S1.2A, suggesting the absence of moderate to large facilitative effects in studies with low sample sizes and/or large error (Sterne *et al.*, 2005). Because Morales & Traveset (2009) included both published and unpublished records in their analysis, we investigated whether there was any significant difference between published and unpublished outcomes; a two-sample t-test found no difference in outcomes in published versus unpublished results (Welch's two-sample t-test: t = -0.648, P = 0.521), but variance was higher in the published data (F-test of heteroscedacity: F = 3.554, $df_{1,2}$ = 25,16, P = 0.011; ratio of variances 3.554), and published studies reported significantly higher absolute values (i.e., more extreme outcomes) than unpublished studies (bootstrapped estimate of difference in means between published and unpublished outcomes: 95%CI = [-0.1295, -0.0116]). We take these results together to suggest the likelihood of publication bias and/or reporting bias (i.e., the file drawer effect) favouring large effects, particularly competitive ones, prior to 2009. Because the funnel plot of all outcomes (including pre- and post-2009 data), has no evident gaps or asymmetry, we conclude that this set of results is comparatively unbiased.

Reproductive success outcomes seem to reflect a different story: one obvious difference between these and the visitation outcomes is the relative scarcity of results with small sample sizes and/or large error (Figure S1.2C & S1.2D), particularly for results published since 2009. Neither funnel is particularly asymmetrical and there are insufficient unpublished results in the Morales & Traveset (2009) dataset (n = 15 published and n = 1 unpublished) to conduct meaningful comparisons. The scarcity of reproductive success outcomes with small sample sizes and/or large error may be due to the file drawer effect or publication bias, but may also be an artefact of pseudoreplication; in many studies, individual plants are treated as replicates, even when some plants come from the same experimental plots and so are not independent. Any of these could have contributed to the relative scarcity of effects with low sample sizes in studies reporting plant reproductive success in the presence or absence of an alien neighbour. We believe that the change in conclusions since 2009 is primarily the result of larger sample size; although Morales& Traveset found that alien plants tended to impose competitive impacts on the pollinator-mediated reproductive success of natives, the upper bound of this estimate was not far from zero and so, we conclude that the addition of more results has shifted that estimate sufficiently that it now overlaps with zero.

Part of the shift in study outcomes may be the result of changes in the way study systems are selected; neither dataset represents a random sample of possible pairwise interactions between alien and native species. Consequently, the average outcome reported prior to 2009 may be different from those published since due to a shift in the systems chosen to study. We found no significant difference in mean phylogenetic distance among species pairs depending on when the data were collected for the visitation (Welch's two-sample t-test: $t = -0.701$, $P = 0.486$) or reproductive success data (Welch's two-sample t-test: $t = 1.693$, $P = 0.102$), but the variance in the two data sets is different for visitation outcomes ($F = 2.393$, $P = 0.007$; pre-2009 data $n(sd) = 42(61.465)$, post-2009 data $n(sd) = 39(95.088)$) although not for reproductive success outcomes ($F = 0.657$, $P = 0.400$, pre-2009 data $n(sd) = 20(94.555)$, post-2009 data $n(sd) = 17(76.633)$). The significantly greater variance in phylogenetic distance between species pairs examined for visitation in the post-2009 data than in the pre-2009 data suggests the possibility of increased phylogenetic variability in systems selected for research; this shift in research interest to a more varied range of relatedness between pairs could partially account for the change in published outcomes.

We conclude that our analysis finds different outcomes from previous analyses (e.g., Bjerknes *et al.*, 2007, Morales & Traveset, 2009) for several reasons: first, reported visitation outcomes in alien-native interaction studies have changed significantly since 2009, which may be due to publication and/or reporting bias; second, estimates may have changed partly due to an increase in the overall sample size; and third, systems selected for study since 2009 may be more variable (in terms of phylogenetic distance among focal species), than those selected prior to that period.

