Pollinator-mediated interactions between native plants and the invasive alien Himalayan balsam

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The candidate confirms that the work submitted is her own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Appendix E has been submitted to Molecular Ecology Resources as a primer note:

Isolation and characterization of microsatellite markers for white deadnettle, *Lamium album.* C. A. Horsley, A. Telford and S. Cavers.

Author contributions:
C. A. Horsley performed the research, analysed the data, co-wrote the paper
A. Telford analysed the data
S. Cavers designed the research, analysed the data, co-wrote the paper

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Abstract

Invasive alien species threaten global biodiversity and can impose severe economic costs. Some invasive alien plants can be strong competitors for pollinators due to a high abundance of attractive flowers, which can disrupt native plant-pollinator interactions and reduce native plant reproductive success. This thesis explores pollinator-mediated competition between native plants and the invasive alien *Impatiens glandulifera*, Himalayan balsam. Previous studies have found conflicting effects of *I. glandulifera* on native plant-pollinator interactions. This study aimed, using a combination of direct field observations and controlled experiments, to explore the reasons behind these differences by examining the relationship between impact and the abundance of *I. glandulifera* at multiple spatial scales, and the responses of a wide range of co-occurring species.

Chapters two and three tested the hypothesis that the composition of plant and pollinator communities and bumblebee-flower visitation vary in response to *I. glandulifera* abundance and spatial scale of invasion. Chapter four tested the hypothesis that *I. glandulifera* pollen will reduce the reproductive success of the native *Lamium album*. Chapter five tested the hypothesis that pollinator-mediated competition will alter the genetic quality of pollen received by co-flowering *L. album.*

I found a relationship between the plant and pollinator community composition and the abundance of *I. glandulifera*, which was generally stronger at a broad scale. Responses to invasion differed according to pollinator taxa and plant traits, which could be useful for identifying and protecting potentially vulnerable native species. Impacts differed according to the mechanism used to examine its effects: bumblebee-flower visitation patterns changed, and *L. album* experienced reduced seed set and disruption to its mating system; however alien pollen did not prevent *L. album* from setting seed. In conclusion, the direction and magnitude of pollinator-mediated effects varied with *I. glandulifera* abundance, spatial scale, and the way in which impact was assessed. A wide range of approaches are necessary to understand the impact of invasive alien plants.

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Chapter One:

General Introduction

1.1 Overview

Invasive alien species can pose a serious threat to global biodiversity via the replacement of endemic species with widespread species (McKinney & Lockwood 1999), and can outcompete native species for resources (Gerber et al. 2008) which can result in species extinctions (Baider & Florens 2011). Invasive alien species can also impose considerable economic costs in their management and control (Pimentel et al. 2005). Some invasive alien plants can disrupt native plant-pollinator interactions (Lopezaraiza–Mikel et al. 2007) due to their attractive and abundant flowers (Stout & Morales 2009), which can reduce native plant reproductive success (Chittka & Schürkens 2001). This thesis explores pollinator-mediated competition between native plants and the invasive alien *Impatiens glandulifera* Royle (Himalayan balsam; Balsaminaceae) with implications for native plant reproductive success.

1.2 Definitions

Terminology used in the field of invasive plant ecology is inconsistent between studies, and should be clearly defined to avoid confusion (Richardson et al. 2000b). This study adopts the terminology recommended by Richardson et al (2000b) (Table 1.1).

Table 1.1: Terminology used to describe invasive plant ecology, as recommended by Richardson et al. (2000b), Diversity and Distributions, 6(2), 93-107. Table adapted from Richardson et al (2000b).

Alien plants	Plant taxa in a given area whose presence is due to intentional or accidental introduction as a result of human activity. Synonyms: exotic, non-native, non-indigenous
Casual alien plants	Alien plants, introduced as a result of human activity, that may flourish and reproduce but do not form self-sustaining populations. They rely on repeated introductions to persist. Synonyms: waifs, transients, occasional escapes, persisting after cultivation
Naturalised plants	Alien plants that reproduce consistently and sustain populations over many life cycles without (or in spite of) direct intervention by humans: they often recruit offspring freely, usually close to the adult plant, and are not necessarily invasive (see below)
Invasive plants	Naturalised plants that produce reproductive offspring, often in very large numbers, at considerable distances from the parent plant and thus have the potential to spread over a considerable area and cause widespread impact
Weeds	Not necessarily alien species. Plants that grow where they are unwanted, and usually have economic or environmental effects. Synonyms: plant pests, harmful species, problem plants.

1.3 Background

Historically, the dispersal of species across biogeographic barriers was rare (summarised in Elton 1958). More recently, increased human movement, commerce and improved transportation has led to both accidental and deliberate routine movement and introduction of species between continents (di Castri 1989). It is estimated that only a small fraction of introduced species will establish, and of these only a small fraction will become invasive (Williamson 1996) because there are several barriers preventing their spread (Richardson et al. 2000b). However, those that do become invasive can cause biodiversity loss (Gerber et al. 2008; Spyreas et al. 2010) by replacing endemic species with widespread species (McKinney & Lockwood 1999), or by causing native species extinctions (Cox & Elmqvist 2000, Baider & Florens 2011).

Biodiversity loss as a result of invasion by alien species poses a threat to ecosystem stability because biodiversity is predicted to buffer ecosystems against environmental perturbations, due to a high level of functional redundancy (Memmott et al. 2004). Ecosystems are dynamic and subject to much natural variability and disturbance, but they are defined by thresholds after which they may reach a new alternative state (Laycock 1991, Scheffer et al. 2001). Species loss beyond a critical threshold may cause communities to collapse (Fortuna & Bascompte 2006, Kaiser-Bunbury et al. 2010). Biodiversity is also necessary for maintaining genetic variation, which is essential for species adaptation to changing environmental conditions (e.g. Fitzgerald et al. 2011), and for maintaining ecosystem services such as pollination. Many wild plants and agricultural crops are dependant on pollinators for reproduction (Ollerton, Winfree, & Tarrant 2011), which is of direct consequence for humans since pollination is essential for human nutrition (Eliers et al. 2011). Biodiversity loss also has considerable economic implications. For example, pollination services alone were valued at an estimated $215 billion dollars globally in 2005 (Gallai et al. 2009).

Some invasive alien species can modify the structure and stability of ecological communities by changing disturbance regimes, nutrient cycling and hydrology (reviewed in Mack & D’Antonio 1998; Levine et al. 2003), and because they can be superior competitors for resources (D’Antonio & Mahall 1991, Chittka & Schürkens 2001, Iponga 2010). They can also impose considerable economic costs in terms of the damage caused to ecosystems and in their management and control (Pimentel et al. 2005).

Under predictions for climate change, many invasive alien species are forecast to further expand their range because they posses traits such as a fast growth rate that enables them to expand under new conditions, such as increased nitrogen deposition and elevated carbon dioxide (reviewed by Dukes & Mooney 1999). This could lead to increased threats in the future. Invasive alien species are themselves considered a major driver for global change (Vitousek 1997; Mack et al. 2000), which is reflected in increasing policy and legislation on invasive species management (McGeoch et al. 2010).

Invasion processes are key to understanding biological questions of evolution and adaptation, because invasion can result in rapid evolutionary changes in the invader in response to novel conditions, and in native species in response to invasion (Sakai et al. 2001); and in addressing ecological processes of community structuring such as succession (Simberloff 2010).

1.4 The invasion process

Invasion can be described according to a simple process that involves overcoming several barriers which prevent spread (Richardson et al. 2000b), summarised in Figure 1.1. First, introduction occurs, meaning that an individual or propagule has surmounted geographic barriers through human-mediated transportation (**A**, Figure 1.1). Some introduced taxa survive as casuals, that is, they fail to maintain populations over long periods. When environmental barriers (**B**, Figure 1.1) and barriers to reproduction (**C**) are also overcome, taxa can sustain populations, signifying that they have become naturalised. To become invasive, i.e. to spread outside of their point of introduction, taxa must overcome barriers to dispersal (**D**) and be able to persist with the abiotic environment and biota within the new region (**E**) (Richardson et al. 2000b). Factors contributing to species invasiveness and the resistance of communities to invasion are described by a range of hypotheses (discussed in Section 1.5).

The tens rule is a rule of thumb used to illustrate that few introduced species are likely to become invasive. It predicts that 10 % of imported species will become casual, 10 % of which will become naturalised, and 10 % of these will become invasive (Williamson 1996). Thus only 0.1 % of introduced species are predicted to become invasive. This can translate to a substantial number however. Around 6,000 plant species have been classified as aliens in Europe (Lambdon et al. 2008), and according to the tens rule, around 600 of these are predicted to be invasive. The tens rule has been criticised since it largely lacks experimental support (e.g. Jeschke & Strayer 2005) and is without theoretical basis. However, it is intended for use as a reference point rather than to calculate the expected proportion of invasive species with any precision (Williamson 2006). More accurate estimates are often difficult to determine, being hindered by a lack of detailed information, such as the number of failed introductions, which can lead to an overestimation of the proportion of alien species that become established (Rodriguez-Cabal et al. 2012).

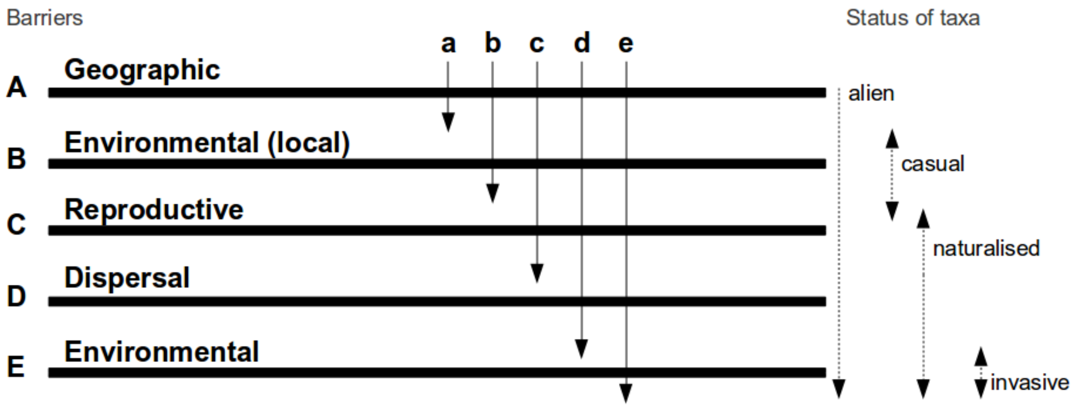

Figure 1.1: Schematic representation of barriers limiting the spread of introduced alien plant species. **A** Major geographic barrier (continental scale, > 100 km), **B** Environmental barrier (biotic and abiotic) at site of introduction, **C** Reproductive barrier (preventing long-term and persistent vegetative and/or generative reproduction, **D** Local/regional dispersal barriers, **E** Environmental barriers in human-modified or alien-dominated vegetation, or in natural or semi-natural vegetation. Arrows **a** to **e** indicate paths followed by taxa to reach different states from introduction to becoming invasive in natural vegetation. Adapted from Richardson et al. (2000b), Diversity and Distributions, 6(2), 93-107.

1.5 Predicting invasiveness

Predicting invasiveness has been subject to intensive study because theoretically, it could enable early targeted eradication of potentially problematic species and restrictions on their introduction could be imposed, and it is of immense scientific interest. There are many apparently contradictory hypotheses in predicting invasiveness however, which may reflect interactions between propagule pressure, the characteristics of the invader and the invaded community, which are not always accounted for within a single study (Lonsdale 1999; Catford, Jansson, & Nilsson 2009). Differences may also be due to the way in which comparisons are drawn (reviewed in van Kleunen et al. 2010). For example, many studies compare invasive species with native species (e.g. Leishman et al 2010), whereas others compare alien non-invasive with alien invasive species (Reichard & Hamilton 1997; Muth & Pigliucci 2006). Comparisons between invasive alien species in the native and introduced range are frequently made to test the enemy-release hypothesis (described below) (e.g. DeWalt et al. 2004) or less commonly, to explore genetic differences (Schlaepfer et al. 2008) or ecological differences such as in pollination biology (Ollerton et al. 2012).

Some of the most widely recognised hypotheses that aim to predict both the invasiveness of alien species and the invasibility of communities are discussed below and listed in Table 1.2.

1.5.1 Predicting invasive alien plant species

Classifying species according to their life history traits has been explored to predict invasiveness (Elton 1958; Drenovsky et al. 2012). Baker (1974) proposed a set of criteria based on life history and reproductive characters to predict which species may be considered a weed. The use of such criteria alone has been of limited success in predicting plant invasiveness, as species are not consistent in their life history traits or reproductive behaviour (Williamson & Fitter 1996; Goodwin, McAllister, & Fahrig 1999). For example, Williamson and Fitter (1996) found that invasive alien plants tend to be insect-pollinated, which may reflect an introduction bias towards species imported for their attractive flowers. However, Milbau and Stout (2008) found that animal-pollinated alien plants had a lower probability of being invasive, although ornamental plants were more likely to be invasive. Common traits of invasive alien species are high seed output, and fast germination and growth rates (e.g. Goergen & Daehler 2001; Flory & Clay 2009; Schlaepfer et al. 2010), although some invasive species do not posses these traits (Williamson & Fitter 1996; van Kleunen et al. 2010).

The "enemy release hypothesis" predicts that plants growing outside of the range of their natural enemies (e.g. herbivores or pathogens) are more likely to be invasive, although there is evidence both for and against (Wolfe 2002; Lake & Leishman 2004; Chun, van Kleunen, & Dawson 2010). The closely related "evolution of increased competitive ability" (EICA) hypothesis predicts that in the absence of herbivores, plants can evolve to reallocate resources previously used in defence to increase reproduction and growth, improving their ability to compete with native species (Blossey & Notzold 1995). There is experimental evidence to support the theory (Uesugi & Kessler 2013), although there are also counter-examples (Cripps et al. 2009). Invasive alien plants can show evolutionary changes in their new range compared to native populations, demonstrating increased competitive ability in terms of their growth rate and survival, which could be in response to escape from natural enemies (Blair & Wolfe 2004). Evolutionary changes following introduction can be rapid, over time scales of less than ten years in some instances (Whitney & Gabler 2008). Some species, such as *Verbascum thapsus* L. (Scrophulariaceae) which is an invasive alien species in the USA, do not exhibit evolutionary adaptation however, but demonstrate a high level of phenotypic plasticity that enables their occurrence over a wide environmental gradient (Parker et al. 2003). Phenotypic plasticity is predicted to facilitate invasion across different environments, but there is evidence both in support and in opposition of the theory (Molina-Montenegro et al. 2011; Godoy, Valladares, & Castro-Díez 2011). This may reflect the fact that most studies consider only a subset of the full range of environmental conditions that are experienced by the species, meaning that variation across an environmental gradient could lead to different interpretations of its response (Hulme 2007). Plant invasiveness can be predicted by genomic attributes as invasive alien plants have been found to be more likely to be polyploids and have high chromosome counts, although the cause and consequence of this remains unclear (Pandit, Pocock, & Kunin 2011). Polyploidy may however promote phenotypic plasticity.

Another hypothesis related to EICA is that invasive alien plants may be successful through possession of “novel weapons” such as allelopathic exudates, to which native plants are vulnerable due to a lack of evolved mechanisms for tolerance or avoidance (Callaway & Ridenour 2004). Chemical defence can be common in invasive alien plant species (Ren & Zhang 2009). Darwin's “naturalisation” hypothesis predicts that successful invaders will differ taxonomically from native species, reflecting that there is less niche overlap and reduced competition; although conversely, alien species that are similar to natives should be more adapted to the environment (Darwin 1859). There is evidence for both arguments, the outcome of which may depend in part on spatial scale. At a local scale, within habitats, invasive species that are closely related to native species may be excluded by competition but at a broader scale, among habitats, invasive alien species that share traits with native species are more likely to be adapted to the environment (Diez et al. 2008). Taxonomic patterns may also reflect an introduction bias towards particular cultivated species (Chrobock et al. 2011).

The spread of alien plant species can increase with residence time (Ahern et al. 2010; Haider et al. 2010) which suggests that many more introduced species will become invasive over time. This may reflect a lag phase, where there is a time delay between introduction and invasion. The lag phase may be a consequence of factors inherent in population growth; or reflect changing environmental conditions that favour the alien some time after its introduction; or demonstrate an initial lack of genetic variation that means the alien is not adapted to suit its novel environment (Crooks & Soule 1996). The latter requires time for adaptive evolution, which can be achieved through increased genetic diversity resulting from hybridisation between taxa or between isolated populations of the same species (Schierenbeck & Ellstrand 2009). Increased residence time may also increase invasiveness due to the increased probability of recruiting offspring and establishing (Richardson et al. 1994). Plants are more likely to establish and become invasive through increasing propagule pressure (Simberloff 2009), although this does not always impact on invasiveness (Nuñez, Moretti, & Simberloff 2011). Alien plants with a wide native range may be more likely to be invasive (Goodwin et al. 1999; Shah, Reshi, & Lavoie 2011). This could reflect a wider tolerance for environmental conditions (Goodwin et al. 1999), or indicate that they possess traits that promote spread, such as high seed production (Booth, Murphy, & Swanton 2003), or because by being widespread they are more likely to be transported. However, this is not consistent across species, as species with a small native range have also been found likely to be invasive (Milbau & Stout 2008).

1.5.2 Predicting invasible communities

Plant invasiveness may depend on the susceptibility of ecosystems to invasion, which can be described by ecosystem resistance and resilience. The resistance of ecosystems to invasion describes variables that limit population growth of invaders (D'Antonio and Thomsen 2004), such as competition from the resident community; whereas resilience describes the maximum perturbation a system can tolerate and remain in the same state (Holling 1973). The "diversity-invasibility" hypothesis predicts that species diversity will make communities more resistant to invasion because there are fewer vacant "niches" (Elton 1958). Diverse communities are also generally considered to be more ecologically stable and less prone to change (Tilman et al. 2006, Dovčiak & Halpern 2010), and so should demonstrate resilience to invasion. There is experimental evidence in support (Naeem et al. 2000) and in opposition (Lonsdale 1999; Collins et al. 2006) to the "diversity-invasibility" theory however, and the issue has been much debated (reviewed by Levine & D'Antonio 1999; Fridley et al. 2007).

Habitat types can show variation in their susceptibility to invasion. For example, mature undisturbed forests have shown resistance to invasion (Richardson et al. 1994), whereas riparian habitats can have large numbers of invasive species (Planty-Tabacchi et al. 1996). This may reflect levels of disturbance, which can facilitate invasion (e.g. D'Antonio 1993). Disturbance, such as fire, flood or nutrient enrichment, may increase invasibility because it creates new habitats or niches, although the extent to which alien species can outcompete natives may depend on their taxonomic similarities (Darwin's naturalisation hypothesis, described above). Although ecosystems are naturally subject to disturbance, such disturbance may facilitate invasion as invasive species can be more opportunistic than native species in capitalising on resources (Davis et al. 2000). The theory of "fluctuating resource availability" predicts that habitats with high resource variability, as caused by disturbance, will be more invasible than those with low resource variability (Davis et al. 2000). The invasive alien *Fallopia* spp. experienced a tripling of its biomass when nutrients were applied in pulses rather than uniformly, although there was no change in the total biomass of the plant community (Parepa, Fischer, & Bossdorf 2013), which provides empirical evidence in support of the theory. *Fallopia* may be a superior competitor by responding more quickly to nutrients or by having a faster growth rate than native species (Parepa, Fischer, & Bossdorf 2013). Disturbance could also lead to invasion by removing or reducing populations of native competitors or enemies (reviewed in Hobbs & Huenneke 1992). Also, if native species cannot adapt to human-modified conditions, the arrival of alien species that are adapted is predicted to lead to invasion (Mack et al. 2000).

Species invasiveness can depend on local growing conditions (Daehler 2003), and invasion can increase with the availability of soil nutrients for example (Maron & Jefferies 1999). The invasibility of habitats could change over time if invasive alien species themselves alter the habitat conditions. The "invasional meltdown" hypothesis predicts invasive alien species can facilitate one another's invasion by modifying the habitat, by fixing nitrogen for example, which favours other aliens over native species (Simberloff & Von Holle 1999). There is evidence for both facilitative and detrimental interactions between invaders however (Simberloff 2006). Many alien plant species rely on mutualists, such as mycorrhizal fungi, insect pollinators or animals for seed dispersal, but this commonly does not act as a barrier to becoming invasive as most can readily form new associations with mutualistic partners in their new range (Richardson et al. 2000a).

Mechanisms driving patterns of invasions are predicted to vary according to spatial scale (Pauchard & Shea 2006; Milbau & Stout 2008). Climate has long been recognised as a controlling factor in the spread and naturalisation of plant species (Lindsay 1953), and at regional scales, climate can be important for predicting areas vulnerable to invasion (Ohlemüller, Walker, & Bastow Wilson 2006; Vicente et al. 2010). At a broad scale, the spatial configuration of the landscape is expected to influence invasion due to in part to factors that may facilitate dispersal (With 2002). This can depend on the size and fragmentation of habitats (Vilà & Ibáñez 2011) and on land use (Vicente et al. 2010). At finer scales, local resources such as soil nutrients are important in determining invasibility (Maron & Jefferies 1999).

Table 1.2: Key hypotheses in predicting the invasiveness of alien species and the invasibility of communities, and some examples of literature exploring these hypotheses. Hypotheses and evidence (in support or against) are detailed in the text.

	Description of hypothesis	**References**
Invasive species	Darwin's naturalisation hypothesis; invasive species are less phylogentically related to native species than non-invasive aliens	Diez et al. (2008)
	Enemy-release; freedom from natural enemies (e.g. herbivores, pathogens) gives invasive species a competitive advantage	Woolfe (2002); Chun et al. (2010)
	Evolution of increased competitive ability; plants can reallocate resources previously used in defence to improve ability to compete with natives	Blossey & Notzold (1995); Cripps et al. (2009)
	Introduction bias; human preference for specific characteristics biases the traits of introduced species	Williamson & Fitter (1996); Chrobock et al. (2011)
	Life history traits can be used to predict which alien species will be invasive e.g. growth rate, mode of pollination	Williamson & Fitter (1996); Milbau & Stout (2008)
	Novel weapons; e.g. allelopathic exudates, that natives lack co-evolved mechanisms to tolerate or avoid	Callaway & Ridenour (2004)
	Phenotypic plasticity; greater in invasive species and considered to facilitate invasion across different environments	Godoy et al (2011); Molina-Montenegro et al. (2011)
	Propagule pressure; number of individuals introduced and frequency of introductions	Simberloff (2009); Nunez et al (2011)
	Residence time	Schierenbeck & Ellstrand (2009)
	Wide native range; wider tolerance for environmental conditions	Goodwin et al. (1999); Milbau & Stout (2008)
Invaded communities	Climate; controls distribution at large scales	Ohlemuller et al. (2006)
	Diversity-invasibility; species diversity will make communities more resilient & resistant to invasion	Elton (1958); Naeem et al (2000); Lonsdale (1999)
	Fluctuating resource availability favours invasives because they can respond to resources quicker than natives	Davis et al. (2000)
	Growing conditions, e.g. nutrients, disturbance	Maron & Jeffries (1999); Daehler (2003)
	Habitat fragmentation; spatial distribution of suitable habitats may influence dispersal	Vila & Ibanez (2011); With (2002)
	Invasional meltdown; invasive species facilitate further invasion by other invasives	Simberloff & Von Holle (1999)
	Land use; reflecting land management regimes	Vicente et al. (2010)
	Presence of mutualists; such as pollinators	Richardson et al. (2000)
	Similar climatic conditions to native range	Ohlemuller et al. (2006)

Predicting species invasiveness is a fundamental question in invasive species biology. However, as described, there are exceptions to each rule which makes generalisations difficult. Rather than treating all invasive species as a homogeneous group and attempting to find characteristics applicable to all, an alternative and more successful approach may be to describe them according to taxonomic, biogeographic or ecological groupings (Kolar & Lodge 2002). However, quantitative assessments relating to the impact of alien species have been carried out for fewer than 200 alien species (Hulme et al. 2013), which represents a small fraction of all known alien species introduced (Lambdon et al. 2008). This demonstrates a need for more species-specific studies which are currently lacking, even for widespread species.

1.6 Impacts of invasive plants

The introduction of alien species can reduce global biodiversity through the replacement of endemic species with widespread species (McKinney & Lockwood 1999). Invasive alien animals can cause native plant species extinctions (Cox & Elmqvist 2000), but rarely have alien plants been documented as causing native plant extinctions (Baider & Florens 2011). However, some invasive alien plants can reduce local biodiversity (e.g. Hulme & Bremner 2006; Gerber et al. 2008; Spyreas et al. 2010). Biodiversity is important in maintaining ecosystem resilience by buffering against environmental perturbations (Elton 1958, Tilman et al. 2006, Memmott et al. 2004), and in providing resistance to invasion (Naeem et al. 2000). Species loss beyond a critical threshold could cause communities to collapse (Fortuna & Bascompte 2006, Kaiser-Bunbury et al. 2010). However, there are counter-examples where biodiversity increases due to increased habitat heterogeneity and complexity (Crooks 2002), or because the addition of the alien species itself increases biodiversity (Sax et al. 2002).