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FIGURES

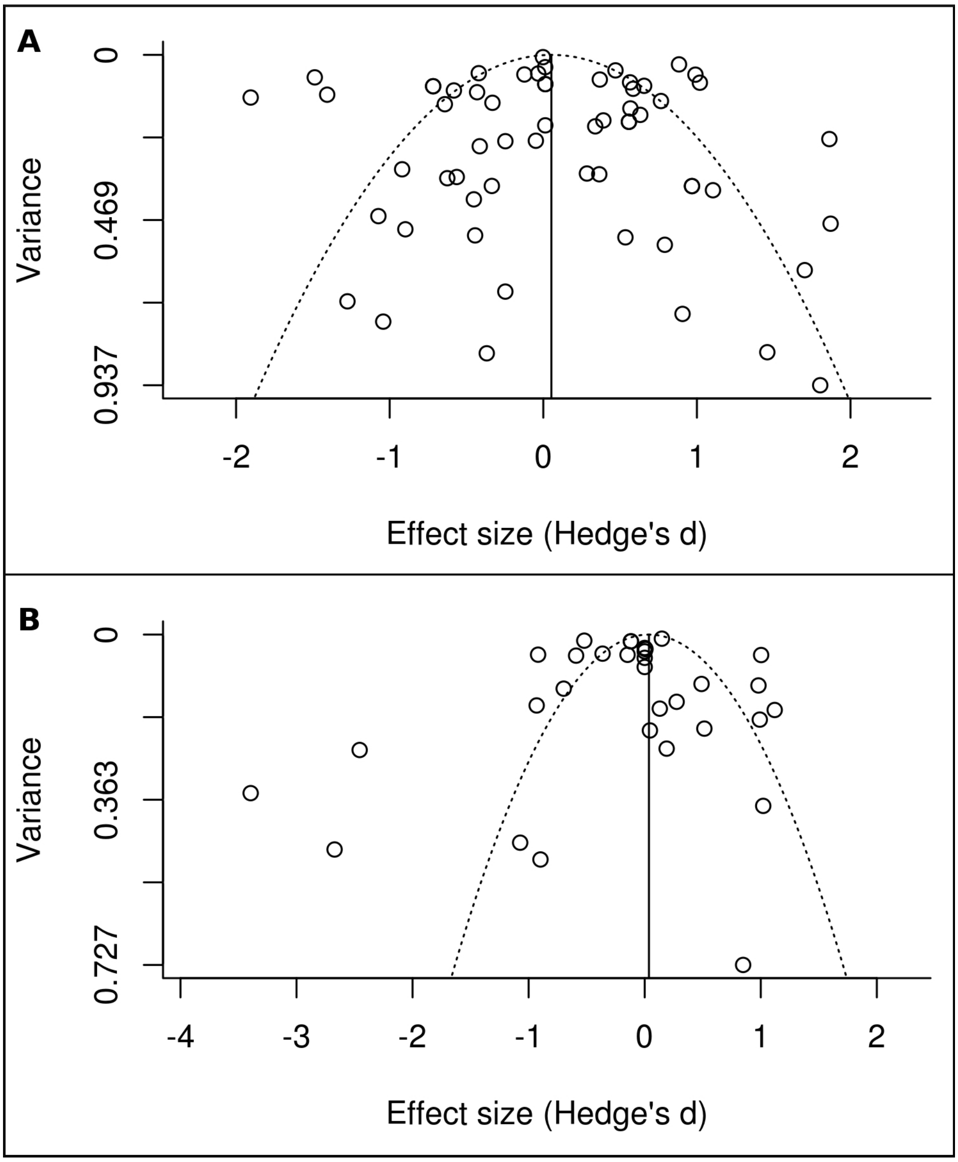

Figure S1.1 Funnel plots showing study outcome (Hedge’s *d*) by sampling variance for the data used to produce the models presented in the visitation (A) and reproductive success (B) results section of the present study.

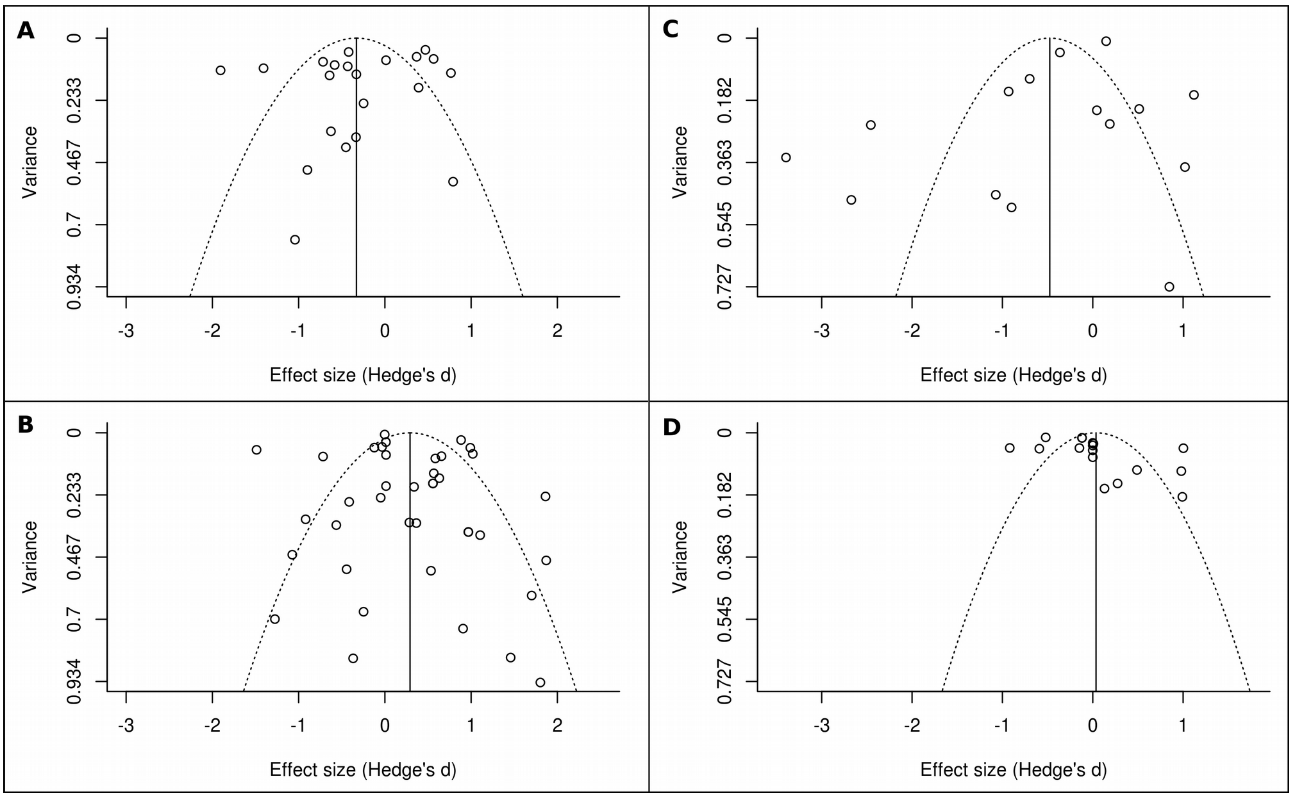

Figure S1.2. Funnel plots showing effect size (Hedge’s *d*) by sampling variance, collected prior to 2009 (A,C), or subsequently (B,D), for visitation (A,B), and reproductive success (C,D) outcomes

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SUPPLEMENT 3: TABLE OF COFLOWERING SYMPATRIC PLANTS AT THE FIELD SITE

Table S3.1. List of species present and flowering at site, their estimated frequency, their prevalence throughout the site, and a summary of visits observed to each species.