The impact of invasive alien plants can vary enormously. Some native plants can coexist with invasive alien plants (Hejda et al. 2009), and can demonstrate adaptive changes in response to invasion (Mealor & Hild 2007; Goergen et al. 2011). Alien plants can sometimes be beneficial, providing new resources to animals which can increase their populations (Graves & Shapiro 2003) or even lead to speciation (Schwarz et al. 2005). Others can be harmful, being toxic to larvae that feed on them for example (Graves & Shapiro 2003). The introduction of alien plants can provide new functions, such as accumulating toxic waste from contaminated soil (Ma et al. 2001). Some invasive alien plants may exert little effect, and may instead be a symptom of degraded habitats (MacDougall & Turkington 2005). Such variation in their effects suggests that species should be considered according to their impacts rather than their nativeness (Ewel & Putz 2004; Davis 2011).

Invasive alien plants can modify ecological communities by changing disturbance regimes, nutrient cycling and hydrology (reviewed in Mack & D'Antonio 1998; Levine et al. 2003). They can also modify communities through competitive interactions for resources such as light (Iponga 2010), water (D'Antonio & Mahall 1991), nutrients (Wardle et al. 1994) and pollinators (Chittka & Schürkens 2001). The disruption of plant-pollinator interactions is of particular concern given that many wild plants and agricultural crops depend on pollinators for reproduction (Ollerton, Winfree, & Tarrant 2011). Most introduced alien plants arrive without the pollinators from their native range, and it is predicted that species with highly specialised pollination systems are less likely to be pollinated than generalists (Richardson et al. 2000a). Plant-pollinator networks are characterised by weak and asymmetrical dependencies where specialist species interact with generalists and vice versa (Bascompte 2003), which facilitates the inclusion of alien species. There are many examples of the integration of alien species into plant-pollinator networks since first noted by Darwin (in Stauffer 1975), (Traveset & Richardson 2006; Vila et al. 2009), and they can form more associations with pollinator species with increasing residence time (Pyšek et al. 2011). Some invasive alien species have been found to be "supergeneralists", receiving even more visits from pollinators than native species (Vila et al. 2009). Invasive alien plants pose a particular threat as they can be strong competitors, attracting pollinators to rewarding flowers (Chittka & Schurkens 2001) which often occur at high abundance (Bjerknes et al. 2007).

Competition for pollinators between co-flowering native plants is predicted to reduce reproductive success (Levin & Anderson 1970; Waser 1978b) by changing the *quantity* (amount) of pollen reaching the stigma or by changing the *quality* of pollen received, that is the purity of the pollen load (sensu Waser 1983). These mechanisms have begun to be explored between competing alien and native species. The quantity of pollen received can decrease through reduced pollinator visitation (Brown et al. 2002), or through pollen wastage as pollinators move between species and deposit pollen on interspecific flowers (Brown & Mitchell 2001). This can reduce seed set (Brown et al. 2002), but not always (Grabas & Laverty 1999). Reduced pollen quality through the receipt of alien pollen can sometimes reduce native plant seed set, although relatively few studies have isolated this from confounding factors such as visitation rates (Brown & Mitchell 2001; Kasagi & Kudo 2005; Matsumoto, Takakura, & Nishida 2010). Pollen quality can also refer to its genetic properties, such as the diversity of pollen donors and their relatedness to the recipient (e.g. Price & Waser 1979; Aigner 2004). Co-flowering native species competing for pollinators can experience a reduction in the diversity of pollen donors received through pollen wastage caused by interspecific movement (Bell, Karron, & Mitchell 2005). Pollinator-mediated competition from invasive alien species has the potential to exert a similar response in native plants, but has yet to be explored. Changes in pollinator behaviour also have the potential to alter the distance that native pollen is dispersed, with implications for the relatedness of pollen received. This may alter plant mating systems, changing the proportion of progeny produced from self versus outcross pollen. The genetic quality of pollen is an important consideration, since reduced genetic diversity can reduce plant fitness through inbreeding depression (the reduction of fitness of inbred relative to outcrossed progeny) (Charlesworth & Charlesworth 1987). Disturbance to native plant-pollinator interactions caused by invasive alien plants has implications for the demography of local native plant populations, gene flow and metapopulation dynamics, as well as having evolutionary significance for mating systems (reviewed in Eckert et al. 2010).

Rather than competing for pollinators, co-flowering native plant species can sometimes facilitate each other's pollination. Plants with morphologically similar floral displays can attract shared pollinators (Moeller 2004), but equally, facilitation can occur between plants that differ in their floral displays, perhaps being attracted by a range of complementary floral rewards (Ghazoul 2006). Sequentially flowering co-occurring plant species may indirectly facilitate each other's pollination by maintaining populations of shared pollinators (Waser & Real 1979). Relatively few of the reported studies demonstrate facilitative interactions between invasive alien and native species however (Moragues & Traveset 2005; Lopezaraiza–Mikel et al. 2007), and fewer still report increased native plant seed set (Muñoz & Cavieres 2008). Other studies have found that invasive alien species have no detectable effect on visitation to co-flowering native plants (Nienhuis et al. 2009; Bartomeus et al. 2010). Invasive alien plants could however facilitate native plant pollination in sequentially flowering species by supporting populations of shared pollinators, but this has yet to be explored.

Whether invasive alien plants exert a competitive, facilitative or neutral effect on the pollination of co-flowering native plants is expected to depend in part on their abundance in the community. This is because floral density has been found to contribute to pollinator visitation rates. At low density, plants receive few pollinator visits which can lead to reduced reproductive success (e.g. Kunin 1993). Fewer pollinators are expected at low plant density because according to optimal foraging theory, pollinators are more attracted to high density patches to minimise foraging costs (Dreisig 1995). At high floral density, plants can experience increased pollinator visitation rates (Bosch & Waser 2001, Feldman et al. 2004), although this does not always translate into increased seed set if interspecific pollen transfer inhibits pollination (Feinsinger et al. 1991). By contrast, other studies have found decreased visitation at high floral abundance (Dauber et al. 2010), or that density does not affect pollinator visitation rate (Bosch & Waser 1999).

Apparently conflicting results of the effects of floral abundance on visitation rates may be unified by Rathcke's density-visitation model (1983, Figure 1.2). The model predicts that pollinator visitation rates experienced by a given plant will depend on the floral density of conspecifics, or equally, on the combined floral density of all co-flowering plants in the community. At low floral density, co-flowering plants should facilitate each other's pollination because the size of the floral display is greater than if they flowered alone. This should attract more shared pollinators. Once floral density reaches beyond some optimum, co-flowering plants should compete for pollinators which have become a limiting resource (Rathcke 1983).

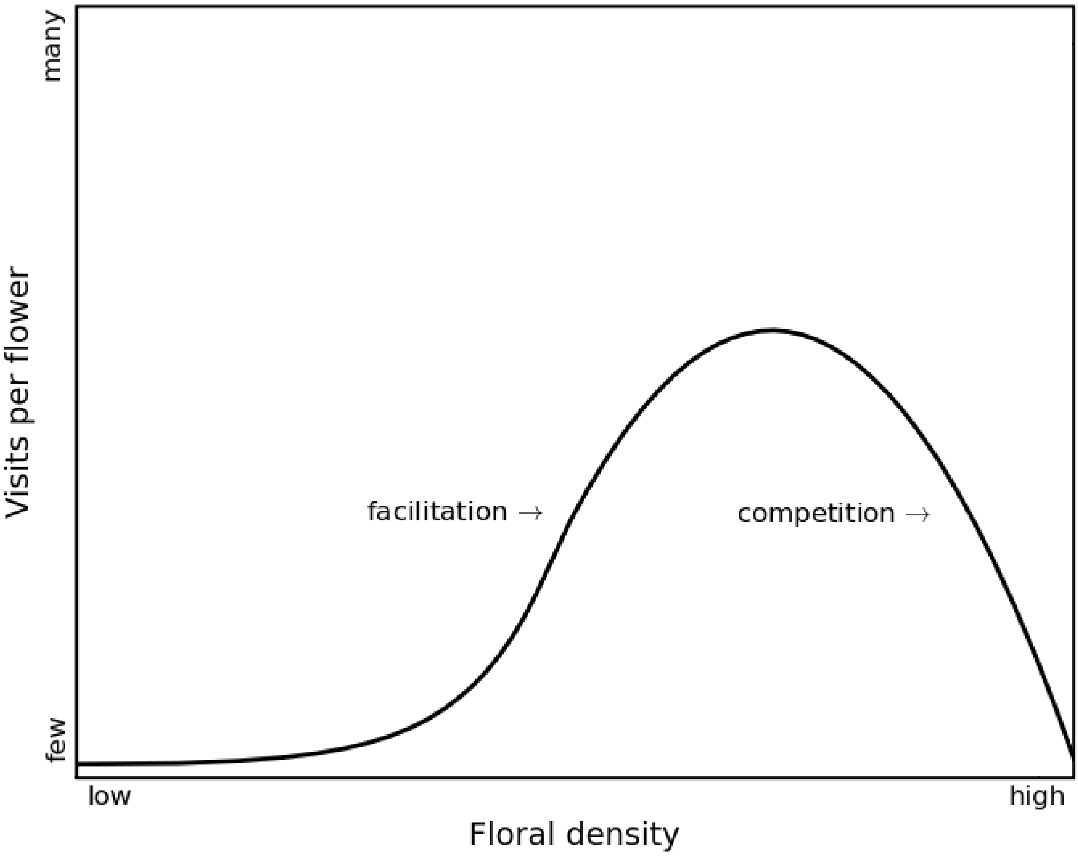

Figure 1.2: Rathcke's density-visitation curve, showing the change in pollinator visitation according to floral density in a plant community. Interspecific interactions between plant species will be facilitative to the left of the maximum point on the visitation axis, and competitive to the right as floral density increases. Reproduced from Rathcke, B. (1983) Competition and facilitation among plants for pollination. *Pollination Biology* pp. 305–329. Editor L. Real. Academic Press, New York.

Rathcke's predictions have been re-examined using an improved model which includes a number of parameters to describe optimal foraging behaviour, such as handling time and floral reward (Essenberg 2012). Essenberg's model confirms Rathcke's predictions, finding facilitation at low floral density and competition at high floral density (Essenberg 2012). This relationship is supported by field experiments examining visitation under changing floral abundance of conspecifics (Sabat and Ackerman 1996, Essenberg 2012). Field studies examining the effect of the floral abundance of heterospecifics on pollinator visitation have not consistently supported Rathcke's density-visitation curve however. For example, Jakobsson et al. (2009) found that the density of heterospecifics had little effect on pollinator visitation, although Dauber et al.

(2010) found low visitation at high floral density which lends support to Rathcke's predictions.

The strength of the relationship between heterospecific floral density and visitation may depend in part on pollinator foraging constancy (Feldman 2008, Stout et al. 2008). Floral constancy, first described more than 2,000 years ago by Aristotle (see Darwin 1876; Bennett 1883), refers to a tendency to visit flowers of one species and overlook alternative rewarding flowers (Wells & Wells 1983; Waser 1986). Constancy varies according to many factors including the pollinator species (Bennett 1883; Christy 1883; Heinrich 1979a; Goulson & Cory 1993). Constancy depends on learning and on handling time involved (Chittka 2002), and on plant density as pollinators will specialise in a frequency-dependent manner (Rathcke 1983; Kunin 1997).

Particularly rewarding plants can act as “magnet” species, meaning that they are highly attractive to pollinators, and this can facilitate visitation to less attractive co-occurring species (Laverty 1992, Johnson et al. 2003). Some invasive alien plant species, such as *Impatiens glandulifera*, have particularly highly rewarding flowers (Chittka & Schürkens 2001) and it has been suggested that this could mean that they act as a magnet species and facilitate visitation to co-flowering plants (Lopezariaza-Mikel et al. 2007). Alternatively, highly rewarding invasive alien plants could exert competitive effects regardless of abundance if pollinators show constancy to this species. Most studies examining the abundance of invasive alien species have found only competitive effects, which can be stronger at high abundance (Takakura et al. 2008; Flanagan et al. 2010, Dietzsch et al. 2011), although facilitative effects at low abundance have been observed (Muñoz & Cavieres 2008).

An alternative model to Rathcke's density-visitation curve is that of Hanoteaux et al. (2013) which predicts that density-visitation should interact with the spatial distribution of co-flowering species and, like Essenberg (2012), should also interact with floral reward. These model predictions are complex, finding that when an attractive species was more abundant, a less attractive species was more successful when uniformly distributed rather than aggregated (Hanoteaux et al. 2013). Although simplistic, Rathcke's model provides an alternative theoretical framework in which to test the relationship between the abundance of invasive alien plants and pollinator visitation in the field. It is also supported by more complex models (Essenberg 2012) and offers a simple and testable hypothesis for examining the impact of the abundance of invasive alien species.

Despite evidence for a relationship between floral density and pollinator visitation, few studies have considered the effect of the abundance of invasive alien plants on native plant pollination (Muñoz & Cavieres 2008; Dietzsch, Stanley, & Stout 2011), with most only considering the effect of the presence of invasive alien species on pollinator visitation (Chittka & Schürkens 2001; Lopezaraiza–Mikel et al. 2007).

Of those studies examining the abundance of invasive alien species, most consider the effects on a single focal native species (Takakura et al. 2008; Dietzsch et al. 2011) but the impact on the native plant community as a whole has received much less attention (Kaiser-Bunbury et al. 2011). Whilst single species studies are essential for detailed exploration of the impacts of invasive alien plants and the mechanisms involved, failure to examine multiple species responses could lead to different conclusions on the impact of invasive alien plants, even for the same alien species (e.g. Chittka & Schürkens 2001; compared to Bartomeus et al. 2010). Impact can be strongest for native plants that share pollinators with the invader (Thijs et al. 2012). In particular, native species that share floral colour and symmetry with alien plants can experience more pollinator sharing (Gibson et al. 2012). Species that are more likely to be affected could therefore be identified according to shared floral traits with the invader. Alien plants can alter the environment by reducing light levels by shading (McKinney & Goodell 2010), and altering soil nutrients (Dassonville et al. 2008; Blanck et al. 2011). Other traits may also be important therefore in determining whether native species experience an effect, such as plant height, or light or nitrogen preference.

Impacts on the native plant community may depend on the spatial scale at which species interactions are examined. Spatial scale is expected to influence the effects that are detected, and reveal different information regarding the mechanisms driving the relationship. For example, competition between the invasive alien *Impatiens glandulifera* and the native plant community for abiotic resources, such as light, have been observed at fine scales of 1 m^2 (Hulme & Bremner 2006) but such local scale studies may not detect pollinator-mediated effects that have been detected at a broader scales of 200 m^2 (Bartomeus et al. 2010). Pollinator-mediated effects can themselves occur over a range of spatial scales. Jakobsson et al. (2009) found that the effect of the presence of the invasive alien *Oxalis pes-caprae* on pollinator visitation to the co-flowering native *Diplotaxis eurocoides* varied according to the spatial scale used to measure the effect, between local scales of metres and broad scales of hectares. Effects of spatial scale may differ according to pollinator taxa. This is because the spatial scale at which pollinators perceive their environment depends on their dispersal ability and foraging range (Steffan-Dewenter et al. 2002). This demonstrates the importance of using both a range of pollinator taxa and multiple spatial scales to examine the potentially wide ranging impacts of invasive alien species. Pollinators that tend to forage over short distances, such as solitary bees which forage up to 600 m (Gathmann & Tscharntke 2002; but see Zurbuchen et al. 2010), may be influenced by local scale invasion. Pollinators that can forage over wide distances, such as bumblebees (Carvell et al. 2012) or hoverflies (Jauker et al. 2009), could respond to invasive alien plants at broader scales. Taxa that respond to their environment at broad scales may be less vulnerable to local disturbance caused by invasion than taxa with small foraging ranges.

Invasive alien plants may impact on the composition of the pollinator community, which in turn is expected to impact on the pollination of the plant community with long-term consequences for plant community composition. The presence of profusely flowering alien invaders can increase local pollinator abundance (Lopezaraiza–Mikel et al. 2007; Bartomeus et al. 2010), and it has been speculated that pollinator populations could increase as a result of the abundant floral resources available (Starý & Tkalcú 1998; Lopezaraiza–Mikel et al. 2007) or because alien species fill a phenological gap in floral resources (Stout & Morales 2009). Mass-flowering crops, which can be considered as ecological equivalents to invasive alien plants, can increase early colony growth in bumblebees, although this did not increase reproductive success (Westphal et al. 2009). Such effects have yet to be examined for invasive alien plants. Not all pollinators are likely to respond equally to invasive alien species. The extent to which pollinators visit the invasive alien plant will depend on a range of interacting factors, such as pollinator species (Bennett 1883; Christy 1883; Heinrich 1979a; Goulson & Cory 1993), the individual within a species (Heinrich 1976a), learning and handling time involved (Chittka 2002), and plant density and spatial arrangement (Rathcke 1983; Kunin 1997). Most visitors to alien species are generalist pollinators, that is they visit many plant species (Memmott & Waser 2002), which includes bumblebees (Heinrich 1976) and most hoverflies (Branquart & Hemptinne 2000). Other pollinators may not visit the invader if the rewards are inaccessible (Nienhuis et al. 2009) as foraging choice can be restricted by the morphology of the insect's mouthparts (Gilbert 1981). It is expected that pollinators that do not visit the invader, or that have close associations with native plant species, will be more affected by changes in the native plant community composition than those that feed on the invader (de Groot et al. 2007; Stout & Morales 2009).

The impact of invasive alien plants on the pollinator community is of particular importance given the evidence for global changes in pollinator range (Williams 1982; Cameron et al. 2011), relative species abundance (Bommarco et al. 2011; Cameron et al. 2011) and species diversity (Biesmeijer et al. 2006) although rates of change are slowing (Carvalheiro et al. 2013). Changes are attributed to a combination of habitat loss, agrochemicals, pathogens, climate change and invasive species (reviewed by Potts et al. 2010). We currently lack knowledge on the relative contribution of invasive alien plants to the pollinator community composition; how such changes may affect native plant pollination; and how this may vary with spatial scale (Bjerknes et al. 2007).

1.7 Study species

This thesis focuses on pollinator-mediated competition between native plant species and the invasive alien *Impatiens glandulifera* Royle (*Impatiens roylei* Walp., Himalayan balsam; Balsaminaceae). It is native to the Himalayas (Gupta 1989), and since its introduction into the UK in 1839 (Coombe 1956 in Beerling & Perrins 1993) it has become naturalised and a widespread invader of primarily riparian habitats. *Impatiens glandulifera* is considered to be an invasive plant in at least 15 European countries (CABI 2004) and in Canada and the USA (Clements et al. 2008). Figure 1.3 demonstrates that *I. glandulifera* has expanded its range across the UK since first introduced and is now found over much of the mainland as well as isolated islands. Given that it is restricted by frost sensitivity (Beerling & Perrins 1993), this range expansion could be a function of climate change making conditions suitable for invasion, or else could be indicative of a lag phase.

1.7.1 Features that may contribute to *I. glandulifera*'s invasiveness

Height

Impatiens glandulifera is the tallest annual in the UK, and can grow to 3 metres (Clements et al. 2008). Combined with branching from the main stem (Beerling & Perrins 1993) it can dominate the aerial environment, which may enable it to compete with co-occurring plant species for light and space. Despite the importance of waterways as vectors for dispersing seeds (Richardson et al. 2007), *I. glandulifera* performs well under low water availability and has not been found to demonstrate intraspecific competition (or interspecific competition with other *Impatiens* species) for water (Skálová et al. 2013).

Flowers

Impatiens glandulifera is well-integrated into native plant-pollinator networks (Lopezaraiza–Mikel et al. 2007). In particular, it is highly attractive to bumblebees (Lopezaraiza–Mikel et al. 2007; Nienhuis et al. 2009; Bartomeus et al. 2010). *Impatiens glandulifera* is primarily bumblebee-pollinated, due to their mechanical fit inside the flowers (Titze 2000; Nienhuis & Stout 2009), but it is also visited by a wide range of insects including the honeybee (*Apis mellifera* L.), and various social wasp and moth species (Dunn 1977; Valentine 1978; Titze 2000), which may also be pollinators. It is characterised by a large floral display of pink, purple or white flowers which are 2.5 - 4 cm long, and held in axillary racemes of 3 - 12 flowers (Beerling & Perrins 1993) (Figure 1.4 a). In the UK, the peak flowering period is between July and October (Beerling & Perrins 1993). Flowers are highly rewarding, producing a large quantity of pollen (Titze 2000) and nectar which is secreted from a spur at the flower's base (Beerling & Perrins 1993). Nectar sugar concentration is within the range of native bumblebee-pollinated plants at 48 % (Chittka & Schürkens 2001) but its rate of nectar secretion at 11312 µg of sugar per 24 hours (Raine & Chittka 2007a) exceeds the rate of native species, for those which have data available, which mostly produce an order of magnitude less (Comba et al. 1999; Raine & Chittka 2007a) (Figure 1.5).

Seed production and dispersal

Plants can produce up to 2500 seeds per individual (Koenies & Glavac 1979 in Clements et al. 2008). Seeds germinate synchronously, which can lead to the formation of dense stands (Beerling & Perrins 1993) that are characteristic of this species (Figure 1.4 b); *I. glandulifera* also often grows gregariously in its native range (Polunin & Stainton 1984). Seeds are dispersed up to 6 metres away from the parent plant by explosive dehiscence of the seed capsule (Figure 1.4 c), depending on prevailing winds and the position of the seed capsule (Beerling & Perrins 1993; Chapman & Gray 2012). Seeds are dispersed over long distances by humans, either as deliberate introductions into gardens or by accidental transportation (Perrins et al. 1993); and can be spread via waterways (Trewick & Wade 1986; Pysek & Prach 1993). It is tolerant of a wide variety of soil types, but is restricted by frost sensitivity (Beerling & Perrins 1993).

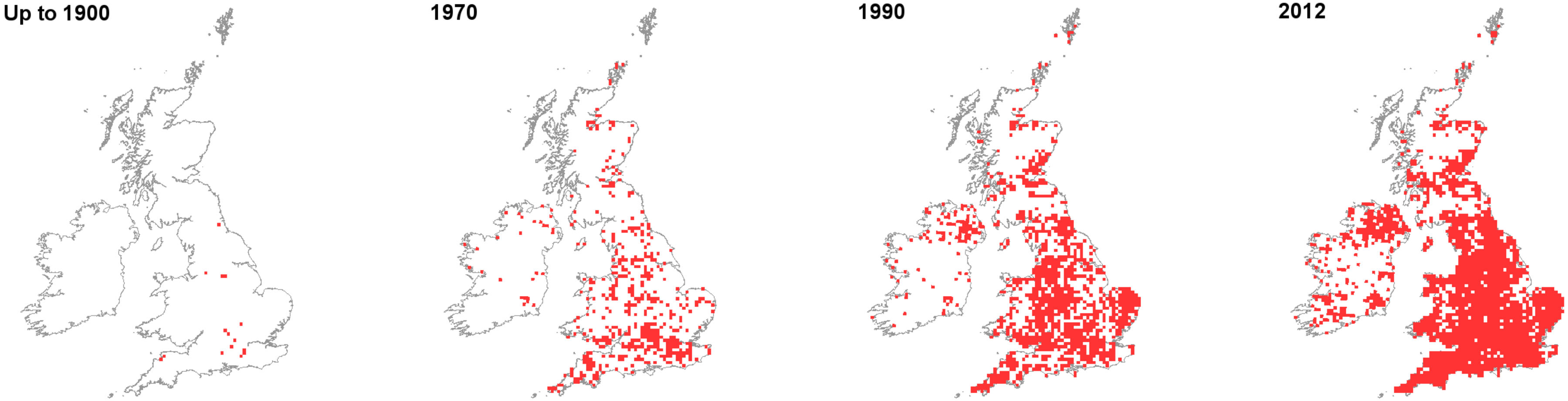

Figure 1.3: The UK distribution of *Impatiens glandulifera* between 1900 and 2012. Data points represent its presence in 10 km grid squares. Data was sourced from the NBN Gateway website.

Figure 1.4: a) *Impatiens glandulifera* flowers, **b)** a typical stand of *I. glandulifera* plants, and **c)** its seed capsules.

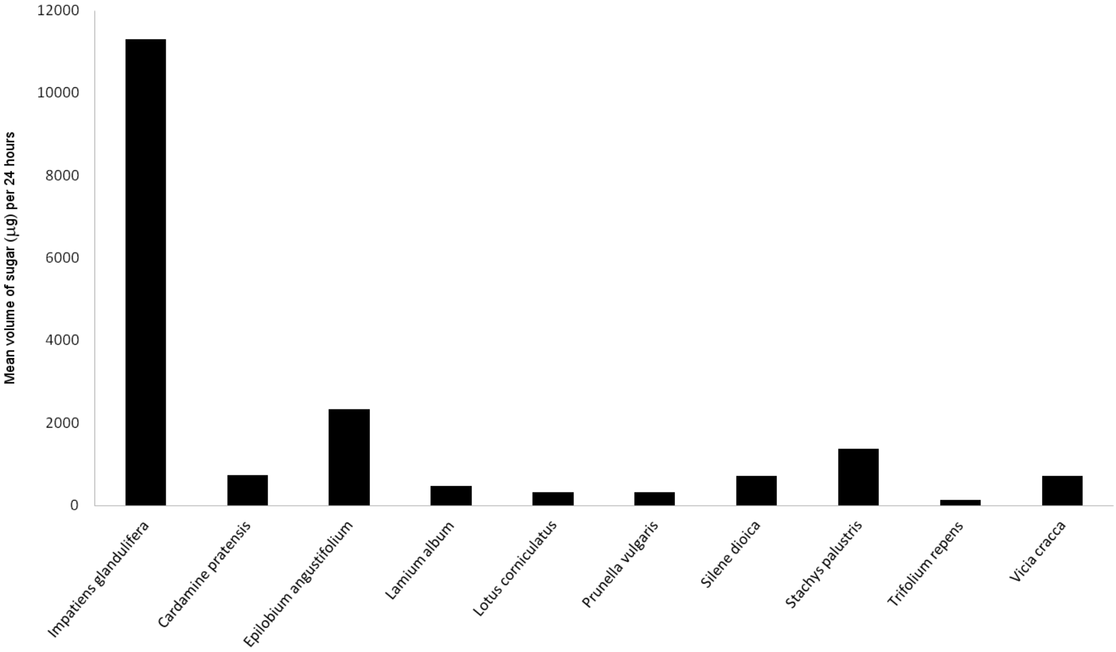

Figure 1.5: The mean volume of sugar (µg) produced per 24 hours by *Impatiens glandulifera* and a selection of co-occurring plants native to the UK. Data reproduced from Raine and Chittka (2007) Entomol Gener 30 (2): 191-192. Standard error information is not available in the published manuscript (Raine & Chittka 2007a).

1.7.2 Impact of *I. glandulifera*

The impact of *I. glandulifera* varies between previous studies. In the UK, the presence of *I. glandulifera* led to reduced native plant species diversity and richness, in particular for light-demanding species (Hulme & Bremner 2006), although in the Czech Republic it was found to have little effect (Hejda & Pyšek 2006; Hejda et al. 2009).

Pollinator-mediated competition between *I. glandulifera* and native plants has received increasing attention due to its large and highly rewarding floral displays, and increasing geographic spread. However, these studies show conflicting results. Some studies have found that *I. glandulifera* competes with native plants for pollinator visitation (Chittka & Schürkens 2001; Thijs et al. 2012), whereas others have found no detectable effect (Nienhuis et al. 2009; Bartomeus et al. 2010) or that it facilitates visitation to co-flowering native plants (Lopezaraiza–Mikel et al. 2007).

Gaps in the knowledge of the impact of *I. glandulifera*

Differences in the impact of *I. glandulifera* may be due in part to its abundance, but this relationship has yet to be examined. Based on the predictions of Rathcke's density-visitation model (1983) (Figure 1.2), *I. glandulifera* may facilitate visitation to co-flowering plants at low floral abundance by attracting shared pollinators, but compete for visits at high abundance when pollinators become limiting. This is expected to impact on the native plant community composition if these interactions alter native plant reproductive success. The composition of the pollinator community should also change as a consequence, with different responses expected for pollinators attracted to *I. glandulifera* and those associated with native plants.

The spatial scale at which invasion is assessed may also contribute to discrepancies between previous studies as different mechanisms may prevail. In fine scale studies, competition for abiotic resources such as light may be strong (Hulme & Bremner 2006); whereas pollinator-mediated effects are likely to occur at broader scales (Lopezaraiza-Mikel et al. 2007), with the observed spatial scale dependant on pollinator foraging range and dispersal ability (Steffan-Dewenter et al. 2002).

The choice of native plant species used to examine the impact of *I. glandulifera* varies between studies (Chittka & Schürkens 2001; Bartomeus et al. 2010; Thijs et al. 2012), but may be important in determining the outcome of the interaction. Pollinator-mediated effects are likely to be greatest for native species that show the most overlap in pollinator sharing (Thijs et al. 2012) and in their flowering period. This demonstrates the necessity of using a wide range of species in the community to establish the full extent of its effects.

Yet to be addressed is the impact of *I. glandulifera* pollen on native plant reproduction. This is despite its pollen being found to dominate pollen networks (Lopezaraiza–Mikel et al. 2007), and evidence in other systems that interspecific pollen transfer (IPT) can reduce native plant reproductive success (Brown & Mitchell 2001). *Impatiens glandulifera* is known to be highly attractive to pollinators (Lopezaraiza–Mikel et al. 2007). Resulting changes in pollinator foraging behaviour could influence patterns of pollen dispersal, which will determine the genetic quality of pollen received by co-flowering native plants, although this effect has yet to be examined for invasive alien species.

Genetic quality of pollen can change via pollen wastage through interspecific movement (Bell et al. 2005) or via the distance that native pollen is dispersed (Campbell & Motten 1985), which has implications for the diversity of pollen donors and the relatedness of pollen received. Despite implications for changing plant mating systems, reducing individual plant fitness and influencing local plant population demographics (Ellstrand & Elam 1993), this approach of examining the genetic quality of pollen has not been previously explored.

1.8 Aims

The aim of this study was to examine pollinator-mediated effects of *Impatiens glandulifera* on native plant pollination. This research explored reasons governing apparent discrepancies between previous studies. Studies examining the effect of the presence of *I. glandulifera* on the community composition of co-occurring plants have found that it reduces native plant species diversity and richness (Hulme & Bremner 2006), but others have found little effect (Hejda & Pyšek 2006; Hejda et al. 2009).

Similarly, studies on the effect of *I. glandulifera* on pollinator visitation have found contrasting results. In the presence *I. glandulifera*, pollinator visitation to co-flowering plants has been found to decrease (Chittka & Schürkens 2001), increase (Lopezaraiza-Mikel et al. 2007) or demonstrate no change (Bartomeus et al. 2010). This study attempted to resolve these apparent discrepancies by investigating the relationship between the abundance of *I. glandulifera* and its impact on the plant and pollinator community composition, and on pollinator visitation. The study also aimed to explore whether this relationship varied with the spatial scale at which invasion was assessed. This is because the mechanism determining the observed relationship in previous studies could be due to spatial scale. At local scales, competition for abiotic resources such as light may be strong (Hulme & Bremner 2006); whereas at broad scales pollinator-mediated effects may dominate (Lopezaraiza-Mikel et al. 2007).

This study also aimed to investigate previously unexplored impacts of *I. glandulifera*. *Impatiens glandulifera* produces large quantities of pollen (Titze 2000), which dominate pollen networks (Lopezaraiza–Mikel et al. 2007). However, its effect on native plant pollination has yet to be studied. This was addressed by examining the effects of interspecific pollen transfer on a co-flowering native species. Pollinator foraging behaviour can influence the genetic quality of pollen received (Campbell & Motten 1985, Bell et al. 2005), which has important implications in reducing individual plant fitness and influencing local plant population demographics (Ellstrand & Elam 1993). Despite this, the impact of pollinator-mediated competition between native and invasive plants on the genetic quality of pollen has not been previously explored. This study therefore investigated the effect of pollinator-mediated competition from *I. glandulifera* on the genetic quality of pollen received by a co-flowering native species.

In Chapter two, I tested the hypothesis that the impact of *I. glandulifera* on the composition of the local plant community varies according to its abundance and the spatial scale at which invasion is assessed. This is because previous studies exploring its impact on the local plant community composition have examined the effect of its presence but found conflicting results (Hulme & Bremner 2006, Hejda & Pyšek 2006; Hejda et al. 2009), which could be an effect of the invader's abundance or a product of the spatial scale at which the effects of invasion were assessed. I tested for a relationship between the abundance of *I. glandulifera* and the composition of the local plant community in terms of its species richness, abundance, and plant traits, at three scales of invasion; 1 m^2, 100 m^2 and a 420 m transect. The aim was to generate a large data set useful for detecting trends and informing future directed study.

In Chapter three, I tested the hypothesis that the impact of *I. glandulifera* on the composition of the local pollinator community and on pollinator visitation to co-flowering plants varies according to its abundance and the spatial scale at which invasion is assessed. This is because previous studies exploring the impact of *I. glandulifera* on these variables have found conflicting effects. To date, studies have examined the effect of the presence of *I. glandulifera* and found that pollinator visitation to co-flowering plants decreased (Chittka & Schürkens 2001), increased (Lopezaraiza-Mikel et al. 2007) or showed no change (Bartomeus et al. 2010). This could be an effect of the invader's abundance or could be due to the spatial scale at which impact was assessed. The pollinator community composition as a function of the presence of *I. glandulifera* has been examined (Lopezariaza-Mikel et al. 2007, Bartomeus et al. 2010) because this has implications for co-flowering plant pollination and thus for plant community composition in the longer term. Therefore, the pollinator community composition was examined in field plots that differed in the abundance of *I. glandulifera*, at local and broad scales, and bumblebee-flower visitation patterns were examined in plots that varied in the relative abundance of *I. glandulifera* flowers.

In Chapter four, I tested the hypothesis that *I. glandulifera* pollen reduces the reproductive success of the co-flowering native species, *Lamium album* L. (white deadnettle; Lamiaceae) via pollinator sharing. This is because interspecific pollen transfer (IPT) from invasive alien species can reduce native plant reproductive success (Brown & Mitchell 2001). This may be an important consequence of *I. glandulifera* invasion, since its pollen is known to dominate pollen networks (Lopezaraiza–Mikel et al. 2007) but its effect on native plant reproductive success is unknown to date. *Lamium album* was chosen as a study species because it is expected to receive *I. glandulifera* pollen under field conditions as they co-flower, co-occur in the same habitat and share bumblebee pollinators. I tested the hypothesis using hand-pollination experiments to isolate the effect of IPT from confounding effects such as pollinator visitation rates.

In Chapter five, I tested the hypothesis that *I. glandulifera* interrupts the mating system and alters the reproductive success of the self-compatible co-flowering native *Lamium album*. This is because pollinator foraging behaviour can influence the genetic quality of pollen received (Campbell & Motten 1985, Bell et al. 2005), which can reduce individual plant fitness and influence local plant population demographics (Ellstrand & Elam 1993). Despite knowledge that *I. glandulifera* alters pollinator foraging behaviour (Lopezaraiza-Mikel et al. 2007), the impact of pollinator-mediated competition between native and invasive plants on the genetic quality of pollen received has not been previously explored. I tested the hypothesis that *I. glandulifera* alters the genetic quality of pollen received by co-flowering plants that share pollinators, by genotyping progeny from *L. album* parents grown either with co-flowering *I. glandulifera* or in removal plots.

In Chapter six, I discuss the main findings of the research in the context of these predictions, and suggest further work.

Chapter Two:

Impact of the abundance of the invasive alien *Impatiens glandulifera* Royle (Himalayan balsam; Balsaminaceae) on local plant community composition: a study at multiple scales of invasion

2.1 Abstract

Invasive alien plants may pose a threat to native plant communities and contribute to local biodiversity loss. We tested for a relationship between the abundance of the invasive alien plant *Impatiens glandulifera* and the composition of the plant community in terms of its species richness, abundance, and plant traits, at multiple scales of invasion. Thirty plots of 100 m^2 were selected from riparian habitat on six rivers in central and south-east Scotland, UK. Plant community data was collected from each plot and related to the abundance of *I. glandulifera* at three scales of invasion: quadrat scale (1 m^2), plot scale (100 m^2), and broad scale (420 m transect). Ordination analyses were used to detect relationships between the plant community composition and the abundance of the invader, at the three scales of invasion. Generalised linear mixed models were used to examine the relationship between invasion at each scale and plot plant species richness, the abundance of bumblebee-pollinated plants, and the abundance of plants grouped by height and by nitrogen and light Ellenberg indicator values, to examine whether plant responses to invasion can be grouped by traits. The composition of the plant community was significantly associated with the abundance of *I. glandulifera* at all three scales of invasion. Plot plant species richness decreased linearly with increasing abundance of *I. glandulifera* at the broad scale of invasion. The plant community was characterised by taller species, and species with high Ellenberg light and nitrogen indicator values, with increasing abundance of the invader at the plot and the broad scale of invasion. There was a higher proportion of bumblebee-pollinated plants in the plot at low *I. glandulifera* abundance, at the plot scale of invasion. In general, the effects of *I. glandulifera* were mostly strongest at a broad scale of invasion. The plant community composition demonstrated the most marked changes with invasion at the broad scale, which could indicate pollinator-mediated effects. Relationships were also found between the abundance of *I. glandulifera* and the abundance of plants grouped by traits, providing evidence that plant traits could be useful in predicting species responses to invasion. Given the reproductive success of *I. glandulifera*, intervention to prevent over-dominance may be important in maintaining plot plant species richness and community structure. The plant community responded to invasion according to both spatial scale and *I. glandulifera* abundance, demonstrating that these effects should be considered when interpreting patterns associated with invasion.

2.2 Introduction

Invasions by alien plants pose a threat to native plant communities by competing for resources such as light (Iponga 2010), water (D'Antonio & Mahall 1991), and pollinators (Brown et al. 2002). Such competition can modify the structure and stability of ecological communities (reviewed in Levine et al. 2003) by reducing local biodiversity (Gerber et al. 2008; but see Hejda & Pyšek 2006) and rarely, by causing native plant extinctions (Baider & Florens 2011). Biodiversity is expected to buffer ecosystems against environmental perturbations as there is a high level of functional redundancy inherent in ecological communities (Memmott et al. 2004), although species loss beyond a critical threshold may result in their collapse (Fortuna & Bascompte 2006, Kaiser-Bunbury et al. 2010). Invasive alien plants also threaten native plant communities by changing disturbance regimes, nutrient cycling and hydrology (reviewed in Mack & D'Antonio 1998; Levine et al. 2003). Although it is estimated that a small fraction of alien plants that have been introduced actually become invasive (Williamson 1996), those that do may be strong competitors. This is because they tend to occur in large and dense populations (Bjerknes et al. 2007), and because native plants lack co-evolved mechanisms to tolerate or avoid them (Callaway & Ridenour 2004). Many invasive alien plants characteristically have large displays of attractive flowers, having been introduced as ornamental species (Stout & Morales 2009), which can enable them to disrupt plant-pollinator networks by usurping pollinators (Memmott & Waser 2002).

The response of native plant communities to the presence of invasive alien plant species has become increasingly studied in recent years (Hulme & Bremner 2006; Hejda et al. 2009; Ramula & Pihlaja 2012), but there remains a paucity of studies that consider the community's response to the abundance of the invader (Kaiser-Bunbury et al. 2011). The abundance of invasive alien plants could be crucial in determining the outcome of studies since theoretically, their impact should be proportional to their abundance. For instance, floral abundance is predicted to regulate pollinator-mediated interactions between co-flowering plants (Rathcke 1983) (Figure 1.2). At low floral density, plants should facilitate each other's pollination since they combine to increase the size of the floral display and attract shared pollinators (Rathcke 1983). Once floral density exceeds the pollinator visitation rate, plants should compete for pollinators (Rathcke 1983).

Kaiser-Bunbury et al (2011) found that invasive alien plants modified plant-pollinator network structure more at high abundance compared to low abundance, but did not find evidence for facilitation; and nor did these changes translate to changes in fruit set. However, where competition from invasive alien plants for pollinators reduces seed set in focal native species (Brown et al. 2002), this could lead to shifts in the plant community composition.

The effects of invasive alien species on native plant communities are likely to vary according to the spatial scale at which they are examined. At sub-global scales, invasions can increase species richness due to the arrival of new species, so long as native species loss is offset by the gain of alien species, but may decrease regional scale species richness by homogenising flora (Sax & Gaines 2003; Schwartz et al. 2006). Widespread invasion also has the potential to impact on native plant communities by causing fragmentation of suitable habitats, although such broad scale studies are lacking (Bartomeus et al. 2010). Fragmentation can lead to reduced gene flow and increased inbreeding (reviewed in Aguilar et al. 2008), which has negative implications for individual plant fitness and persistence of local populations. Resource competition between invasive alien and native species is also expected to vary with spatial scale. Competition for abiotic resources such as light and space is expected to act most strongly at local scales. For example, competition between an invasive alien and the native plant community for light was demonstrated at a scale of 1 m^2 (Hulme & Bremner 2006). Competition for pollinators may act at broader scales. In particular, pollinators that are able to forage over wide distances, such as bumblebees (Carvell et al. 2012), could respond to the large floral displays of invasive alien plants at broad scales. The spatial scale at which pollinators respond to their environment varies according to their dispersal ability and foraging range (Steffan-Dewenter et al. 2002), so pollinator-mediated effects of invasion should vary with pollinator taxa.

Studies that examine plant communities have the potential to detect the responses of a wide range of plant species to competition from invasive aliens. Most studies to date consider the effect of invasive alien plant abundance on a focal native species, or a small number of species (Muñoz & Cavieres 2008; Takakura et al. 2008; Flanagan et al. 2010). Although single species studies are important for determining mechanisms involved, failure to examine multiple species responses could lead to differences between studies, even those examining the same invasive alien plant species. For example, competition from the invasive alien *Impatiens glandulifera* reduced seed set in the native *Stachys palustris* (Chittka & Schürkens 2001) but had no effect on seed set in *Raphanus sativus* (Bartomeus et al. 2010). These studies may have differed in the abundance of the invader, and differed in the floral traits of symmetry and colour of the native plant species used to detect impact. Interactions between plant species may be predicted by life history traits (Hodgson et al. 1999; Eskelinen 2010). In particular, floral traits have often been used to predict pollinator-mediated interactions between native plant species (e.g. Lazaro et al. 2008), and recently it has been demonstrated experimentally that floral traits of symmetry and colour (in the visible spectrum) can also predict such interactions between native and invasive alien plant species (Gibson et al. 2012). Other plant traits yet to be considered may also be important in predicting interactions between native and invasive alien species. For example invasive alien plants can increase shading (McKinney & Goodell 2010), which could have the greatest impact on light-demanding species in the community. In another study, short plants were displaced by tall invaders whereas there was no effect on plants of comparable height, which could indicate competition for light (Thiele et al. 2010), and suggests that the height of plants may contribute to their vulnerability to invasion. Invasive alien plants can cause an increase in nutrient pools and fluxes (Dassonville et al. 2008; Blanck et al. 2011), meaning that the response of the native plant species may vary according to their tolerance of different nutrient levels.

Impatiens glandulifera Royle (Himalayan balsam; Balsaminaceae) is a widespread alien species that is invasive across Europe (Pysek & Prach 1995), and considered to be in the top 20 most invasive plants in the UK (Crawley 1987). *Impatiens glandulifera* is the tallest annual in the UK, and has a large floral display (Beerling & Perrins 1993) of highly rewarding flowers which secrete nectar at a faster rate than that recorded for any native species (Raine & Chittka 2007a). *Impatiens glandulifera* is primarily bumblebee-pollinated (Titze 2000; Nienhuis & Stout 2009), but is also visited by a wide range of insects including the honeybee (*Apis mellifera* L.), and various social wasp and moth species (Dunn 1977; Valentine 1978; Titze 2000) which may also act as pollinators. *Impatiens glandulifera* can therefore disrupt plant-pollinator interactions (Lopezaraiza–Mikel et al. 2007; Thijs et al. 2012), and can reduce native plant reproductive success (Chittka & Schürkens 2001). Despite this, there are few studies focussing on how the native plant community composition changes in response to invasion (Hulme & Bremner 2006; Hejda & Pyšek 2006; Hejda et al. 2009), and results are conflicting. Differences between studies have been postulated to be caused by differences in the abundance of *I. glandulifera* (Hejda & Pyšek 2006), but it has yet to be established whether a relationship exists between *I. glandulifera* abundance and its effect on the native plant community composition. This study examines the relationship between *I. glandulifera* abundance and the plant community composition in riparian habitat across central and south-east Scotland, at multiple scales of invasion. Specifically, we focussed on pollinator-mediated effects by targeting insect-pollinated plant species, and addressed the following questions: i) What is the relationship between *I. glandulifera* abundance and local plant species richness and community composition? ii) Does this vary with the spatial scale at which invasion is assessed? iii) Do plant traits determine their vulnerability to invasion?

2.3 Methods

2.3.1 Selection of study area

The study was carried out in riparian habitat on the rivers Almond, Eden, Esk, Tay, Tyne and Tweed in central and south-east Scotland (Figure 2.1). In April 2010, 30 plots separated by at least 1 km and measuring 20 x 5 m were selected, and marked by wooden stakes, to represent a gradient of *I. glandulifera* abundance (Table 2.1, Figure 2.2). To isolate the effect of *I. glandulifera* on the plant community from other common invasive alien plants, plots were selected to exclude *Fallopia japonica* Houtt and *Heracleum mantegazzianum* Sommier and Levier, as far as possible. Some plots in which *I. glandulifera* was absent at the start of the study were subsequently invaded, and others showed an increase in abundance over the season. This suggests that the habitat was similar across plots as they did not differ in their suitability for invasion.

In order to minimise differences between plots, they were selected so that the abundance of *I. glandulifera* was unrelated to its position in the river catchment, to minimise associations between abundance and co-varying factors such as river width and surrounding land use. Plots were selected from accessible riparian habitat that was less than 100 m in elevation, and surrounding land cover did not differ significantly between plots (ANOVA, $p \geq 0.32$; full results are in Appendix A, Table A.1). This was determined by creating a 1 km radius circular buffer zone around each plot using Arc GIS (ESRI 2010) and land cover classes in this area were found using the Centre for Ecology and Hydrology (CEH) Land Cover Map 2000 (Fuller et al. 2002). Classes were then redefined into broader classes of arable, grassland, improved grassland, urban or woodland. An analysis of variance (ANOVA) was used to test whether the amount of each land cover class varied between plots.

2.3.2 Vegetation survey

Plots were visited monthly from May to September 2010 in a random order by proceeding from either upstream or downstream. For practical reasons, plots were grouped by rivers closest together, but the order in which these groups were visited was randomised. Sampling frequency varied among plots (Table 2.1) due to inaccessibility caused by flooding or farming practices. Also a subset of 17 plots were visited more frequently to increase the size of the data set as time restrictions prevented more frequent visits to all plots. This was accounted for in the statistical analysis (see Section 2.3.4) by using GLMM models which handle unbalanced data, and by analysing each month separately in the ordination analyses.

Vegetation was sampled along a 20 m transect through the middle of each plot, using 1 m^2 quadrats spaced evenly along the transect at 5 m intervals. These five quadrats per plot were used to calculate a plot mean for each sampling occasion. The abundance of each plant species was estimated by counting the number of stems or estimating its percentage cover. Most species were measured by the former, but the latter was used to record plant species where stem number was difficult to determine, such as creeping plants *Calystegia sepium* L. (hedge bindweed, Convolvulaceae) or *Vicia sepium* L. (bush vetch, Fabaceae); or where stems were extremely numerous, such as *Chrysosplenium oppositifolium* L. (opposite-leaved golden saxifrage, Saxifragaceae). Measuring the abundance of some species by percentage cover maximised recorder efficiency, which was necessary given the large number of plots in the study and their wide geographic distribution. Plant abundance data from these two methods were combined using a Bray-Curtis dissimilarity index, which was used to quantify the compositional dissimilarity between plots. Additionally, the percentage cover of bare ground in each quadrat was estimated as a measure of disturbance, and the number of trees in the plot were counted as a measure of disturbance and shading. Plants that were entirely or mostly non-entomophilous were excluded, because the focus of the study was to explore possible pollinator-mediated effects of *I. glandulifera* on the plant community. Plant abundance was measured as a proxy for floral resources.

Table 2.1: Locations of thirty experimental plots in riparian habitat on six rivers, with the maximum *Impatiens glandulifera* abundance recorded in each 20 x 5 m plot during the study (May-September 2010), and its abundance at a broad scale of a 420 m transect (recorded in September 2010). Abundance was measured as an estimate of its percentage cover, and classed as absent (0 %), low (0.1-30 %), medium (30.1-60 %) or high (> 60 %), shown in parentheses. Missing values for broad cover estimates and variation in sampling frequency were due to inaccessibility caused by flooding or farming practices. Also a subset of 17 plots were visited more frequently to increase the size of the data set as time restrictions prevented more frequent visits to all plots. This was accounted for in the analysis by using GLMM models which can handle unbalanced data, and by analysing each month separately in the ordination analyses.