Flowering plant	Frequency	Prevalence	Origin	In plots	Visits received	% of total observed visits
Achillea millefolium	uncommon	near road	native	Y	4	0.03
Apocynum androsaemifolium	rare	patchy	native	Y	5	0.04
Arctium minus	extremely rare	patchy	alien	N	N/A	N/A
Asclepias syriaca	rare	along road	native	N	N/A	N/A
Aster umbellatus	common	entire site	native	Y	199	1.62
Aster vimineus	common	entire site	native	Y	57	0.46
Calystegia sepium	rare	along road	native	N	N/A	N/A
Centaurea jacea	dominant species	patchy	alien	Y	3085	25.15
Cichorium intybus	rare	along road	alien	N	N/A	N/A
Cirsium arvensis	rare	entire site	alien	Y	10	0.08
Clematis virginiana	locally common	ditches	native	N	N/A	N/A
Erigeron philadelphicum	extremely rare	along road	native	N	N/A	N/A
Eutrochium purpureum	common	patchy	native	Y	223	1.82
Gallium palustre	common	low, moist areas only	native	Y	6	0.05
Hypericum perforatum	rare	patchy	alien	Y	10	0.08
Impatiens capensis	rare	ditches	native	N	N/A	N/A
Iris versicolor	rare	patchy, in wet area only	native	N	N/A	N/A
Lamiaceae (spp.)	rare	low, moist areas primarily	N/A	Y	3	0.02
Lathyrus latifolia	rare	ditches	alien	N	N/A	N/A
Leucanthemum vulgare	rare	entire site	alien	Y	0	0.00
Lotus corniculatus	rare	along road	alien	Y	9	0.07
Lythrum salicaria	extremely rare	N/A	alien	N	N/A	N/A
Melilotus albus	rare	along road	alien	N	N/A	N/A
Oenothera biennis	rare	along road	native	N	N/A	N/A
Pastinaca sativa	locally common	patchy	alien	Y	243	1.98
Platanthera psycodes	extremely rare	N/A	native	N	N/A	N/A
Prunella vulgaris	rare	along road	alien	Y	4	0.03
Ranunculus acris	rare	along road	alien	Y	2	0.02
Rhinanthus alectorolophus	rare	near wet areas	alien	N	N/A	N/A
Scutellaria lateriflora	rare	patchy	native	N	N/A	N/A
Solidago canadensis	dominant species	entire site	native	Y	7330	59.77
Solidago graminifolia	locally frequent	patchy	native	Y	570	4.65
Spiraea alba	common	northwest quarter of site	native	Y	443	3.61
Taraxacum officinale	rare	along road	alien	N	N/A	N/A
Trifolium hybridum	rare	along road	alien	N	N/A	N/A
Trifolium pratense	rare	along road	alien	Y	6	0.05
Trifolium procumbens	rare	along road	alien	N	N/A	N/A
Verbena hastata	rare	near wet areas	native	N	N/A	N/A
Vicia cracca	rare	along road	alien	Y	6	0.05

SUPPLEMENT 4: CHAPTER 3 STATISTICAL MODEL DETAILS

Statistical model details

Initial models

Models including more variables (e.g. pollinator size estimate, plant species visited) failed to converge, likely due to zero-inflation of the data and insufficient sample size.

Models without pooled factors

Models that were run without pooling visitor functional groups together and/or without pooling distances into distance classes were heteroscedastic and overdispersed.

Models with pooled factors

Models in which visitor functional group was pooled down to 6 groups, and distance was pooled down to 3 distance classes, successfully converged, were homoscedastic, and were normally distributed after log-transformation.

I checked for heteroscedasticity of the model by examining a plot of the residuals plotted against their fitted values (Figure S2.1). The absence of any evident pattern indicates that the fit is reasonably homoscedastic. A histogram of the frequency of visitation rates in the log-transformed data shows no serious departure from the normal distribution (Figure S2.2).

TABLES

Table S4.1. Least squares means and standard errors for distance, functional group, then each distance for each, as generated in log-normal model.

	LS mean visits/flower/hour	**SE**
Visitor functional group		
Ants	0.42	0.28
Dipterans	0.19	0.28
Honeybees	-0.06	0.29
Solitary bees & wasps	-0.49	0.31
Other visitors	-1.03	0.35
Bumble bees	-1.72	0.42
Distance from the *C. vulgare* array		
1-10m	-0.56	0.29
15-30m	-0.49	0.29
40-50m	-0.29	0.29
Distance from *C. vulgare* array for each visitor functional group		
Ants		
1-10m	0.36	0.32
15-30m	0.25	0.33
40-50m	0.66	0.31
Dipterans		
1-10m	0.15	0.34
15-30m	0.33	0.33
40-50m	0.08	0.34
Honeybees		
1-10m	0.05	0.35
15-30m	-0.51	0.40
40-50m	0.29	0.33
Solitary bees & wasps		
1-10m	-0.86	0.45
15-30m	-0.29	0.38
40-50m	-0.31	0.38
Other visitors		
1-10m	-1.25	0.52
15-30m	-1.22	0.52
40-50m	-0.61	0.42
Bumble bees		
1-10m	-1.78	0.65
15-30m	-1.51	0.58
40-50m	-1.85	0.67

FIGURES

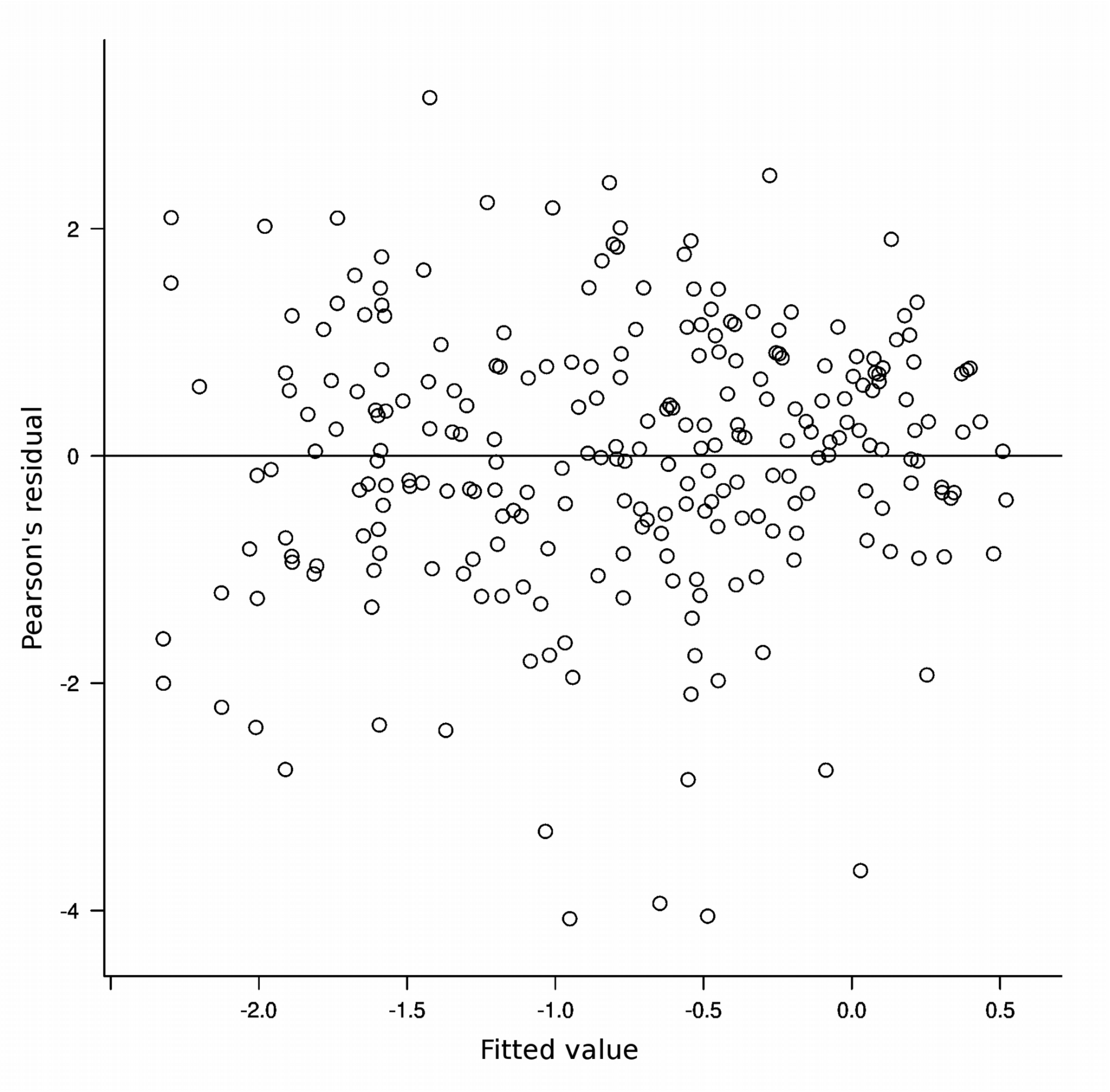

Figure S4.1. Plot of fitted values by their residuals in the pooled model presented in the results section of Chapter 3.