Plot	**River**	**Grid reference**	**Maximum *I. glandulifera* abundance, plot scale (%)**	***I. glandulifera* abundance, broad scale (%)**	**No. of sampling occasions**
1	Tay	NO0895139555	Medium (35)	*Missing value*	2
2	Tay	NO1231833069	Low (0.5)	*Missing value*	2
3	Tay	NO1019530507	High (68)	*Missing value*	3
4	Tay	NO1038229613	Absent (0)	*Missing value*	1
5	Eden	NO1848909070	Absent (0)	Low (2)	3
6	Eden	NO2840408544	Absent (0)	Low (15)	6
7	Eden	NO3086908947	Low (26)	Medium (53)	6
8	Eden	NO3291809700	Medium (48)	Medium (53)	6
9	Almond	NT0929369582	Medium (38)	Low (15)	6
10	Almond	NT1333373855	Low (7)	Medium (35)	6
11	Almond	NT1415374077	Medium (53)	Medium (35)	6
12	Esk	NT3303662037	Absent (0)	Absent (0)	5
13	Esk	NT3247363781	Absent (0)	Absent (0)	5
15	Esk	NT3373668973	High (76)	Low (22)	5
15	Esk	NT3420769342	Low (6)	Low (17)	5
16	Esk	NT3453271846	High (60)	Medium (37)	6
17	Tyne	NT4998171647	Low (3)	Low (18)	6
18	Tyne	NT5661875668	Low (6)	Low (16)	6
19	Tyne	NT5754375917	Absent (0)	Low (22)	4
20	Tyne	NT5875576556	Medium (41)	Medium (33)	6
21	Tyne	NT5956277824	Low (3)	Low (21)	6
22	Tyne	NT6081178110	Low (7)	Medium (39)	5
23	Tweed	NT5487834608	High (69)	Medium (31)	2
24	Tweed	NT5785134504	Absent (0)	Absent (0)	1
25	Tweed	NT5895931833	Medium (56)	Low (16)	3
26	Tweed	NT6638031082	Low (15)	*Missing value*	2
27	Tweed	NT7580835820	Medium (47)	Low (25)	3
28	Tweed	NT8493940216	Medium (36)	Low (24)	3
29	Tweed	NT8954445697	Low (13)	Low (18)	3
30	Tweed	NT9345551053	High (62)	Medium (36)	3

Figure 2.1: Thirty experimental plots in central and south-east Scotland, UK that varied in the abundance of *Impatiens glandulifera*.

Figure 2.2: Plot 27 (NT 75808 35820) in **a)** April, **b)** July and **c)** September 2010. Abundance of *Impatiens glandulifera* is classed as low, low and medium respectively.

2.3.3 Invasion at multiple spatial scales

Impatiens glandulifera abundance was measured at quadrat, plot and broad scales using percentage cover estimates.

Quadrat scale invasion (**1 m^2**) was measured using the mean number of *I. glandulifera* stems in five 1 m^2 quadrats in each plot (using the same method as the aforementioned vegetation survey).

Plot scale invasion (**100 m^2**) was measured as the mean percentage cover from 100 1 m^2 quadrats which covered the plot area, to explore the effects of abundance. Percentage cover estimates were then classified as absent (0 % *I. glandulifera* cover), low (sparse cover, 0.1-30 %), medium (noticeably invaded, 30.1-60 %) or highly invaded (predominantly *I. glandulifera*, more than 60 % cover) for significance testing of non-linear effects of abundance.

Broad scale invasion (**420 m transect** of variable width, as determined by the width of the river bank) was calculated by measuring *I. glandulifera* abundance along a 200 m transect each side of the 20 m long plot, on both sides of the river. At 5 m intervals along the transect abundance was estimated as absent, low, medium or high, within a 1 m wide section of river bank. This was converted into a percentage cover estimate, by using the median percentage for each class (class ranges described above). A mean was the calculated for each transect to find a single value for the whole broad scale at each plot. No plots were classed as high abundance at this scale.

2.3.4 Statistical analysis

All statistical analyses were done in R v2.10.1 (R Development Core Team, 2011). The plant community composition was examined by fitting a Bray-Curtis dissimilarity index calculated from abundance data for the whole plant community, excluding *I. glandulifera*. The relationship between the community composition and explanatory variables of *I. glandulifera* abundance at three spatial scales, the number of trees in the plot and the mean percentage cover of bare ground in the plot were examined using a partial Constrained Analysis of Principal Co-ordinates (partial CAP) (vegan package v2.0-2; Oksanen et al. 2011). The partial CAP removed variance associated with date, to first examine the effect of invasion independent of temporal effects. The model was tested for significance using analysis of variance (ANOVA). Each term in the model was then tested for significance using permutation tests (999 times), stratified by plot to avoid pseudoreplication. To examine temporal effects of *I. glandulifera* invasion, a CAP was used to analyse the plant community composition and explanatory variables described above, for data from each month separately. Model significance was determined as described above. Associations between *I. glandulifera* and individual plant species were examined using an unconstrained PCoA ordination (with two axes) on the Bray-Curtis dissimilarity index, which included *I. glandulifera*, and was calculated from species' average abundance over the season. Only species occurring in more than five plots were included, as it was considered that estimates obtained for rarer species would be unreliable. Association was measured using species scores derived from the unconstrained PCoA ordination, weighted by the species' abundance.

The relationship between *I. glandulifera* abundance and plant species richness was examined using generalised linear mixed effects models (GLMMs) (function lme, v0.999375-35; Bates et al. 2011), with a Poisson error distribution. Plot and river were treated as random effects, the latter because plots on the same river were expected to be more similar to each other than to plots drawn at random. Data was tested for overdispersion. *Impatiens glandulifera* abundance and a quadratic term (included to detect a curved rather than linear relationship with the response variable to test the Rathcke (1983) density-visitation model predictions, to investigate pollinator-mediated effects), date and its interaction with *I. glandulifera* abundance, the number of trees in the plot, and the mean percentage cover of bare ground in the plot were all treated as fixed effects. Minimum adequate models (Crawley 2002) were obtained using lowest AIC values, by removing non-significant terms in a backwards stepwise manner. *Impatiens glandulifera* abundance at the quadrat and plot scales, and bare ground and the number of trees, were log transformed to improve conformity to normal distributions. This was achieved by examining the frequency distribution of the residuals. Models were repeated to examine each measure of *I. glandulifera* abundance. Variance explained by the model was estimated using a pseudo-R^2 value (hereafter simply referred to as R^2), calculated by correlating the observed data with the model values for the fixed effects. Unlike linear models, it is not possible to extract a true R^2

value from GLMM models due to their nested structure (Xu 2003).

The effect of invasion on the functional composition of the community was examined by grouping plants by height (log transformed), Ellenberg light and Ellenberg nitrogen indicator values, which were taken from the 'PlantAtt' database (Hill et al. 2004) because they could not be measured in the field for logistical reasons. Plant species' mean height estimates (Hill et al. 2004) were used in place of direct field measurements due to time constraints. Although plants are expected to vary around this mean, this approximation of species height was considered useful for detecting general trends which could inform future studies. Ellenberg indicator values (Ellenberg et al. 1992) are used to classify plants according to their ecological behaviour. They indicate conditions under which species are usually found in the wild, without necessarily indicating ideal growth conditions, and reflect a range of environmental factors integrated into a single value (Ellenberg et al. 1992). For example, Ellenberg nitrogen indicator values describe soil fertility, or productivity, since they encompass many variables including soil nutrients, moisture availability and disturbance (Schaffers & Sykora 2000; Hill et al. 2000). Ellenberg indicator values were employed in this study for practical reasons, since obtaining the wide range of field measurements encapsulated by Ellenberg indicator values is both costly and time consuming. Short-term field measurements are also of limited use since they can fluctuate strongly over time and space, whereas indicator values represent conditions over the long-term.

Plants were also grouped by whether they were bumblebee-pollinated or non-bumblebee pollinated, which was determined from a database (Ellis & Ellis-Adam 1993). The abundance of bumblebee-pollinated plants in each plot was examined using the same models as described for species richness, but according to a Binomial error distribution because abundance values were converted into proportions. Community weighted averages (CWA) were calculated for plant height, Ellenberg light and nitrogen indicator values and bumblebee-pollinated plants using the proportion of each group within each plot, weighted by their abundance. The same GLMMs were used as for species richness tests described above but according to a Gaussian error distribution. Therefore, randomisation tests were used to determine significance values (Bates 2006). The data was randomly permuted (10,000 times), where the environmental data was randomly associated with the species data. The model was re-fitted to this random data and the coefficients extracted to give a null distribution. The real data was compared to this null distribution and significance assessed using a 2-sided *p* test. Correlation between plant traits were tested using Pearson's product-moment correlation.

2.4 Results

One hundred and six plant species were recorded, 18 of which could not be fully identified since they were encountered during a non-flowering stage and therefore lacked features necessary for identification. A full species list is in Appendix A, Table A.2. The most common species were *Symphytum x uplandicum* and *Aegopodium podagraria* which were found in more than half of the plots (19 and 25 respectively); and *Petasites hybridus* and *Cruciata laevipes* which were found in more than a third of the plots (11 and 13 respectively).

The abundance of individual plant species in the community was significantly associated with the abundance of *I. glandulifera* at both the plot and broad scales of invasion, when the effects of temporal variation were removed (Table 2.2). The effect of *I. glandulifera* was low, explaining 15.57 % of the variation in the data. Of the variance caused by *I. glandulifera*, broad scale invasion accounted for the biggest effect, explaining the most variance in the data (Table 2.2).

Table 2.2: Result of partial Constrained Principal Co-ordinates Analysis (partial CAP), using Bray-Curtis dissimilarity index on plant abundance data collected between May and September 2010, with variance caused by date removed. Overall model significance was determined through analysis of variance (ANOVA) using permutation tests. The significance of environmental variables tested (*I. glandulifera* abundance at the quadrat, plot and broad scales, bare ground (log), and number of trees (log)) was determined using permutation tests, stratified by plot, and permuted 999 times. Plot scale invasion represents the maximum abundance recorded in the plot over the season.

Explained variance (%)	Axis 1	Axis 2	Model *F*	Model *p*	Significant variables	Axis 1	Axis 2	*p*	R^2
15.57	4.114	1.238	4.841	0.005	Plot scale (log)	-0.819	-0.574	0.001	0.299
					Broad scale	-0.955	-0.297	0.001	0.966
					Number of trees (log)	0.788	-0.616	0.001	0.578
					Percentage cover of bare ground (log)	0.025	0.999	0.001	0.296

Table 2.3: Result of CAP (Constrained Analysis of Principal Coordinates) using Bray-Curtis dissimilarity index using species abundance data collected in each month. Model significance values were determined through analysis of variance (ANOVA) using permutation tests. The significance of environmental variables tested (*I. glandulifera* abundance at the quadrat, plot and broad scales, bare ground (log), and number of trees (log)) was determined using permutation tests, with data permuted 999 times.

Month	**Explained variance (%)**	**Axis 1**	**Axis 2**	**Model *F***	**Model *p***	**Significant variables**	**Axis 1**	**Axis 2**	***p***	**R^2**
May	25.2	0.782	0.599	1.515	0.035	Quadrat (log)	-0.074	-0.997	0.001	0.634
						Plot (log)	0.326	-0.945	0.001	0.562
						Broad	-0.813	-0.582	0.001	0.717
						Number of trees (log)	0.980	-0.199	0.002	0.544
June	27.7	0.750	0.334	0.956	0.560	Number of trees (log)	-0.429	0.903	0.001	0.696
July	24.6	0.770	0.416	2.057	0.005	Quadrat (log)	-0.972	0.235	0.010	0.252
						Broad	-0.831	0.557	0.001	0.917
						Bare ground (log)	-0.695	-0.719	0.001	0.633
August	40.0	1.175	0.396	1.663	0.022	Quadrat (log)	-0.128	0.992	0.001	0.923
						Plot (log)	-0.249	0.968	0.001	0.996
						Broad	-0.822	0.570	0.001	0.849
Sept.	30.3	1.194	0.536	1.849	0.005	Quadrat (log)	-0.912	-0.409	0.002	0.525
						Plot (log)	-0.874	-0.487	0.001	0.704
						Broad	-0.998	-0.067	0.001	0.950
						Number of trees (log)	0.679	-0.735	0.002	0.627

Plant community composition was examined month by month but there was no clear temporal effect on the relationship with *I. glandulifera* abundance, although the strongest effect was in August (Table 2.3). In general, species abundance showed a significant relationship with invasion at all scales (Table 2.3, Figure 2.3 showing July data as an example). At a broad scale, *I. glandulifera* abundance was greater in more open habitats, as it was negatively correlated with the number of trees and unrelated to the amount of bare ground in the plot (Figure 2.3).

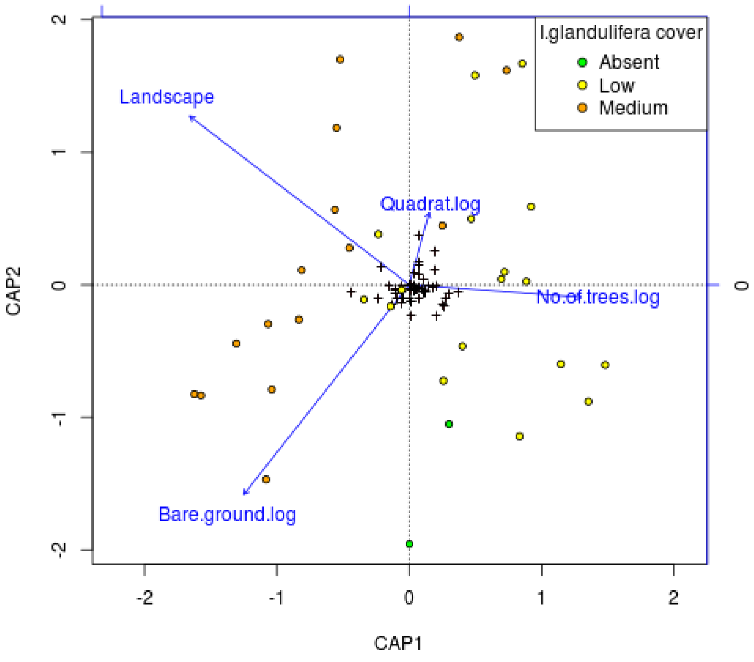

Figure 2.3: CAP (Constrained Analysis of Principal Coordinates) ordination diagram using a Bray-Curtis dissimilarity index using species abundance data, collected in July 2010. Environmental variables fitted are *Impatiens glandulifera* abundance at the quadrat scale (log) and broad scale, bare ground in the plot (log), and the number of trees in the plot (log). Each point represents a plot, coloured according to *I. glandulifera* abundance at the broad scale (green = absent, yellow = low, orange = medium). The maximum cover at this scale was medium. Each cross represents a plant species. Plot scale *I. glandulifera* abundance is not shown due to its correlation with invasion at the quadrat scale.

The species that were most abundant where *I. glandulifera* was most abundant were *Chamerion angustifolium* (Ellenberg light indicator value 6, nitrogen 5, mean height 150 cm), *Calystegia sepium* (Ellenberg light indicator value 7, nitrogen 7, mean height 200 cm), and *Tanacetum vulgare* (Ellenberg light indicator value 7, nitrogen 7, mean height 120 cm) (Figure 2.4). Species that were least associated with high *I. glandulifera* abundance were *Petasites hybridus* (Ellenberg light indicator value 6, nitrogen 7, mean height 120 cm), *Myrrhis odorata* (Ellenberg light indicator value 7, nitrogen 7, mean height 180 cm), and *Cruciata laevipes* (Ellenberg light indicator value 6, nitrogen 5, mean height 60 cm) (Figure 2.4). Significance tests of plant species traits and invasion are considered below.

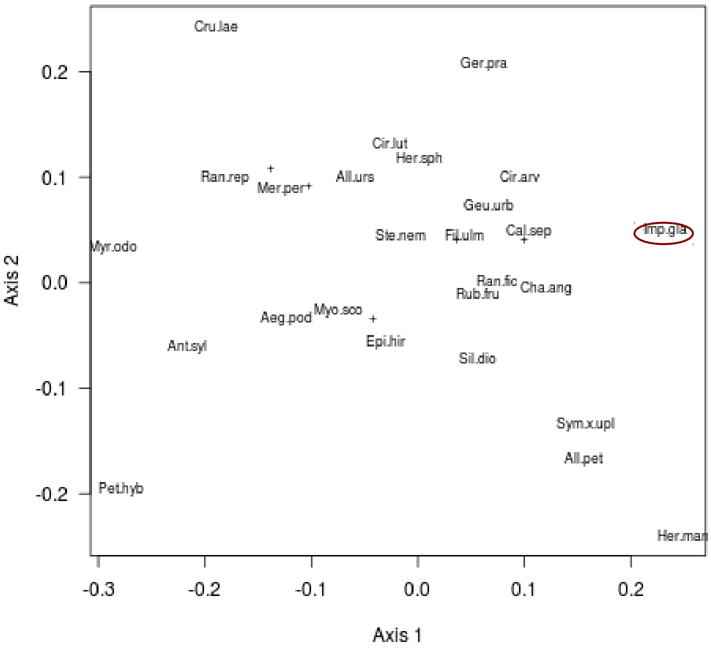

Figure 2.4: Associations of the most common species with *Impatiens glandulifera* ("Imp.gla", circled) using species scores derived from the Bray-Curtis dissimilarity index, calculated from species' mean abundance from the sampling period of May to September 2010. Species full names are in Appendix A Table A.2. Species appear at the mode of their abundance, and their proximity to *I. glandulifera* indicates their association with the mode of its abundance.

Plot plant species richness significantly decreased with increasing *I. glandulifera* abundance at the broad scale of invasion, but there was no significant relationship at quadrat and plot scales (Table 2.4, Figure 2.5).

Table 2.4: Plant species richness in 30 experimental plots sampled multiple times between May and September 2010, as a function of *Impatiens glandulifera* abundance. Generalised linear mixed models (GLMMs) were used, according to a Poisson error distribution. Fixed effects were *I. glandulifera* abundance, date, number of trees (log), and bare ground (log). A quadratic term, and an interaction term between *I. glandulifera* abundance and date were removed to find the minimum adequate model. Random effects were plot and river. Full details of each model's other significant fixed effects can be found in Appendix A, Table A.3.

***I. glandulifera* abundance**	**Estimate**	**Std error**	***z***	***p***	**R^2**
Quadrat scale (log)	0.035	0.079	0.445	0.656	0.041
Plot scale (log)	-0.113	0.089	-1.276	0.202	0.034
Broad scale	-0.010	0.004	-2.320	0.020	0.165

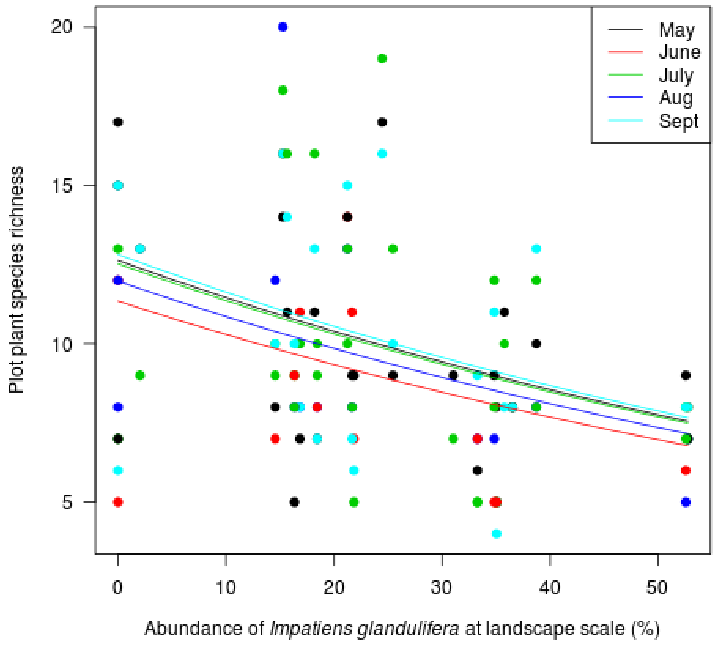

Figure 2.5: Plant species richness in 30 experimental plots, sampled multiple times between May and September 2010, that varied in the abundance *Impatiens glandulifera* at the broad scale of invasion. Abundance was measured as the percentage cover of *I. glandulifera* along a 420 m transect.

There was a significant curved relationship between the proportion of bumblebee-pollinated plants and *I. glandulifera* abundance (Table 2.5, plot scale (log)). Categorical rather than continuous measures of invasion then enabled significance testing to elucidate the relationship between abundance and the proportion of bumblebee-pollinated plants (Table 2.5, rank abundance). The proportion of bumblebee-pollinated plants was significantly higher at low abundance of *I. glandulifera* (plot scale invasion) compared to in its absence (Table 2.5, Figure 2.6). This decreased with increasing abundance of *I. glandulifera*, but this relationship was not significantly different to plots where *I. glandulifera* was absent (Table 2.5, Figure 2.6).

Table 2.5: The proportion of bumblebee-pollinated plants in 30 experimental plots as a function of *Impatiens glandulifera* abundance. Generalised linear mixed models (GLMMs) were used, according to a Binomial error distribution with a linear term (L), and a quadratic term (Q) added for continuous explanatory variables where appropriate. Fixed effects were *I. glandulifera* abundance, date, percentage cover of bare ground (log) in the plot and number of trees (log) in the plot. Plot and river were treated as random effects. The interaction between *I. glandulifera* abundance and date, and in some models the quadratic term, were removed to find the minimum adequate model. Full details of each model's other significant fixed effects are in Appendix A, Table A.4.

***I. glandulifera* abundance**	**Term**	**Estimate**	**Std error**	*z*	*p*	**R^2**
Quadrat scale (log)	L	0.605	0.381	1.590	0.112	0.132
	Q	-0.323	0.216	-1.499	0.134	
Plot scale (log)	L	1.554	0.527	2.948	0.003	0.181
	Q	-0.932	0.310	-3.005	0.003	
Plot scale: Rank max. abundance	L					0.185
Low		0.695	0.233	2.990	0.003	
Medium		0.332	0.229	1.453	0.146	
High		0.161	0.245	0.660	0.509	
Broad scale	L	0.009	0.006	1.531	0.126	0.193

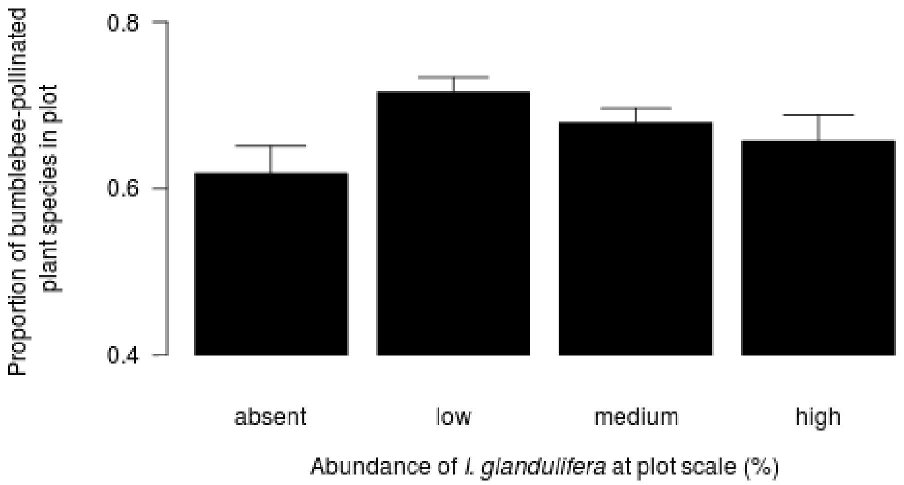

Figure 2.6: Proportion of bumblebee-pollinated plant species in 30 experimental plots, sampled multiple times between May and September 2010, where *Impatiens glandulifera* was absent, or at low, medium or high abundance at the plot scale of invasion. Values are means + SE. N = 26, 47, 35, 19 for the number of samples in plots where *I. glandulifera* was absent, or at low, medium or high abundance respectively.

Species that were most abundant where *I. glandulifera* was most abundant did not suggest a relationship between invasion and plants grouped by Ellenberg light- and nitrogen indicator values and by plant height. However, general trends showed that the abundance of species with high Ellenberg light indicator values significantly increased with *I. glandulifera* abundance at the quadrat, plot and broad scales (Table 2.6, Figure 2.7 a). There was a significant increase in the abundance species with high Ellenberg nitrogen values and for taller species as *I. glandulifera* abundance increased at the plot and broad scales (Tables 2.7 and 2.8, Figures 2.7 b and 2.7 c, for nitrogen indicator values and plant height respectively). Ellenberg light was significantly positively correlated with plant height, which in turn was significantly positively correlated with Ellenberg nitrogen indicator values (Table 2.9).

Table 2.6: Community weighted average of plant abundance grouped by Ellenberg light indicator values, in 30 experimental plots sampled multiple times between May and September 2010. Generalised linear mixed models (GLMMs) were used, according to a Gaussian error distribution. Fixed effects were *I. glandulifera* abundance, at the quadrat, plot and broad scales, percentage cover of bare ground in the plot (log) and the number of trees in the plot (log). River and plot were treated as random effects. Date was removed to find the minimum adequate model. Significance values were calculated by permutation tests (data permuted 10,000 times). Full details of each model's other significant fixed effects are in Appendix A, Table A.5.

***I. glandulifera* abundance**	**Estimate**	**Std error**	***t***	***p***	**R^2**
Quadrat scale (log)	0.200	0.065	3.09	0.001	0.308
Plot scale (log)	0.078	0.076	1.03	0.250	0.315
Broad scale	0.007	0.004	1.68	0.023	0.351

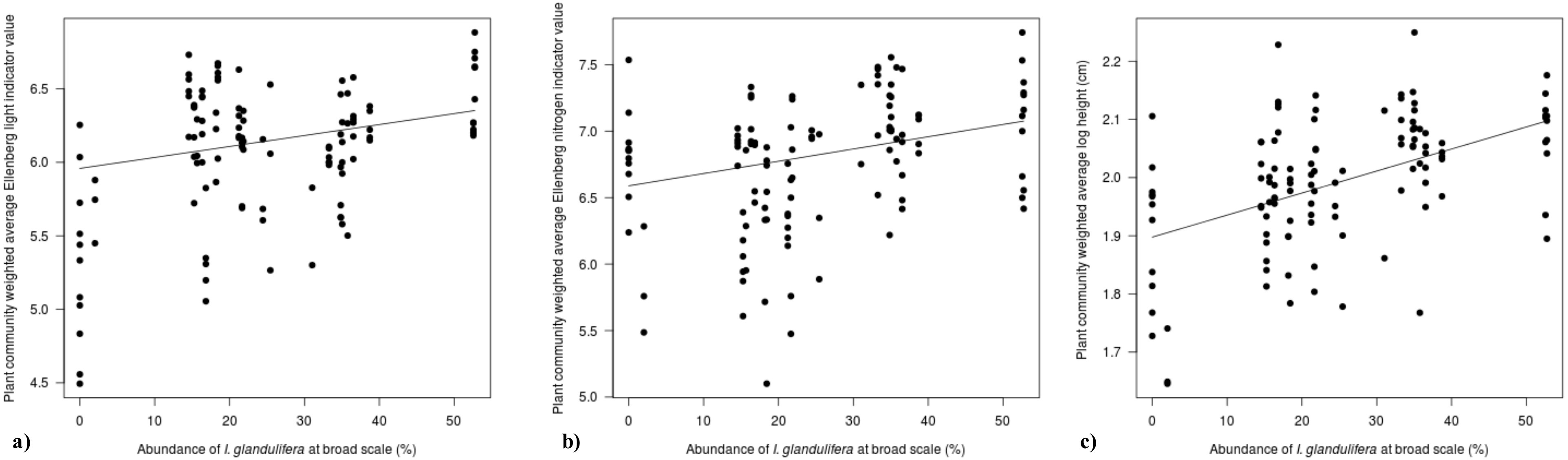

Figure 2.7: Community weighted average of **a)** Ellenberg light indicator values **b)** Ellenberg nitrogen indicator values and **c)** plant height (log) in 30 experimental plots sampled multiple times between May and September 2010, that varied in the abundance of *Impatiens glandulifera* at the broad scale. Abundance was measured as the percentage cover of *I. glandulifera* along a 420 m transect.

Table 2.7: Community weighted average of plant abundance grouped by Ellenberg nitrogen indicator values, in 30 experimental plots sampled multiple times between May and September 2010. Generalised linear mixed models (GLMMs) were used, according to a Gaussian error distribution. Fixed effects were *I. glandulifera* abundance, at the quadrat, plot and broad scales, percentage cover of bare ground in the plot (log) and the number of trees in the plot (log). River and plot were treated as random effects. Date was removed to find the minimum adequate model. Significance values were calculated by permutation tests (data permuted 10,000 times). Full details of each model's other significant fixed effects can be found in Appendix A, Table A.6.

I. glandulifera **abundance**	**Estimate**	**Std error**	***t***	***p***	**R^2**
Quadrat scale (log)	-0.097	0.010	-0.97	0.173	0.056
Plot scale (log)	-0.010	0.114	-0.08	0.902	0.058
Broad scale	0.009	0.006	1.63	0.011	0.167

Table 2.8: Community weighted average of plant abundance grouped by log plant height (cm), in 30 experimental plots sampled multiple times between May and September 2010. Generalised linear mixed models (GLMMs) were used, according to a Gaussian error distribution. Fixed effects were *I. glandulifera* abundance, at the quadrat, plot and broad scales, percentage cover of bare ground in the plot (log) and the number of trees in the plot (log). River and plot were treated as random effects. Date was removed to find the minimum adequate model. Significance values were calculated by permutation tests (data permuted 10,000 times). Full details of each model's other significant fixed effects can be found in Appendix A, Table A.7.

I. glandulifera **abundance**	**Estimate**	**Std error**	***t***	***p***	**R^2**
Quadrat scale (log)	-0.012	0.020	-0.62	0.429	0.135
Plot scale (log)	0.051	0.021	2.41	0.002	0.240
Broad scale	0.004	0.001	3.55	<0.001	0.353

Table 2.9: Correlations of plant attributes; light preference, soil fertility preference, and log plant height (cm). Significance values were calculated using Pearson's product-moment correlation. Light and soil fertility preferences are Ellenberg light and nitrogen indicator values.

Plant attributes	**Correlation coefficient**	**df**	***t***	***p***
Light preference x Soil fertility preference	-0.013	124	-0.15	0.884
Plant height x Light preference	0.207	124	2.36	0.012
Plant height x Soil fertility preference	0.624	124	8.90	<0.001

2.5 Discussion

In this study, there was a significant relationship between the composition of the plant community and the abundance of *Impatiens glandulifera*, at multiple spatial scales. This could account for differences between previous studies examining the impact of *I. glandulifera* on plant community composition (Hulme & Bremner 2006; Hejda & Pyšek 2006; Hejda et al. 2009). Taking these effects into account could better enable comparison between studies in the future. The relationship between the abundance of *I. glandulifera* and the abundance of plant species in the community was strongest at a broad scale which could indicate pollinator-mediated effects, and suggests that local *I. glandulifera* invasion could impact over wide spatial scales. Plants grouped by traits varied in their response to *I. glandulifera* invasion, which could be useful in predicting species most likely to be affected by invasion. We found a greater proportion of bumblebee-pollinated plants in the plot at low *I. glandulifera* abundance, and species that were taller and that had high Ellenberg light and nitrogen indicator values were found with increasing abundance of the invader. The contrasting responses of different species highlights the importance of using a community-level approach to examine the impact of invasive alien plants.

There are limitations in using space-for-time substitutions to mimic invasion processes, since it is not possible to separate out differences due to the level of invasion from differences in the habitat, such as soil nutrients, that may cause this level of invasion (Hejda & Pyšek 2006). The experimental design of this study inevitably yields correlative data, but invasive species studies are restricted by ethical constraints preventing their introduction, and by the difficulties associated with studying invasion at a large spatial scale. Despite these limitations, the results could indicate a real effect. This is because plots did not appear to differ in their suitability for invasion, which suggests similarities across habitats: the abundance of *I. glandulifera* changed over the season, and some plots in which *I. glandulifera* was initially classed as absent were subsequently invaded. This could be due to late germination of seeds in the seed bank. *Impatiens glandulifera* germinating later in the season were characteristically short and thus distinguishable from plants missed though observer error. Measures were also taken to minimise the effects of the wider habitat in the experimental design. Plots were of similar elevation and surrounding land cover, and the abundance of *I. glandulifera* was unrelated to its position in the river catchment to minimise associations with co-varying factors such as river width.

An alternative approach to space-for-time substitutions would be to use removal experiments (Hulme & Bremner 2006; Lopezaraiza–Mikel et al. 2007), but this may cause disturbance that in itself influences species composition, and was impractical in this study due to the spatial arrangement and large number of plots involved. It also takes time for the community to respond to the treatment. Space-for-time substitutions have an advantage over removal experiments therefore, because plant communities represent the effects of invasion from previous years. Although there are drawbacks in this study's design, it is rarely possible to monitor an ongoing invasion from the start (Müllerová et al. 2005) nor over the long term (Kwiatkowska et al. 1997). Observational studies do not reveal the mechanisms involved in the invasion process, but they do generate large data sets that are useful in detecting patterns and informing directed study.

2.5.2 Effect of *I. glandulifera* abundance on the plant community composition

The effect of the abundance of *I. glandulifera* on the composition of plant communities has not previously been considered. Instead, studies have examined the impact of its presence and have found it had little effect (Hejda & Pyšek 2006; Hejda et al. 2009), or that it significantly reduced plant species richness and diversity (Hulme & Bremner 2006). The abundance of plant species varied with the abundance of *I. glandulifera*, and at the broad scale, plant species richness decreased with increasing *I. glandulifera* abundance. This suggests that a relationship exists between the abundance of *I. glandulifera* and the plant community composition, which could have contributed to the differences between previous studies. However, it is possible that the relationship we observed may not be the result of invasion, but a symptom of communities that are more suitable for invasion. Elton (1958) hypothesised that community diversity promotes resilience to invasion from alien species because there are fewer vacant “niches”. This has been much debated (reviewed by Levine & D’Antonio 1999), and there is experimental evidence in support (Naeem *et al.* 2000) and in opposition of the theory (Collins et al. 2006). From our study, it is not possible to distinguish the effects of the invader from the effects of the habitat on species distributions and associations, but our results are suggestive of a relationship. Plot plant species richness was lowest at high *I. glandulifera* abundance. By occupying more space, theoretically a high abundance of *I. glandulifera* should exert more impact. Individual *I. glandulifera* plants have been found to form clusters, leaving space for native species to co-occur (Hejda & Pyšek 2006). However, *I. glandulifera* can produce up to 2500 seeds per plant (Koenies & Glava 1979 in Clements et al. 2008) which suggests that habitats may become heavily invaded without intervention. At high abundance, *I. glandulifera* may reduce species richness at a local spatial scale through resource competition or by reducing habitat heterogeneity. However, all plant species found in the study are widespread across the UK, in agreement with Hulme and Bremner (2006), who suggest that the threat posed by *I. glandulifera* at a regional spatial scale is low.

2.5.3 Effect of the spatial scale of *I. glandulifera* invasion

A relationship between invasion and the plant community composition was detected at quadrat, plot and broad scales, being mostly strongest at the latter. Whilst efforts were made to minimise possible effects of habitat, this could be due to abiotic conditions in the broad scale, such as disturbance. Alternatively, the broad scale effects could indicate pollinator-mediated effects. *Impatiens glandulifera* is primarily bumblebee-pollinated (Titze 2000; Nienhuis & Stout 2009) and, given that bumblebees can forage over large distances, with conservative estimates of 755 m 775 m for *Bombus lapidarius* and *B. pascuorum* respectively for example (Carvell et al. 2012), this suggests that they can respond to *I. glandulifera* invasion over broad scales. *Impatiens glandulifera* has a large floral display (Beerling & Perrins 1993) and offers a copious nectar reward (Raine & Chittka 2007a). As such, it is highly attractive to bumblebees, which have been found to increase in abundance in its presence (Lopezaraiza–Mikel et al. 2007; Bartomeus et al. 2010). Invasion may therefore have wide-ranging effects, and exert an impact on plant communities at a broad scale. However, contrary to expectation, there was no clear seasonal effect of *I. glandulifera* invasion (that is before and during flowering) on the plant community composition, although effects were strongest in August which is the peak flowering period. This suggests that pollinator-mediated effects may be the strongest effects, but that there must also be competition for abiotic resources occurring outside of the flowering period. Abioitic resource competition is expected to act more strongly at a local scale. As the tallest annual in the UK (Beerling & Perrins 1993), *I. glandulifera* can out-compete plants for light at a local scale (Hulme & Bremner 2006). *Impatiens glandulifera* was expected to confer a competitive advantage over most species at the plot scale in this study, since the majority recorded were shorter than *I. glandulifera* (as estimated from mean height values).

2.5.4 Predicting effects of *I. glandulifera* using plant traits

Plant traits were measured using Ellenberg indicator values. Ellenberg indicator values have been criticised as they simplify the ecological responses of plants, and thus field measurements should be preferable (Dierschke 1994 in Diekmann et al. 2003). However, Ellenberg indicator values are still widely used (e.g. Pysek & Pysek 1995; Fanelli et al. 2006, Duprè et al. 2010), and in a study similar to ours (Hulme & Bremner 2006). This is because they reflect a range of integrated information (Ellenberg et al. 1992) that would be time consuming and costly to measure, and which would require repeated long-term field measurements. The reliability of Ellenberg indicator values is subject to continued debate however (Diekmann et al. 2003). Ellenberg indicator values were developed using field observations in Western Germany (Ellenberg et al. 1992), and do not consistently match with different locations (Hill et al. 2000). However, they can reliably reflect field measurements if a range of interacting variables are measured (Schaffers & Sykora 2000), because indicator values represent a range of environmental factors that must be accounted for when examining their reliability.

A relationship was found between *I. glandulifera* invasion and plant species abundance grouped according to their traits. This may have been due either to the effects of *I. glandulifera* invasion, or the result of correlation with environmental variables also responsible for the abundance of the invader. The plant community was characterised by an increasing abundance of species indicative of high soil fertility (high Ellenberg nitrogen indicator values) with increasing *I. glandulifera* abundance. Invasive alien plants can cause an increase in nutrient pools and fluxes (Dassonville et al. 2008). Their influence can depend on initial site conditions (Blanck et al. 2011), although the extent to which *I. glandulifera* abundance changes soil nitrogen or is a symptom of it cannot be determined in this study because site conditions prior to invasion were unknown. *Impatiens glandulifera* is expected to influence soil nutrients however, because it has a high net uptake of nutrients compared to native species due to its rapid growth rate, and because it enhances nutrient fluxes due to higher returns to the topsoil in dead organic matter (Blanck et al. 2011). The abundance of *I. glandulifera* is unlikely to depend on nitrogen levels alone however, as it is tolerant of a range of habitat types (Beerling & Perrins 1993). Ellenberg nitrogen indicator values were correlated with Ellenberg light indicator values and with plant height. This inter-correlation may be why no relationship was found between Ellenberg nitrogen indicator values and species that were most abundant at high levels of invasion, *Chamerion angustifolium*, *Calystegia sepium* and *Tanacetum vulgare* and species least associated with high abundance, *Petasites hybridus*, *Myrrhis odorata* and *Cruciata laevipes*.

There was a general trend of an increase in plants with high Ellenberg nitrogen indicator values with increasing *I. glandulifera* abundance which could reflect high soil fertility, or else could reflect other conditions that also allow species indicative of high soil fertility to occur. The plot plant community was characterised by taller species, and by species occurring in high light intensity (Ellenberg light indicator values), with increasing abundance of *I. glandulifera* at the plot and broad scales of invasion. This could indicate that sites where there is high light intensity may also be a characteristic of sites with high soil fertility. Contrary to our results, Hulme and Bremner (2006) found fewer species with high Ellenberg light indicator values in the presence of *I. glandulifera*, due to the effects of shading. *Impatiens glandulifera* can grow to 3 m (Clements et al. 2008), and so can dominate the aerial environment. Our finding of more species with high Ellenberg light indicator values with increasing *I. glandulifera* abundance could reflect that *I. glandulifera* was found at higher abundance in more open habitats. Alternatively, it could be due to inter-correlation of Ellenberg light indicator values with plant height and Ellenberg nitrogen indicator values; and these traits may have interacted more strongly with *I. glandulifera* abundance than light preference.

Taller species are more able to compete for light (Pajunen, Oksanen, & Virtanen 2011), and so the plant community may be characterised by taller species with increasing abundance of *I. glandulifera* as a consequence of this competition. Plant height was estimated using mean values for each species from the PlantAtt database (Hill et al. 2004). Whilst the drawbacks of using these values is acknowledged, since true plant height is expected to vary around this mean, they offer a proxy for field measurements when time constraints prevent direct measurement and can be useful for determining general trends. This is illustrated by their widespread use in place of field measurements (e.g. Keith et al. 2009).

The relationship between mean plant species height and the abundance of *I. glandulifera* could indicate pollinator-mediated effects. Pollinators can show a preference for foraging over a horizontal plane (Waddington 1979; Makino 2008), as it should be more efficient to move between neighbouring plants by maintaining a similar height rather than moving over large distances vertically (Levin & Kerster 1973). At 3 metres, *I. glandulifera* was taller than many of the co-occuring plant species recorded in the study. This could result in some aerial stratification in pollinator foraging between those visiting *I. glandulifera* and those visiting native species closer to ground level (see Chapter three). Pollinators attracted to *I. glandulifera* may be more likely to encounter plants of a similar height to *I. glandulifera,* which could select for taller plant species. There is experimental evidence for vertically stratified foraging among bees (Roubik 1993, Nuttman et al. 2011), even over small vertical distances of between 0 and 1.8 m (Tuell & Isaacs 2009). However, some bee species do not demonstrate such stratification, suggesting that they forage opportunistically or that they show different responses to other environmental variables such as temperature and wind speed that differ with height (Roubik 1993). This suggests that a combination of pollinator-mediated effects and abiotic conditions (described above) may contribute to the observed positive correlation of mean plant height and *I. glandulifera* abundance.

The proportion of bumblebee-pollinated plants in the plot showed a curved relationship with invasion, peaking at low *I. glandulifera* abundance, which demonstrates support for Rathcke's density-visitation model (1983) (described in Figure 1.2) and provides support for the predicted pollinator-mediated effects. However, only the increase in the proportion of bumblebee-pollinated plants at low *I. glandulifera* abundance, compared to in its absence, was statistically significant. Increased local populations of these plants could be caused by correlation with unmeasured environmental variables such as soil nutrients, or could be indicative of increased pollinator visitation via facilitation caused by *I. glandulifera*. There is evidence from other studies in support of the latter, because native plants that share pollinators with invasive alien species are more likely to experience an effect (Gibson et al. 2012). We thus specifically targeted bumblebee-pollinated plant species because they share pollinators with *I. glandulifera*. Thijs et al. (2012) found that plant species sharing pollinators with *I. glandulifera* experienced the greatest impact due to competition for visitation. In contrast, other studies examining the effect of the presence of *I. glandulifera* on visitation to co-flowering plants have found increased visitation (Lopezaraiza–Mikel et al. 2007), decreased visitation (Thijs et al. 2012) or no effect (Bartomeus et al. 2010): differences between these studies could in part reflect the overlap of pollinators between plant species.

We did not find competitive effects at high *I. glandulifera* abundance, contrary to the predictions of Rathcke's density-visitation curve (1983). This may indicate that the abundance of *I. glandulifera* flowers did not exceed the maximum floral abundance after which competition occurs, particularly if *I. glandulifera* attracts more bumblebee pollinators, as discussed above. Alternatively, bumblebee-pollinated plants temporally isolated from *I. glandulifera* would not experience direct effects of pollinator-mediated competition. Changes in the proportion of bumblebee-pollinated plants supports our suggestion that stronger effects at a broad scale indicate pollinator-mediated effects between *I. glandulifera* and the plant community.

2.6 Conclusion

Despite the drawbacks and limitations associated with correlative studies, our results add to existing evidence indicating that *I. glandulifera* exerts an impact on native plant communities. This has implications for plant-pollinator network structure. A significant relationship was found between the composition of the plant community and the abundance of *Impatiens glandulifera*, which varied with spatial scale. Taking these factors into account could be important in enabling comparisons between studies. The plant community composition varied with the abundance of *I. glandulifera* most strongly at a broad scale. This could be indicative of abiotic conditions or pollinator-mediated effects. The latter suggests that local invasion can have wide-ranging impacts. Plant species responded to *I. glandulifera* invasion according to their traits, which could be useful in predicting species most likely to be affected by invasion, and enable targeted conservation efforts. The contrasting responses of different species highlights the importance of using a community-level approach to examine the impact of invasive alien plants. Controlled experiments will help untangle the underlying processes from site-specific variation (Chapter six). Future research should also be directed towards long term monitoring of invasions to differentiate between impact caused by the invader and correlations with co-varying factors; and to explore changes in the plant community associated with the time since invasion.

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Chapter Three:

Impact of *Impatiens glandulifera* on the pollinator community depends on its relative abundance and spatial scale

3.1 Abstract

The presence of the invasive alien plant *Impatiens glandulifera* has in past studies been found to elicit conflicting effects on pollinator visitation to neighbouring native plants. These earlier studies did not address its relative abundance however, which may account for these different results. We assessed the pollinator community composition in field plots that differed in the abundance of *I. glandulifera* at local and broad scales, and examined bumblebee-flower visitation patterns in plots that varied in the relative abundance of *I. glandulifera* flowers. The impact of *I. glandulifera* on the pollinator community composition varied according to its abundance, and according to the spatial scale at which impact was assessed. The response of pollinators varied across taxa, emphasising the importance of using a range of species to examine the effects of invaders. Bumblebee foraging behaviour showed a stronger response to the presence or absence of *I. glandulifera* rather than to its relative abundance, which suggests that it is highly attractive. In the presence of the invader, bumblebee visitation to co-flowering Lamiaceae and purple-flowered plants was reduced, although one purple-flowered species, *Epilobium hirsutum*, received more visits. In the presence of *I. glandulifera*, classed as tall, visitation to short plants was reduced whereas plants of medium stature were unaffected. This suggests that the traits of co-flowering plants used to assess the impact of invasive alien plants should be considered, as they may determine the outcome of the interaction.

3.2 Introduction

Invasive alien plants can displace native plants through competition for abiotic resources such as water (D’Antonio & Mahall 1991), light (Iponga et al. 2008) and nutrients (Wardle et al. 1994). In recent years, there has been increasing interest in competition for biotic resources, as invasive alien plants have been found to disrupt plant-pollinator networks by competing for shared pollinators (Memmott & Waser 2002; Lopezaraiza–Mikel et al. 2007). Pollinator sharing is common in plant communities (Waser et al. 1996). However, some invasive aliens pose a particular threat to native plant-pollinator interactions because they can be highly attractive to pollinators due to prolific rewards (Chittka & Schürkens 2001), large floral displays, and a propensity to occur in dense and extensive populations (Bjerknes et al. 2007). Although only a small fraction of introduced alien plant species are expected to become established (Williamson 1996), some have become invasive partly due to their ability to compete for pollinators (Traveset & Richardson 2006; Bjerknes et al. 2007), such as *Impatiens glandulifera* (Chittka & Schürkens 2001) which is invasive across Europe (Pysek & Prach 1995). Pollinator-mediated competition presents a major concern since many wild plants and agricultural crops depend on pollinators for reproduction and for maintaining genetic diversity (Ollerton, Winfree, & Tarrant 2011). Competition between native and invasive alien plants for pollinators can reduce visitation and seed set in native plants (e.g. Brown et al. 2002), and may impact on the genetic diversity of pollen received (Chapter five) which has implications for individual plant fitness (Charlesworth & Charlesworth 1987) and gene flow (Eckert et al. 2010). There is evidence for global shifts in pollinator communities in terms of relative species abundance (Bommarco et al. 2011; Cameron et al. 2011), species diversity (Biesmeijer et al. 2006), and range (Williams 1982; Cameron et al. 2011) (but see Carvalheiro et al. 2013), which has implications for agricultural production and ecosystem function (Potts et al. 2010). Crops, in particular species that are entirely dependent on animal pollinators, are vulnerable to pollinator loss which could impact on human food production (Klein et al. 2007). In the UK, animal-pollinated wild plants have declined more than self- or wind-pollinated species, in parallel with pollinator declines (Biesmeijer et al. 2006). Plant-pollinator networks may be buffered against some species loss as they are made up of weak and asymmetrical dependencies (Bascompte 2003) and have a high level of redundancy (Memmott et al. 2004). However, theoretical models predict that severe disturbance will cause plant-pollinator networks to reach a tipping point and collapse (Fortuna & Bascompte 2006, Kaiser-Bunbury et al. 2010). However, we currently lack knowledge as to the extent to which alien invasions impact on the pollinator community composition and the consequences of this change for native plant pollination, and how these impacts vary with spatial scale (Bjerknes et al. 2007).