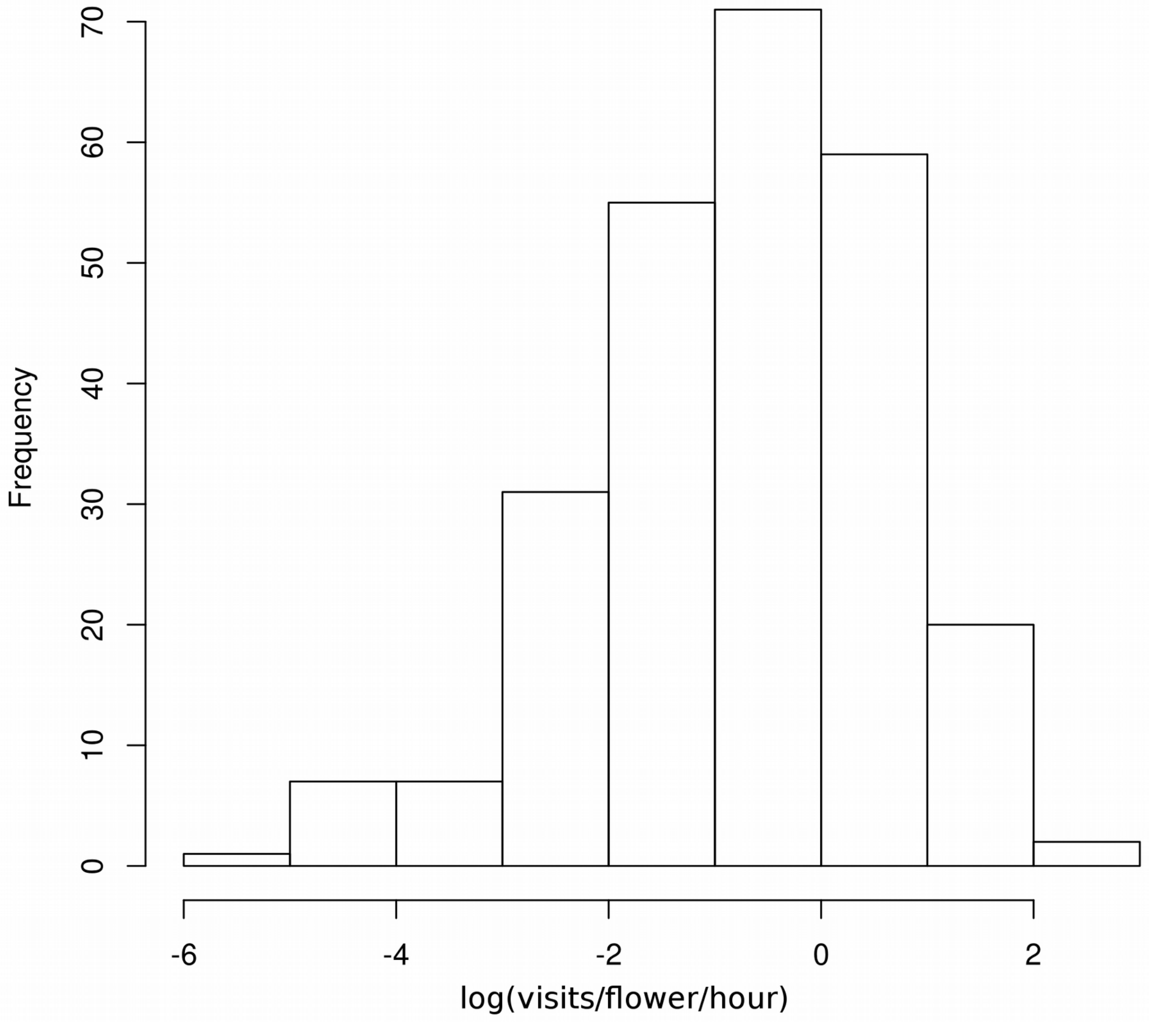

Figure S4.2. Histogram of frequency of log-transformed visitation rates.