Pollinator taxa respond to their environment at different spatial scales, depending on their dispersal ability and foraging range (Steffan-Dewenter et al. 2002; Gabriel et al. 2010), and so pollinator-mediated interactions between invasive aliens and native plants should also be scale-dependent. This is an important consideration because the strength of the interaction, and whether it is competitive or facilitative, has been found to vary with spatial scale (Bjerknes et al. 2007; Nielsen et al. 2008; Jakobsson et al. 2009). Pollinators that mostly forage over short distances, such as small solitary bees which forage up to 600 m, are mainly influenced by local scale floral resources (Gathmann & Tscharntke 2002; but see Zurbuchen *et al.* 2010). Such pollinators benefit from heterogeneous habitats, which provide all the necessary resources for the duration of their lifecycle (Steffan-Dewenter et al. 2002). Local changes in native plant species richness and diversity could have the greatest impact on taxa with small foraging ranges as they may be unable to compensate for loss of resources (that are not provided by invasive alien species) by foraging further away. Social bees (bumblebees and honeybees) have broader foraging ranges (Visscher & Seeley 1982; Walther-Hellwig & Frankl 2000; Carvell et al. 2012). This enables them to benefit from mass-flowering crops and dispersed local resources across the landscape (Westphal et al. 2009; Carvell et al. 2012). In contrast to central place foragers (that is, insects restricted by their nest location), hoverflies are highly mobile and respond to their environment at an even broader scale (Jauker *et al.* 2009). Taxa that respond to their environment at broad scales may be less vulnerable to local impacts of invasive alien plants.

Several studies have examined how the presence of an invasive alien plant affects insect visitation to focal native plant species (Chittka & Schürkens 2001) or communities (Lopezaraiza–Mikel et al. 2007). These studies have observed contrasting patterns, finding that the invader facilitated pollinator visitation to co-occurring native plants (Lopezaraiza–Mikel et al. 2007), or that the invader competed with native plants for visitation (Chittka & Schürkens 2001; Brown et al. 2002; Tscheulin et al. 2009), or that it had no effect (Aigner 2004; Bartomeus et al. 2010). Although the presence of the invader was considered, these studies may have differed from each other in terms of the invader's abundance. This is of note because whether pollinator-mediated interactions between invasive alien and native plant species are facilitative or competitive is predicted to vary with the invader's abundance. Rathcke's density-visitation model (1983, Figure 1.2) predicts that a community of co-flowering native plants should facilitate each other's pollination at low floral density, since species combine to increase the size of the floral display and attract shared pollinators. This should switch to a competitive interaction once pollinators become a limiting resource (Rathcke 1983).

Essenberg (2012) developed a model building on Rathcke's predictions which included parameters to describe pollinator foraging behaviour, assuming that they distribute themselves in the environment to maximise foraging success. Essenberg's model (2012), and accompanying field study, confirm Rathcke's predictions of facilitation at low floral density and competition at high floral density. Other field studies have not consistently supported Rathcke's density-visitation curve however. Jakobsson et al. (2009) found that the density of heterospecifics had little effect on pollinator visitation, although Dauber et al. (2010) found low visitation at high floral density which does support Rathcke's predictions. Hanoteaux et al. (2013) offer an alternative to Rathcke's model, predicting that density-visitation should also interact with the spatial distribution of co-flowering species and, like Essenberg's model (2012), should interact with floral reward. Although simplistic, Rathcke's model offers a theoretical framework in which to test the relationship between the abundance of invasive alien plants and pollinator visitation in the field, and is supported by more complex models (Essenberg 2012).

Particularly rewarding plants can act as “magnet” species, meaning that they are highly attractive to pollinators, and this can facilitate visitation to less attractive co-occurring species (Laverty 1992, Johnson et al. 2003). Some invasive alien species, such as *Impatiens glandulifera*, have particularly highly rewarding flowers (Chittka & Schürkens 2001), and theoretically could act as a magnet species and facilitate visitation to co-flowering plants (Lopezariaza-Mikel et al. 2007). Some invasive alien plants can facilitate visitation to co-flowering native species at low abundance (Muñoz & Cavieres 2008). However, most studies examining the abundance of invasive alien species have found only competitive effects, which can be stronger at high abundance (Muñoz & Cavieres 2008; Takakura et al. 2008; Flanagan et al. 2010, Dietzsch et al. 2011). This may be a result of floral constancy to the invasive alien species. Floral constancy can influence the strength of the density-visitation relationship (Feldman 2008, Stout et al. 2008). Invasive alien plants could therefore be highly competitive regardless of abundance if pollinators show constancy to this species. Floral constancy (see Darwin 1876; Bennett 1883) describes foraging selectively on flowers of one type despite the availability of alternative rewarding flowers (Wells & Wells 1983; Waser 1986). Constancy varies with a wide range of factors including the pollinator species (Bennett 1883; Christy 1883; Heinrich 1979; Goulson & Cory 1993) and learning and handling time (Chittka 2002).

To date, studies examining how the abundance of invasive alien plants affect plant-pollinator interactions target single or a small number of focal native plant species (Muñoz & Cavieres 2008; Takakura et al. 2008; Flanagan et al. 2010, Dietzsch et al. 2011). Large scale plant community studies are lacking, although some evidence suggests that the impact of invasive alien abundance on plant-pollinator network structure increases with the level of invasion (Kaiser-Bunbury et al. 2011). Invasive alien plants can alter the structure of the pollinator community. In the presence of profusely flowering alien invaders, local pollinator abundance can increase (Lopezaraiza–Mikel *et al.* 2007; Bartomeus *et al.* 2010), which may in part be because pollinators can be attracted to large or densely flowering plant populations (Dreisig 1995; Grindeland et al. 2005). This can increase visitation to co-flowering native plants (Lopezaraiza–Mikel et al. 2007).

The impact of alien invaders has been found to vary between pollinator taxa (de Groot et al. 2007). This means that the outcome of studies may depend in part on the pollinator taxa used to examine the impact of invasive alien plants. Generalist pollinators, such as bumblebees (Heinrich 1976) and most hoverflies (Branquart & Hemptinne 2000), more commonly incorporate alien plants into their diet compared to specialists (Memmott & Waser 2002). Alien plants could therefore provide additional resources to support larger populations, and extend the geographic range and flight season of some insects (Graves & Shapiro 2003). This could facilitate pollination of co-flowering plants that are also visited by these species. Foraging choice is restricted in part by the morphology of the insect's mouthparts (Gilbert 1981), and so rewards provided by invasive alien plants may prove inaccessible to some pollinators (Nienhuis et al. 2009). Invasion may indirectly affect these pollinators because competition from invasive alien plants can cause shifts in the native plant community composition, reducing species diversity (Spyreas et al. 2010) and richness (Hulme & Bremner 2006; but see Hejda & Pyšek 2006). Such effects are predicted to have the greatest impact on pollinators that do not utilise the alien invader (Stout & Morales 2009) because they depend on native plant species. For example, butterflies closely associated with certain native plant species, for food or larval development, were found to experience the greatest changes in terms of reduced abundance, species richness and diversity in the presence of invasive alien plants compared to generalist hoverflies (de Groot et al. 2007).

Impatiens glandulifera Royle (Himalayan balsam; Balsaminaceae) is an invasive alien species that has spread rapidly across Europe since its introduction (Pysek & Prach 1995), and is considered to be one of the top 20 most invasive plants in the UK (Crawley 1987). It is the tallest annual in the UK and has a large floral display (Beerling & Perrins 1993), and is primarily bumblebee-pollinated (Titze 2000; Nienhuis & Stout 2009). Flowers are highly rewarding since the rate of nectar production per flower far exceeds that of any European plant (Raine & Chittka 2007a). Whilst there are a few studies into its impact, results are conflicting showing facilitative, neutral or competitive effects (Chittka & Schürkens 2001; Lopezaraiza–Mikel et al. 2007; Nienhuis et al. 2009; Bartomeus et al. 2010; Thijs et al. 2012). Differences may be due in part to the abundance of the invader, but it has yet to be established whether a relationship exists between *I. glandulifera* abundance and its impact. This study is the first to explore how the pollinator community responds to a gradient of *I. glandulifera* abundance, and at local and broad scales. Specifically, we addressed three questions: i) What is the relationship between *I. glandulifera* abundance and pollinator community composition? ii) Does this vary with the spatial scale at which invasion is assessed? iii) Does bumblebee-flower visitation change with the relative abundance of the invader?

3.3 Methods

3.3.1 Pollinator community survey

To examine the effect of flowering *I. glandulifera* on the pollinator community composition, pollinators were sampled a total of three times – twice in August, at the beginning and end of the month, and once at the end of September. Poor weather conditions prevented collection in July. The pollinator community was sampled using five pan traps coloured blue, yellow, white, pink and purple (Figure 3.1), which were placed at ground level in each of the 30 experimental plots (described in Chapter two). Pan traps were positioned in two adjacent groups, one of three traps and the other of two (in randomised colour combinations) because a maximum of three traps fitted beneath the wire covering used to protect them from disturbance (Figure 3.1). Pan traps were placed at ground level to examine the impact of *I. glandulifera* on pollinator visitation to the ground flora. An assumption of our methodology is that the pan trap catch is biased towards insects foraging at this level, rather than insects foraging on

I. glandulifera in the upper canopy. This is because there is evidence to suggest that pollinators forage in a horizontal stratum (Waddington 1979), and therefore may only encounter pan traps if they are placed at the same height as their preferred flowers (Cane et al. 2000) (see Section 3.5.1). It was not possible to also set pan traps in the upper canopy in this study due to the logistics of carrying additional equipment to the field sites.

Plots were 20 x 5 m stretches of riparian habitat that differed in the abundance of *I. glandulifera* along a gradient, but shared similar surrounding land cover types and elevation (see Chapter two). Traps were plastic bowls, approximately 8.5 cm deep x 20 cm diameter, sprayed with UV-reflecting paint (Sparvar Leuchtfarbe, Spray-Colour GmbH, Merzenich, Germany). Traps were filled with approximately 400 ml of water and a drop of detergent was added to break to surface meniscus. Traps were exposed for 48 hours, during dry and sunny conditions at temperatures ≥ 16 °C to increase the catch by collecting insects when they were most active.

A disadvantage of all pollinator sampling methods is that they do not measure absolute abundance since insects are not collected from a defined area (Westphal et al. 2008). For convenience, we refer to the number of insects collected from pan traps as "abundance", but it should be noted that this is actually some measure of insect activity density, since there should be a bias towards more active species. Pan traps also show a taxonomic bias towards small-bodied bees and thus under-represent large-bodied bees (Cane et al. 2000, Wilson et al. 2008). They are likely to under-represent species with social recruiting to resources (*Apis*) since individuals do not return to their nest to recruit others. Insect orders are attracted to pan traps according to colour (e.g. Nuttman et al. 2011, Vrdoljak & Samways, 2012), reflecting flower colour preferences. Only part of the pollinator community will be sampled if a limited number of colours are used. We therefore used a wide range of colours to sample across groups and limit taxonomic bias. Despite the drawbacks of pan traps, they are widely acknowledged as an effective method for surveying pollinators and are commonly used (Westphal et al. 2008, Lebuhn et al. 2013). They are efficient and cost-effective for large scale studies and unlike transect surveys, they show no collector bias and they are better indicators of species richness than transects (Westphal et al. 2008).

The order in which plots were visited was randomised by proceeding from either upstream or downstream, and by randomising the order in which rivers were visited. For logistical reasons, the latter was restricted according to geographic location – rivers were divided into two groups which contained rivers closest together. Specimens collected from the pan traps were stored in 70 % ethanol, and Hymenoptera (bees and Aculeate wasps; and Symphyta - sawflies), and Diptera (Syrphidae - hoverflies) were identified to species using current taxonomic literature (Perkins 1919; Richards 1980; Lomholdt 1984; Wilmer 1985; Else 1994, Else (unpublished); Stubbs & Falk 2002).

3.3.2 Bumblebee visitation observations

Bumblebee visitation in response to *I. glandulifera* abundance was observed in a subset of 16 of the 30 plots, due to time constraints, which were spread over three of the original six rivers and represented a gradient of *I. glandulifera* abundance. Surveys were adapted from methodology used by Lopezaraiza-Mikel et al. (2007). Between June and August 2010, three 30-minute transects (one per month) were conducted along the 20 m length of each plot. These three transect walks per plot were carried out at temperatures ≥ 16 °C and at low wind velocity, which were both measured using a Kestrel 3000 pocket wind meter, to observe bumblebee visitation when they were most active. To avoid bias associated with diurnal activity patterns (Dafni et al. 2005), the time at which transects were walked was randomised between 0900 hours and 1700 hours. The species identity of every bumblebee-flower interaction was recorded 2 m either side of the transect. To measure floral abundance in each plot, the number of floral units (individual flowers or compound inflorescences) on every bumblebee-pollinated plant species present in the plot were counted within five 1 m^2 quadrats spaced evenly along each transect at 5 m intervals. For subsequent analysis, the floral abundance of each plant species in the plot was estimated using the sum of floral units within these five quadrats. Information on nectar production was not possible to measure in the field due to time constraints, and this information is lacking for most species in the literature (Raine & Chittka 2007a) so could not be considered directly in this study. However, this was taken into account indirectly by calculating a measure of "plant attractiveness" for each species (Section 3.3.4.2). Foraging bumblebees within reach were collected to take pollen samples. Pollen samples were used to consider floral constancy since pollen will reveal interspecific movement between *I. glandulifera* and co-flowering species, unlike the visitation data which does not indicate which plant species were visited previously. Pollen was sampled from the thorax using a 3 mm^3 block of fuchsian-stained jelly on a mounted needle (Figure 3.2). This was melted onto a microscope slide and sealed with a coverslip. The first 200 grains of pollen counted on the slide were identified as being either *I. glandulifera* pollen or from another species. Time constraints prevented further identification.

3.3.3 Invasion at plot and broad scales

In the pollinator community composition analysis, *I. glandulifera* invasion was measured at a plot (local) scale (sampled in 100 m^2) and at a broad scale (sampled in a 420 m transect of variable width, as determined by the width of the river bank) as percentage cover. The methodology is detailed in Chapter two. In the bumblebee-flower visitation analysis, invasion was measured as the proportion of *I. glandulifera* flowers in each plot (here only plot scale invasion was examined), and the methodology is described above (Section 3.3.2). Invasion was measured as percentage cover estimates in the pollinator community analysis rather than as the proportion of *I. glandulifera* flowers because time constraints prevented using the latter. This is because *I. glandulifera* is profusely flowering, meaning that it was impractical to count the number of *I. glandulifera* flowers in thirty plots, nor along 420 m transects of variable width at the broad scale of invasion. Therefore, percentage cover was used as a proxy for representing floral abundance in the pollinator community analysis.

Figure 3.1: Five pan traps, coloured blue, yellow, white, pink and purple, were positioned at ground level in each experimental plot, above showing three traps. Traps were placed under an up-turned hanging basket to prevent them being disturbed and left out for 48 hours in warm, dry and sunny conditions. Traps were placed in two adjacent groups, one of three traps and the other of two (in randomised colour combinations) because a maximum of three traps fitted beneath the hanging basket.

Figure 3.2: Pollen sampled from the thorax of *Bombus pascuorum*, using a $3mm^3$ block of fuchsian-stained jelly on a mounted needle.

3.3.4 Statistical analysis

All statistical analyses were done in R v2.10.1 (R Development Core Team, 2011).

3.3.4.1 Pollinator community in pan traps

The relationship between *I. glandulifera* abundance and insect abundance was examined using generalised linear mixed effects models (GLMMs) (function lme, v0.999375-35; Bates, Maechler, & Bolker 2011), with a Poisson error distribution. Plot was treated as a random factor, and since plots on the same river were expected to be more similar than to plots drawn at random, river was also included as a random factor. Data were overdispersed, so the model was translated to a log-normal Poisson model by including an individual level random factor, that is, a vector from one to the total number of observations (Elston et al. 2001; Bolker 2011). *Impatiens glandulifera* abundance at the plot (local) scale and a quadratic term (included to detect a curved rather than linear relationship to test the Rathcke (1983) density-visitation model predictions) were treated as fixed effects. Date, the number of trees in the plot, the mean percentage cover of bare ground in the plot, plot plant species richness and the number of open flowers in the plot were also treated as fixed effects. Explanatory variables were obtained from the vegetation survey described in Chapter two. Minimum adequate models were obtained (Crawley 2002) using lowest AIC values found by removing non-significant terms in a backwards stepwise manner. *Impatiens glandulifera* abundance at the plot scale, bare ground, the number of trees, plant species richness and the number of open flowers were log transformed to improve conformity to normal error distributions. Models were repeated to examine *I. glandulifera* abundance at the broad scale. Since the date that insects and vegetation data (Chapter two) were collected differed, a linear model was used to predict the values for each explanatory variable on the date that insects were collected. To estimate the variance explained by the model, an R^2 estimate (an analogue of the coefficient of determination, R^2) was calculated by correlating the observed data with the model values for the fixed effects. Unlike linear models, it is not possible to extract a true R^2 value from GLMM models due to their nested structure (Xu 2003).

The relationship between *I. glandulifera* abundance and insect species richness was examined using rarefaction (function vegan, v 2.0-2; Oksanen et al. 2011) to remove the effects of varying sample size (Gotelli & Colwell 2001). This standardised species richness and enabled comparisons between plots. Rarefied richness was log transformed to improve conformity to normal error distributions. GLMMs were repeated as above, but according to a Gaussian error distribution. Randomisation tests were therefore used in Gaussian models to determine significance values (Bates 2006). Species richness was randomised 10,000 times, within month. The model was then re-fitted to this random data, and the coefficients were extracted to give a null distribution. The real data was compared to this null distribution and significance assessed using a 2-sided p test.

3.3.4.2 Bumblebee-flower visitation observations

The relationship between the abundance of *I. glandulifera* and the abundance of bumblebees foraging along transects was examined using GLMMs, as described above for examining insect abundance but with inclusion of additional explanatory variables of wind speed, temperature, and plant height (Hill et al. 2004). Plant height was log transformed to improve conformity to a normal error distribution.

Non-linear least squares (NLS) models were used to determine how bumblebee-flower visitation changed according to the relative abundance of *I. glandulifera* flowers in the plot. Plant "attractiveness" was derived to describe bumblebee-flower visitation for each plant species. This was to calculate a standardised measure to enable comparison of visitation between plots, which differed in the absolute numbers of flowers. Model 1 estimated plant "attractiveness" of a given plant species based on the equation:

$$\mathbf{V} = \mathbf{F^a}$$

where V is the proportion of visits received by a given plant species, and F is the proportion of floral units of the given species (out of the total floral units of all plant species in the plot). F is raised to the power of a, which is taken as a measure of the attractiveness of the plant species. The null expectation was that the proportion of visits received by a given plant species was directly proportional to its floral abundance (dashed line, Figure 3.3), where $a = 1$. This is an assumption for the purposes of the model. The attractiveness of a plant species to bumblebees will in reality depend on a wide range of interrelated factors (Waser 1986). To take these factors directly into account would require repeated and extensive field measurements. However, elucidating the many reasons that govern plant attractiveness are not important for the purposes of this model (see below). Rather, the aim of estimating plant attractiveness was to be able to measure how bumblebee-flower visitation changes with *I. glandulifera* invasion.

Deviation from the null expectation, that visits received by a given plant species will be proportional to floral units, will be due to a wide range of unmeasured factors such as such as floral reward and handling time. The actual value of plant attractiveness, represented by the power, a itself is unimportant here however, instead the relative change found under *I. glandulifera* invasion is of note. Deviation from the null expectation in model 1 was used to indicate the attractiveness of each plant species. Model significance indicated rejection of the null hypothesis. Plants where $a > 1$ were called “unattractive” (curves below the dashed line; Figure 3.3 red line), and plants where $a < 1$ were called “attractive” (curves above the dashed line; Figure 3.3 blue line). Thus the greater the value of the power, the more “unattractive” the species. This is because as a proportion, F is a value between 0 and 1. Thus since F takes a value less than 1, when raised to the power of a greater than 1, the proportion of visits is smaller than the proportion of flowers, and the plant is unattractive. The steeper the initial slope of the curve (the more rapidly the curve approaches 1), that is the smaller the value of the power, the more “attractive” the species.

Model 2 was used to determine whether the presence of flowering *I. glandulifera* altered plant attractiveness (that is, a standardised measure of bumblebee-flower visitation). Model 2 takes a different power, a or b, depending on whether *I. glandulifera* is absent or present:

$$\mathbf{V} = \begin{cases} \mathbf{F^a} & \textbf{absent} \\ \mathbf{F^b} & \textbf{present} \end{cases}$$

where the power a is used when *I. glandulifera* is absent and b is used when it is present. Model 3 was used to examine how attractiveness changes with *I. glandulifera* abundance, that is, to examine the relationship between *I. glandulifera* abundance and bumblebee-flower visitation, and takes a different power depending on whether *I. glandulifera* is at low, medium or high floral abundance:

$$\mathbf{V} = \begin{cases} \mathbf{F^a} & \textbf{absent} \\ \mathbf{F^b} & \textbf{low} \\ \mathbf{F^c} & \textbf{medium} \\ \mathbf{F^d} & \textbf{high} \end{cases}$$

where *a* is the power used when *I. glandulifera* is absent, and *b*, *c*, and *d* are the powers used when the abundance of *I. glandulifera* is low, medium or high. Classes were defined according to the proportion of flowers within the plot: absent, 0 flowers; low, up to 30 % of the total flowers in the plot were *I. glandulifera*; medium, up to 60 % were *I. glandulifera* flowers; high more than 60 % were *I. glandulifera* flowers.

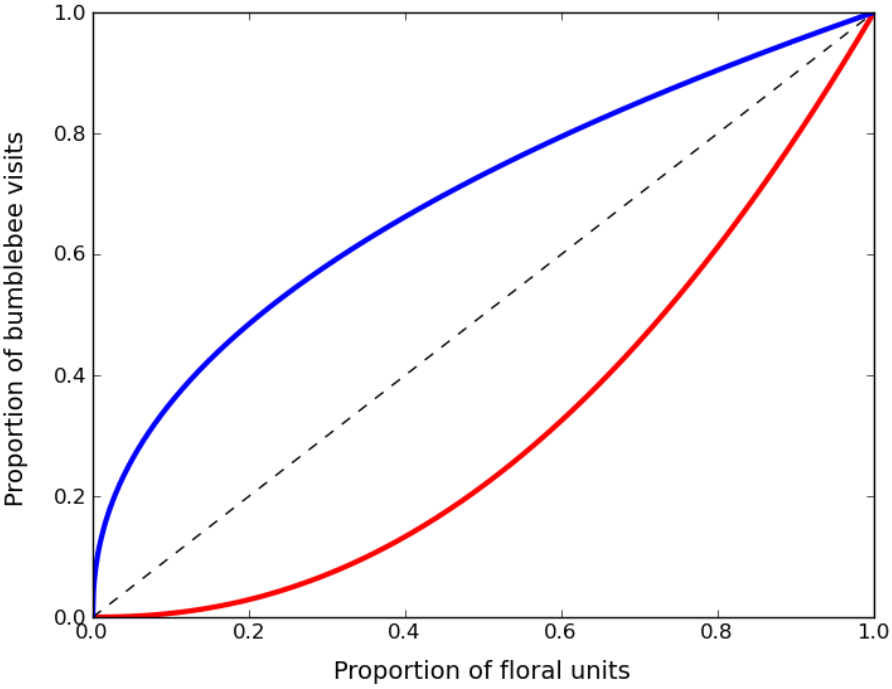


Figure 3.3: The model for assessing plant "attractiveness" is given by the equation $V = F^a$, where the proportion of bumblebee visits (V) received by a given plant species is relative to its floral abundance (F), calculated as the proportion of floral units out of the total present in the experimental plot. The power *a* is a measure of the attractiveness of the plant species. The dashed line represents the null expectation that the proportion of visits received by a given plant species is directly proportional to its floral abundance, and *a* = 1. Deviation from the null expectation indicates plant attractiveness; *a* less than one indicates that it is "attractive" (blue line) and greater than one indicates it is "unattractive" (red line). Plant attractiveness will depend on a wide range of unmeasured variables, however it is the relative change in attractiveness under *I. glandulifera* invasion that is of note for the purposes of this study.

All models were weighted by the number of foraging bumblebees present in the plot. A likelihood ratio test (ANOVA) was used to compare model 2 to model 1; and model 3 to model 2. Model 2 significance indicated that *I. glandulifera* presence significantly changed plant attractiveness (that is, *I. glandulifera* changed bumblebee-flower visitation to a given species), and model 3 significance indicated that the abundance of *I. glandulifera* changed plant attractiveness more than its presence. Model significance resulted in a change to the value of the power, and to the shape of the curve. Model 2 was necessary in addition to model 3 for significance testing of model 3. Model 3 tested for some kind of relationship between *I. glandulifera* abundance and visitation, such as that predicted by Rathcke's density-visitation model (1983). The lowest AIC value was used to find the best fitting model to describe the relationship between *I. glandulifera* and visitation, which may or may not be related to its abundance.

Plants were examined by species and grouped by family, and by traits of colour (in the visible spectrum) and plant height. Height was ranked as short (0-99 cm), medium (100-199 cm), or tall (200+ cm) (data from Hill et al. 2004). Although true plant height is expected to vary around this mean, plant height could not be measured in the field for logistical reasons, given the scale of the study. Therefore, mean plant height was used to indicate general trends. Species or groups present in less than five plots and receiving less than five bumblebee visits in total were not examined as the sample size was too small.

3.4 Results

3.4.1 Effect of flowering *I. glandulifera* on the under-storey pollinator community

The number of species and individuals collected from pan traps at ground level, beneath the *I. glandulifera* canopy, are shown in Table 3.1. Pollinators were sampled three times between August and September 2010 from each of 30 experimental plots. The most abundant species were hoverflies *Syrphus ribesii* (534 individuals), *Heliophilus pendulus* (239 individuals), *Episyrphus balteatus* (222 individuals); and the social wasp *Vespula vulgaris* (203 individuals). After *Syrphus* (607 individuals), *Bombus* was the most numerous genus (273 individuals). A full species list is given in Appendix B, Table B.1.

Of particular conservation interest are the hoverfly species *Eupeodes nielseni* and *Neoascia obliqua*, which are “Notable”, that is they are thought to occur in 100 or fewer 10 km grid squares in Great Britain (Falk, 1991).

Table 3.1: Number of species in the orders Hymenoptera (Aculeates – bees and wasps; and sawflies) and Diptera (hoverflies) from pan traps in 30 experimental plots, each sampled three times between August and September 2010. *Bombus terrestris* and the three species of the *Bombus lucorum* complex are grouped as a single species as they cannot be reliably distinguished.

	Hymenoptera						Diptera
	Aculeates					Sawflies	
	Solitary bees	Social bees		Solitary wasps	Social wasps		Hoverflies
		Bombus	*Apis*				
Number of species	9	7	1	7	5	7	41
Number of individuals	31	273	43	12	233	36	1505

At both the plot and broad scale, the abundance of hoverflies significantly decreased with increasing flowering *I. glandulifera* abundance (Figures 3.4 a and b respectively, Table 3.2; full model results are in Appendix B, Table B.2). At a broad scale, hoverfly abundance showed a unimodal relationship with increasing *I. glandulifera* abundance, peaking at low levels of invasion, although this curved relationship was non-significant. The abundance of social wasps significantly increased with increasing flowering *I. glandulifera* abundance at both the plot and broad scales (Figures 3.5 a and b respectively, Table 3.3; full model results in Appendix B, Table B.3).

There was no significant relationship between the abundance of pan trap-caught bumblebees and a gradient of increasing flowering *I. glandulifera* abundance, at any scale (Appendix B, Table B.4). However, bumblebee abundance in the pan traps was significantly higher when *I. glandulifera* was present in the plot compared to when it was absent (GLMM, $b = 0.817$, $z = 2.206$, $p = 0.03$; Appendix B, Table B.4) (Figure 3.6 a). Equivalent analyses to those reported in Tables 3.4 and 3.5 revealed no significant effect of flowering *I. glandulifera* abundance on sawfly abundance (GLMM, $p \geq 0.2$). Solitary bees, solitary wasps and honeybees (*Apis*) were not included in the analysis due to low numbers collected. There was no significant relationship between flowering *I. glandulifera* abundance and rarefied hoverfly or bumblebee species richness (log) (GLMM, $p \geq 0.12$, $p \geq 0.09$ respectively; results not shown).

Table 3.2: Abundance of hoverflies in 30 experimental plots sampled three times between August and September 2010, as a function of flowering *Impatiens glandulifera* abundance. Generalised linear mixed models (GLMMs) were used, according to a Poisson error distribution. Fixed effects were *I. glandulifera* abundance, number of trees (log), date, bare ground (log), estimated plant species richness (log) and estimated number of open flowers (log). A quadratic term and plant species richness were removed to find the minimum adequate model. Random effects were plot and river, and individual level random effects were included to account for overdispersion in the data. Full details of each model's other significant fixed effects can be found in Appendix B, Table B.2. R^2 is not possible to determine from GLMMs. Therefore an R^2 estimate was calculated, by correlating the observed data with the model values for the fixed effects.

***I. glandulifera* abundance**	**Estimate**	**Std error**	***z***	***p***	**R^2 estimate**
Plot scale (log)	-0.821	0.094	-8.778	<0.001	0.495
Broad scale	-0.034	0.007	-4.780	<0.001	0.336

Table 3.3: Abundance of social wasps in 30 experimental plots sampled three times between August and September 2010, as a function of flowering *Impatiens glandulifera* abundance. Generalised linear mixed models (GLMMs) were used, according to a Poisson error distribution. Fixed effects were *I. glandulifera* abundance, number of trees (log), date, bare ground (log), estimated plant species richness (log) and estimated number of open flowers (log). Bare ground was removed in the broad scale model to find the minimum adequate model. Random effects were plot and river, and individual level random effects were included to account for overdispersion in the data. Full details of each model's other significant fixed effects can be found in Appendix B, Table B.3. R^2 is not possible to determine from GLMMs. Therefore an R^2 estimate was calculated, by correlating the observed data with the model values for the fixed effects.

***I. glandulifera* abundance**	**Estimate**	**Std error**	***z***	***p***	**R^2 estimate**
Plot scale (log)	0.663	0.304	2.180	0.029	0.139
Broad scale	0.041	0.020	2.047	0.041	0.112

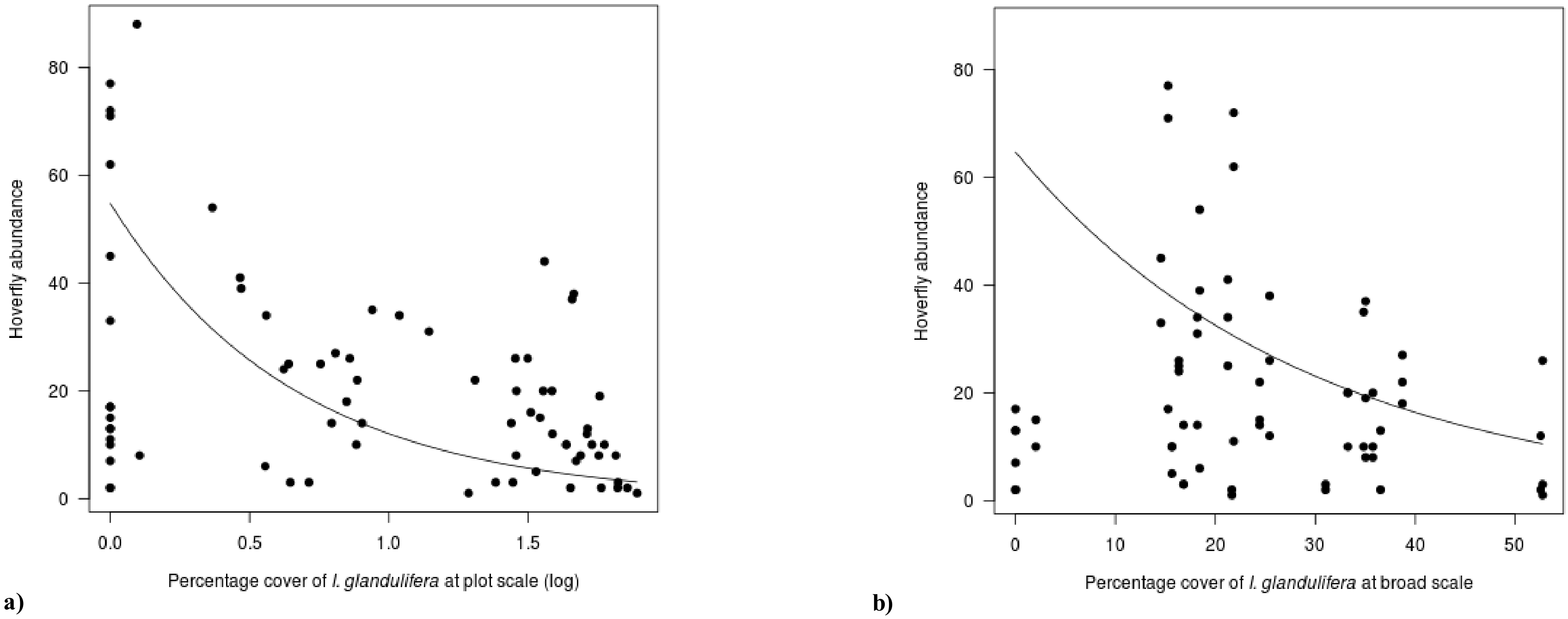
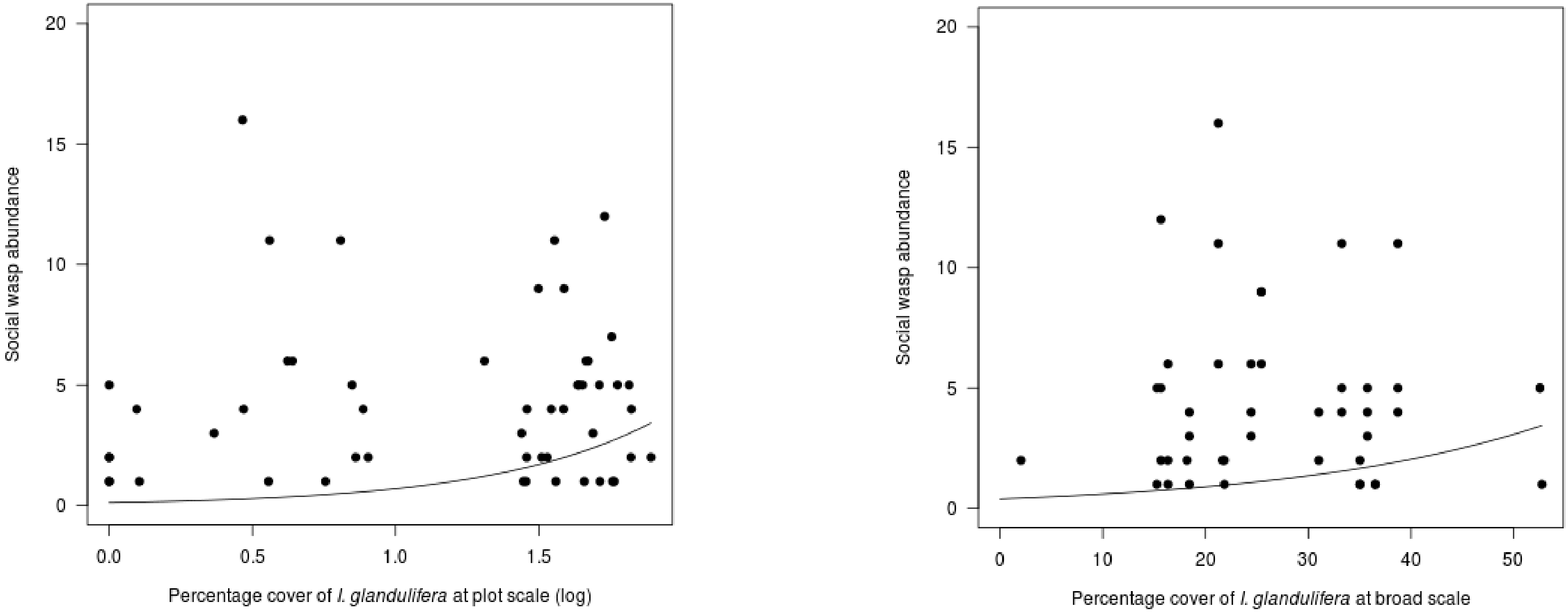


Figure 3.4: Hoverfly abundance in 30 experimental plots sampled three times between August and September 2010, which varied in the percentage cover of flowering *Impatiens glandulifera* at **a)** the plot scale of invasion and **b)** the broad scale of invasion. Hoverflies were collected in pan traps at ground level. Fitted lines were calculated using predicted values for each term in GLMM models fitted to the data. Models (according to a Poisson error distribution) contained fixed effects of *I. glandulifera* abundance, bare ground (log), number of trees (log), date and estimated number of open flowers (log), and random effects of plot, river, and individual level random effects. In b) an additional fixed effect was plant species richness.



a) **b)**

Figure 3.5: Social wasp abundance in 30 experimental plots sampled three times between August and September 2010, that varied in the percentage cover of flowering *Impatiens glandulifera* at **a)** the plot scale of invasion and **b)** the broad scale of invasion. Social wasps were collected in pan traps at ground level. Fitted lines were calculated using predicted values for each term in GLMM models fitted to the data. Models (according to a Poisson error distribution) contained fixed effects of *I. glandulifera* abundance, number of trees (log), plant species richness and estimated number of open flowers (log), and random effects of plot, river, and individual level random effects. In a) an additional fixed effect was bare ground (log).

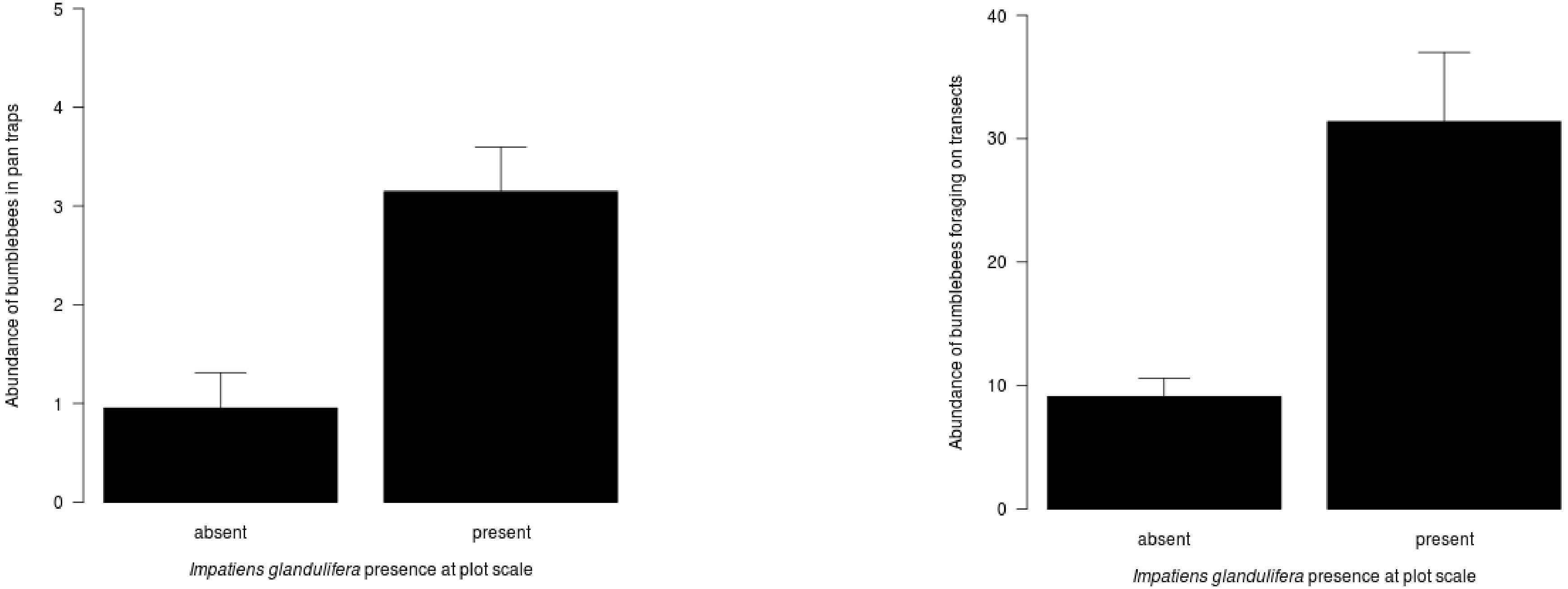
3.4.2 Effect of flowering *I. glandulifera* on bumblebee visitation

The abundance of bumblebees caught foraging along transects was significantly higher in plots where *I. glandulifera* was present compared to where it was absent (GLMM, $b = 1.520$, $z = 3.280$, $p = 0.001$) (Figure 3.6 b), which mirrors the findings in the pan traps (Figure 3.6 a).

The effect of *I. glandulifera* invasion on bumblebee-flower visitation was measured using non-linear least squares (NLS) models. Models were used to standardise bumblebee-flower visitation across plots since absolute numbers of flowers differed. This was achieved by deriving plant "attractiveness" to describe bumblebee-flower visitation. Attractiveness was measured by deviation from the null expectation that visits received by a given plant species will be proportional to floral units, which will be a consequence of a wide range of unmeasured variables. The actual value of plant attractiveness, represented by the power, a, is unimportant in this context, rather the relative change found under *I. glandulifera* invasion is of note.

The relationship between *I. glandulifera* floral density and bumblebee visitation shows that it was attractive, receiving significantly more visits than expected based on the proportion of floral units available (Table 3.4 "all bumblebees", Figure 3.7). *Impatiens glandulifera* received a high proportion of bumblebee visits, even at low abundance (Figure 3.7). It should be noted that since floral abundance was estimated using the sum of five 1 m^2 quadrats (see Methods section), there is a margin of error. Therefore, in some instances bumblebees were recorded as foraging on *I. glandulifera* although none was recorded in the quadrats (Figure 3.7).

The attractiveness of *I. glandulifera* differed for each bumblebee species. The strength of preference for the invader was *Bombus hortorum* > *B. lapidarius* > *B. pascuorum* > *B. lucorum/terrestris* > *B. pratorum* (Table 3.4, power a, and Figures 3.8.1 – 3.8.5 where a steeper initial slope of the curve reflects a stronger preference, and a smaller a). Although all bumblebee species showed a preference for *I. glandulifera* above the null expectation (Table 3.4, power $a < 1$, Figures 3.8.1 – 3.8.5), this was only significant for *B. pascuorum* and *B. lucorum/terrestris* although this may reflect larger sample sizes (Table 3.4). The remaining species showed a greater proportion of individuals choosing not to forage on *I. glandulifera*, even in instances where it represented a high proportion of flowers in the plot (Figures 3.8.1 – 3.8.5). *Bombus pascuorum* had the most diverse diet, visiting nine plant species in addition to *I. glandulifera* (Figure 3.8.2), compared to five species for *B. hortorum*, *B. lapidarius* and *B. lucorum/terrestris*, and four species for *B. pratorum* (Figures 3.8.1, 3.8.3 - 3.8.5 respectively). A high proportion of visits were made to co-flowering plant species (Figures b 3.8.1 – 3.8.5). However, *I. glandulifera* pollen dominated the pollen samples taken from bumblebee thoraxes, with a high proportion of individual bumblebees carrying entirely *I. glandulifera* pollen (Figures c 3.8.1 - 3.8.5).



a)

b)

Figure 3.6: a) Bumblebee abundance in pan traps at ground level in 30 experimental plots sampled three times between August and September 2010, and **b)** abundance of foraging bumblebees along 20 m transects in 16 experimental plots, sampled in June, July and August 2010. Flowering *I. glandulifera* was absent or present in the plot. Values are means + SE. a) N = 22, 102 and b) N = 26, 16 samples in plots where *I. glandulifera* was absent and present respectively.

Table 3.4: Result of non-linear least squares (NLS) models based on the equation $V = F^a$ where V is the proportion of bumblebee visits received by *Impatiens glandulifera* and F is the proportion of its flowers available, which is raised to the power *a*, a measure of its attractiveness. Significance indicates deviation from the null expectation that proportion of visits is directly proportional to floral abundance, where the power $a = 1$. $a < 1$ indicates the plant is more attractive than expected and $a > 1$ less attractive than expected; the bigger the power, the less attractive *I. glandulifera* is. Models were weighted by the number of bumblebees in the plot. Data is from bumblebee-flower visitation recorded from transects in 16 plots, each sampled three times between June and August 2010.

Bumblebee species	**N**	***a***	***t***	***p***	**R²**
B. hortorum	114	0.108	-0.938	0.361	0.141
B. lapidarius	41	0.150	-1.516	0.155	0.149
B. pascuorum	360	0.278	-2.755	0.010	0.107
B. lucorum/terrestris	178	0.381	-2.78	0.011	0.413
B. pratorum	73	0.430	-1.774	0.098	0.209
All bumblebees	766	0.268	-2.845	0.008	0.537

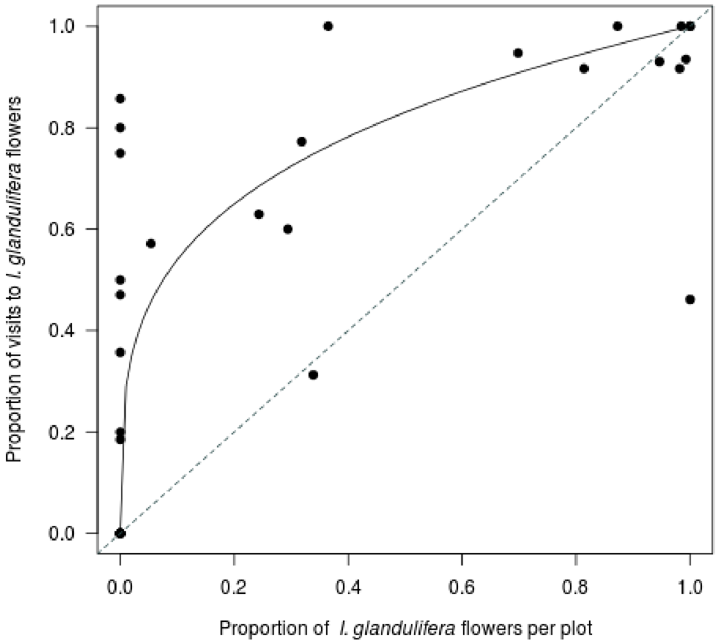
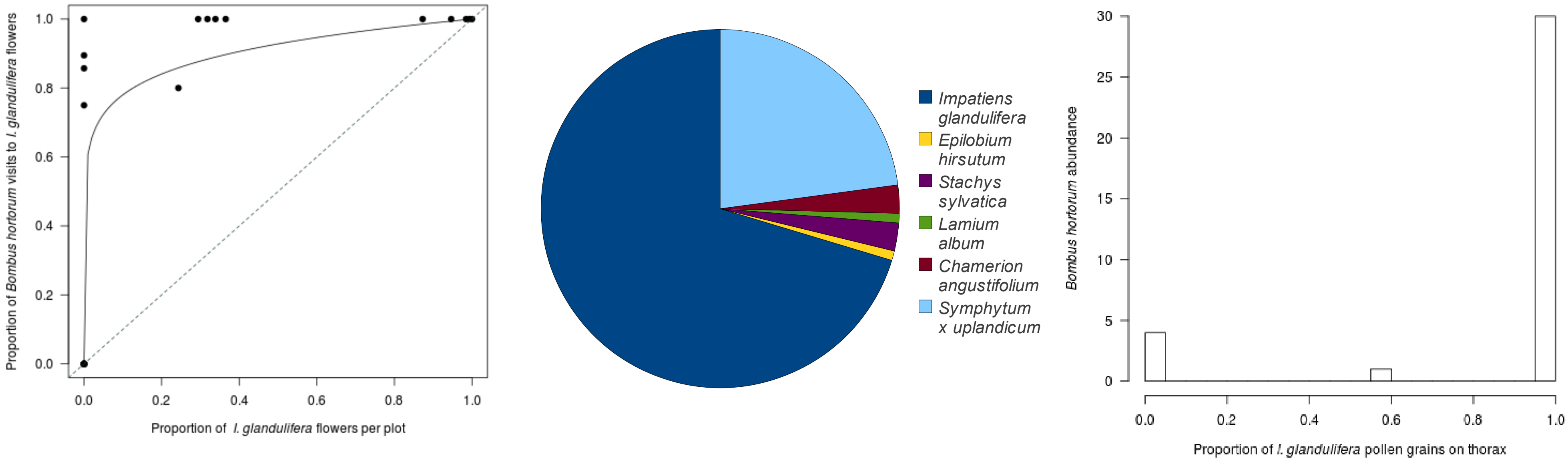
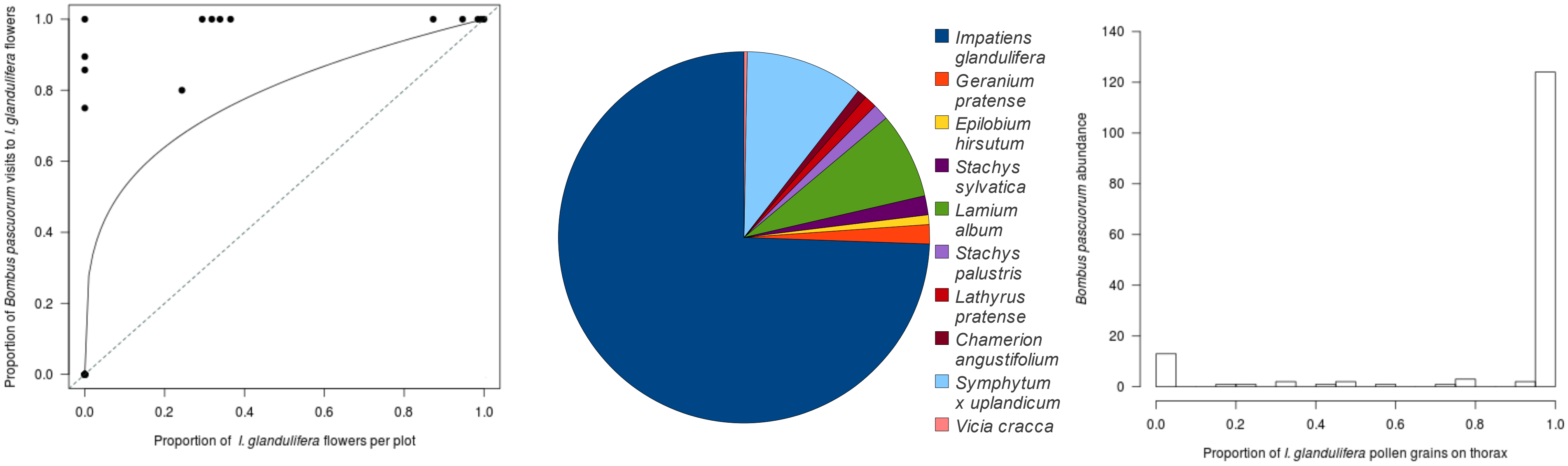


Figure 3.7: Proportion of bumblebees foraging on *Impatiens glandulifera* in 16 experimental plots, sampled from 20 m transects in June, July and August 2010. The dashed line represents the null expectation that the proportion of visits is directly proportional to floral abundance. Floral abundance was measured as the proportion of total flowers present, at the plot scale, in five $1m^2$ quadrats. The fitted line was calculated using predicted values from the NLS model fitted to the data (Table 3.4). The model is weighted according to the number of bumblebees per plot.



a) **b)** **c)**

Figure 3.8.1: a) Proportion of *Bombus hortorum* foraging on *Impatiens glandulifera* in 16 experimental plots, sampled from 20 m transect walks in June, July and August 2010. *Impatiens glandulifera* floral abundance was measured as a proportion of the total floral units present, at the plot scale, in five 1m^2 quadrats. The dashed line represents the null expectation that the proportion of visits is directly proportional to floral abundance. The fitted line was calculated using predicted values from the NLS model fitted to the data (Table 3.4). Models were weighted according to the number of *B. hortorum* individuals per plot. N = 114, total individuals observed. **b)** Proportion of *B. hortorum* individuals recorded foraging on different plant species, N = 114. **c)** Proportion of *I. glandulifera* pollen grains found on the thoraxes of *B. hortorum* individuals collected, N = 35. Non-*I. glandulifera* pollen was not identified to species.



a) **b)** **c)**

Figure 3.8.2: a) Proportion of *Bombus pascuorum* foraging on *Impatiens glandulifera* in 16 experimental plots, sampled from 20 m transect walks in June, July and August 2010. *Impatiens glandulifera* flowers were measured as a proportion of total floral units present, at the plot scale, in five $1m^2$ quadrats. The dashed line represents the null expectation that the proportion of visits is directly proportional to floral abundance. The fitted line was calculated using predicted values from the NLS model fitted to the data (Table 3.4). Models were weighted according to the number of *B. pascuorum* individuals per plot. N = 360, total individuals observed. **b)** The proportion of *B. pascuorum* individuals recorded foraging on different plant species, N = 360. **c)** Proportion of *I. glandulifera* pollen grains found on the thoraxes of *B. pascuorum* individuals collected, N = 151. Non-*I. glandulifera* pollen was not identified to species.

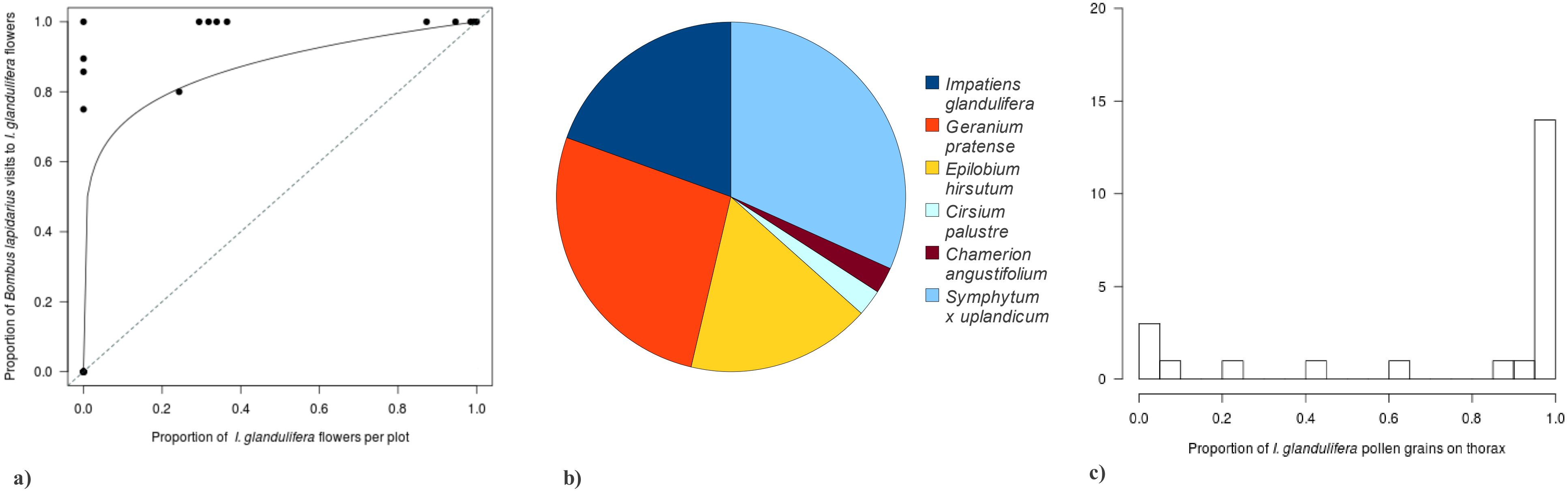
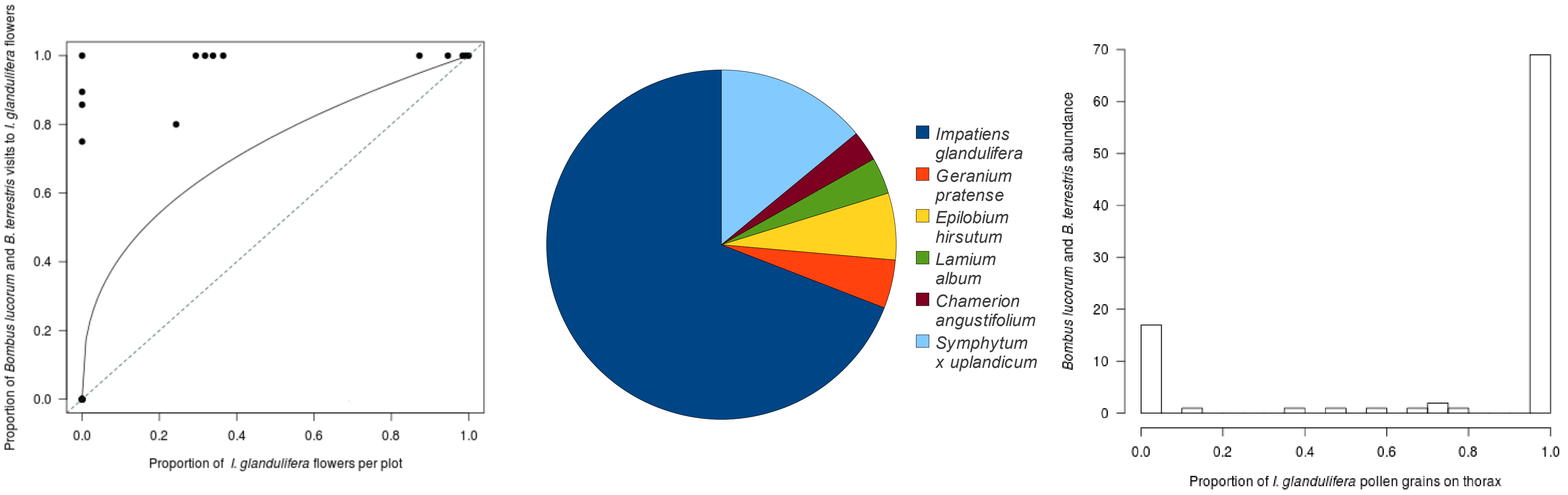


Figure 3.8.3: a) Proportion of *Bombus lapidarius* foraging on *Impatiens glandulifera* in 16 experimental plots, sampled from 20 m transect walks in June, July and August 2010. *Impatiens glandulifera* flowers were measured as a proportion of total floral units present, at the plot scale, in five 1m^2 quadrats. The dashed line represents the null expectation that the proportion of visits is directly proportional to floral abundance. The fitted line was calculated using predicted values from the NLS model fitted to the data (Table 3.4). Models were weighted according to the number of *B. lapidarius* individuals per plot. N = 41, total individuals observed. **b)** Proportion of *B. lapidarius* individuals recorded foraging on different plant species, N = 41. **c)** Proportion of *I. glandulifera* pollen grains found on the thoraxes of *B. lapidarius* individuals collected, N = 23. Non-*I. glandulifera* pollen was not identified to species.



a)

b)

c)

Figure 3.8.4: a) Proportion of *Bombus lucorum* and *B. terrestris* foraging on *Impatiens glandulifera* in 16 experimental plots, sampled from 20 m transect walks in June, July and August 2010. *Impatiens glandulifera* flowers were measured as a proportion of total floral units present, at the plot scale, in five $1 m^2$ quadrats. The dashed line represents the null expectation that the proportion of visits is directly proportional to floral abundance. The fitted line was calculated using predicted values from the NLS model fitted to the data (Table 3.4). Models were weighted according to the number of *B. lucorum* and *B. terrestris* individuals per plot. N = 178, total individuals observed. **b)** Proportion of *B. lucorum* and *B. terrestris* individuals recorded foraging on different plant species, N = 178. **c)** Proportion of *I. glandulifera* pollen grains found on the thoraxes of *B. lucorum* and *B. terrestris* individuals collected, N = 94. Non-*I. glandulifera* pollen was not identified to species.

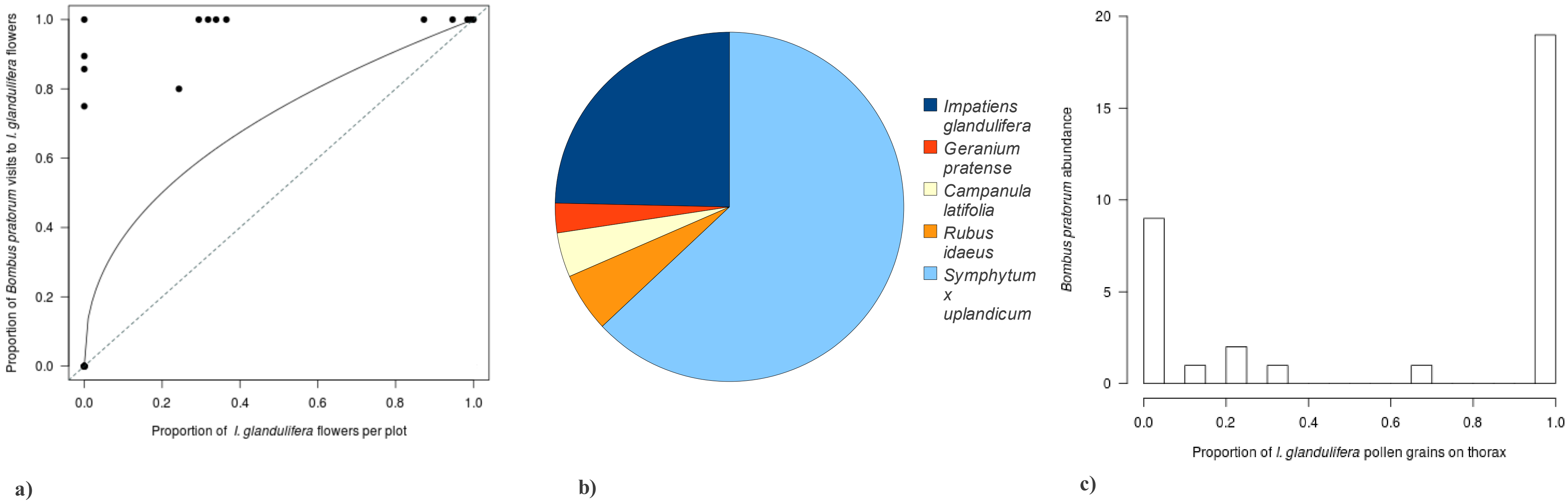


Figure 3.8.5: a) Proportion of *Bombus pratorum* foraging on *Impatiens glandulifera* in 16 experimental plots, sampled from 20 m transect walks in June, July and August 2010. *Impatiens glandulifera* flowers were measured as a proportion of total floral units present, at the plot scale, in five 1m^2 quadrats. The dashed line represents the null expectation that the proportion of visits is directly proportional to floral abundance. The fitted line was calculated using predicted values from the NLS model fitted to the data (Table 3.4). Models were weighted according to the number of *B. pratorum* individuals per plot. N = 73, total individuals observed. **b)** Proportion of *B. pratorum* individuals recorded foraging on different plant species, N = 73. **c)** Proportion of *I. glandulifera* pollen grains found on the thoraxes of *B. pratorum* individuals collected, N = 33. Non-*I. glandulifera* pollen was not identified to species.

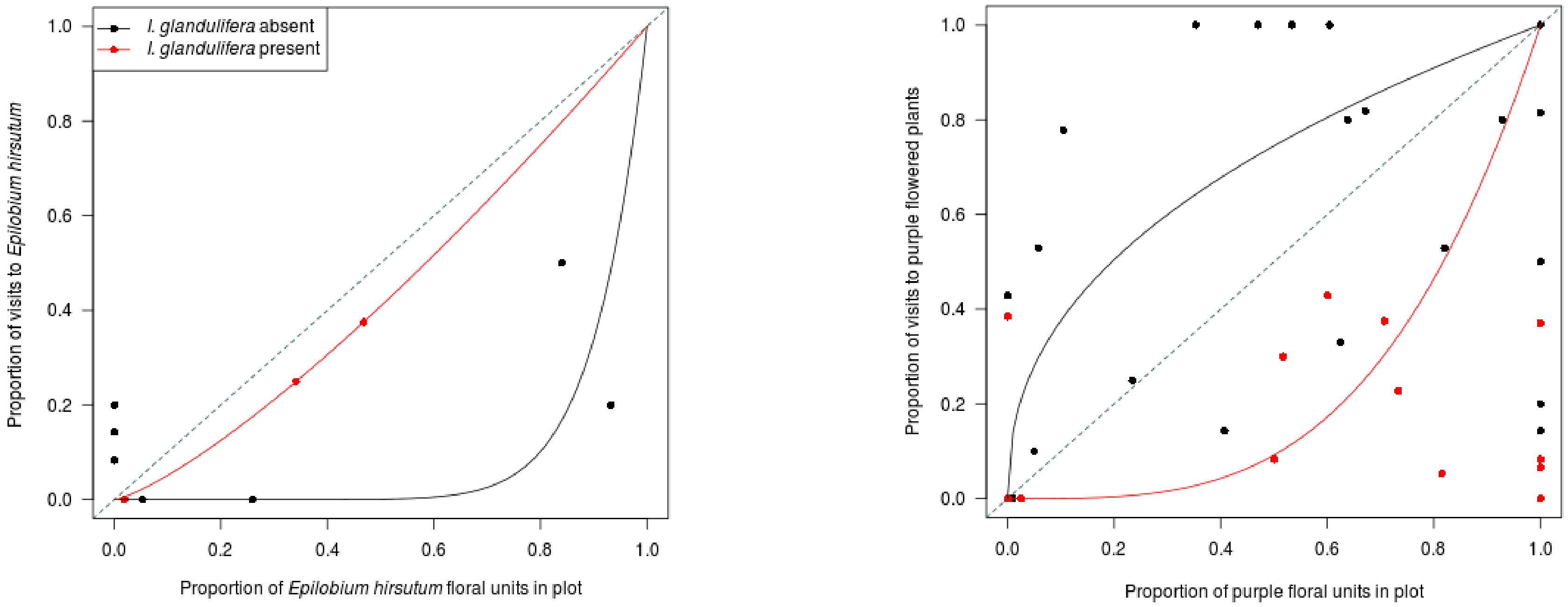
Of the plant species common enough to analyse individually, *Chamerion angustifolium* and *Stachys sylvatica* were significantly less attractive than expected based on the proportion of floral units (Table 3.5) (Appendix B, Figures B.1 and B.2). The number of bumblebee visits received by *Epilobium hirsutum*, *Lamium album* and *Symphytum x uplandicum* did not differ significantly from the null expectation that the proportion of visits is directly proportional to floral abundance (Table 3.5) (Appendix B, Figures B.3-B.5). This attractiveness value in itself is not important, and is the product of a number of unmeasured variables. Attractiveness is used as a reference point to measure how visitation changes under *I. glandulifera* invasion (which is calculated using models 2 and 3, see below).

Table 3.5: Result of non-linear least squares (NLS) models based on the equation $V = F^a$ where V is the proportion of bumblebee visits received, F is the proportion of floral units of a given plant species, which is raised to the power *a*, a measure of its attractiveness. Plant species modelled were those common enough to analyse. Significance indicates deviation from the null expectation that the proportion of visits is directly proportional to the floral abundance, where the power $a = 1$. Power $a < 1$ indicates the plant is more attractive than expected and $a > 1$ less attractive than expected; the bigger the power, the less attractive. N is the number of replicated plots in which a species received a minimum of one visit (out of a possible 48; 16 plots visited three times each). Models were weighted by the number of bumblebees in the plot.

Plant species	**N**	***a***	***p***	**R^2**
Chamerion angustifolium	7	5.613	0.010	0.022
Epilobium hirsutum	10	1.918	0.143	0.368
Lamium album	24	1.864	0.052	0.381
Stachys sylvatica	16	2.581	0.001	0.167
Symphytum x uplandicum	24	0.818	0.518	0.452

Competition between *I. glandulifera* and other plant species for bumblebee visits was measured by examining changes in plant attractiveness in the presence of *I. glandulifera* and with increasing *I. glandulifera* abundance. There was no significant effect of the presence of *I. glandulifera* on the attractiveness of *Chamerion angustifolium*, *Stachys sylvatica*, *Lamium album*, or *Symphytum x uplandicum* (NLS, $p \geq 0.117$) nor with its increasing abundance (NLS, $p \geq 0.247$). In the presence of *I. glandulifera*, *Epilobium hirsutum* became significantly more attractive (Table 3.6, model 1 compared to model 2 - numerically lower AIC value, Figure 3.9.1 a). The measure of attractiveness was 7.94 times greater when the invader was present compared to when it was absent (Table 3.6, model 2 power *a* compared to power *b*).

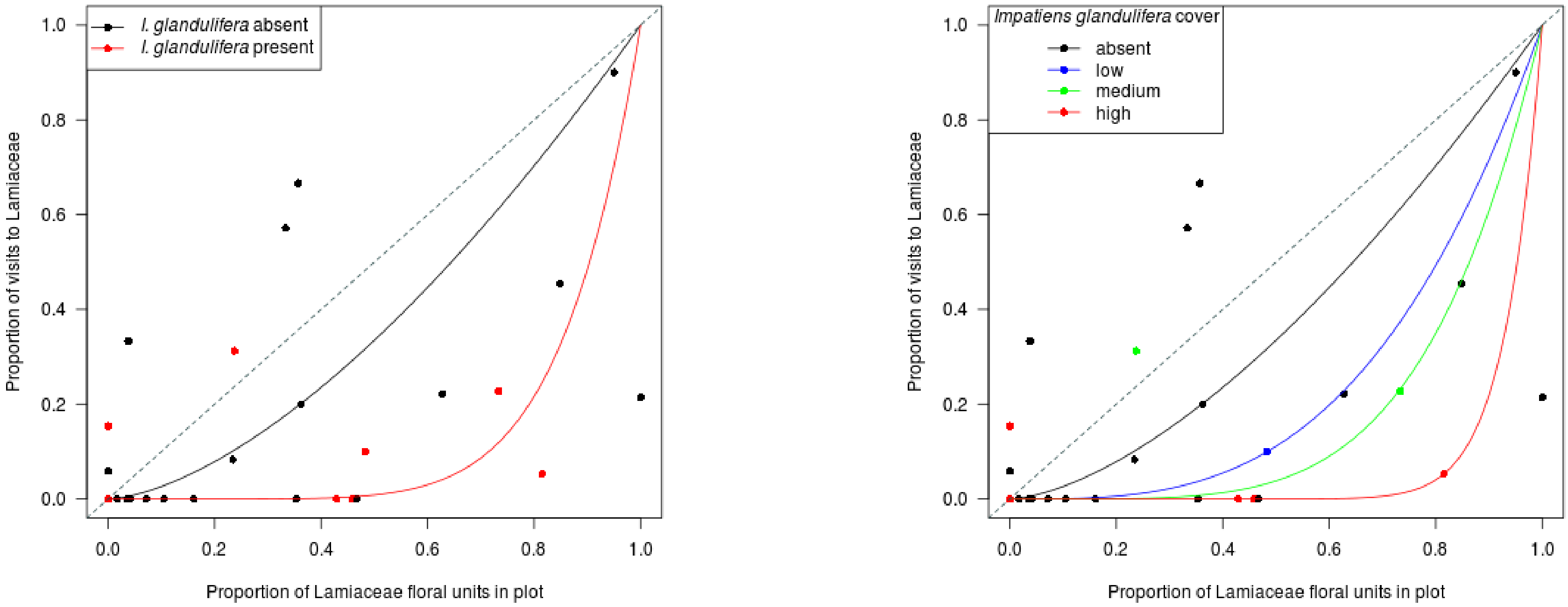
Plants with purple flowers and plants in the Lamiaceae family became significantly less attractive in the presence of *I. glandulifera* (Table 3.6, model 1 compared to model 2; Figures 3.9.1 b and 3.9.2 a respectively), despite the purple-flowered *E. hirsutum* being more attractive in the presence of *I. glandulifera* when considered separately. These groups were chosen as there was enough data to allow analysis - data was too sparse to analyse other colours and plant families. The measure of attractiveness were 8.09 and 4.35 times smaller in the presence of *I. glandulifera* compared to when it was absent, for purple-flowered plants and Lamiaceae plants respectively (Table 3.6, model 2 power *a* compared to power *b*). However the effect was weak for purple flowers (Table 3.6). Both groups became increasingly less attractive along a gradient of an increasing proportion of *I. glandulifera* flowers (Table 3.6, model 3, powers *a*, *b* and *c*, Figure 3.9.2 b), but this was not significantly different to their attractiveness in the presence of *I. glandulifera* (Table 3.6, model 2).



a)

b)

Figure 3.9.1: Proportion of bumblebees foraging on **a)** *Epilobium hirsutum* **b)** purple-flowered plants in the absence and presence of flowering *Impatiens glandulifera*. Data is from bumblebee-flower interactions along 20 m transect walks in 16 experimental plots, repeated in June, July and August 2010. Floral abundance was measured as a proportion of the total floral units present in the plot. The dashed line represents the null expectation that the proportion of visits is directly proportional to floral abundance. The black line is the proportion of visits received in the absence of *I. glandulifera*, and the red line is the proportion of visits received in its presence. Models were weighted according to the number of bees per plot, and fitted lines calculated using predicted values from the NLS model fitted to the data (Table 3.6).



a) b)

Figure 3.9.2: Proportion of bumblebees foraging on **a)** Lamiaceae flowers in the absence and presence of *Impatiens glandulifera*, and **b)** on Lamiaceae flowers in the absence and at low, medium and high abundance of flowering *I. glandulifera*. Data is from bumblebee-flower interactions along 20 m transect walks in 16 experimental plots, repeated in June, July and August 2010. Floral abundance was measured as a proportion of the total floral units present in the plot. The dashed line represents the null expectation that the proportion of visits is directly proportional to floral abundance. The black line is the proportion of visits received in the absence of *I. glandulifera*. In a) the red line is the proportion of visits received in its presence, and in b) the blue, green and red lines are the proportion of visits received in the presence of *I. glandulifera* at low, medium and high abundance respectively. Models were weighted according to the number of bees per plot, and fitted lines calculated using predicted values from the NLS model fitted to the data (Table 3.6).

Table 3.6: Result of non-linear least squares (NLS) models based on the equation $V = F^a$ where V is the proportion of bumblebee visits received, and F is the proportion of floral units of a given plant species/group, which is raised to the power *a*, a measure of its attractiveness. Model 1 describes plant attractiveness (used to measure bumblebee visitation) according to its floral abundance, model 2 includes the additional variable of *Impatiens glandulifera* presence, and model 3 tests for a relationship between *I. glandulifera* abundance and attractiveness (visitation). Analysis of variance (ANOVA) was used to compare model 2 to model 1, and model 3 to model 2. In model 1, significance indicates deviation from the null expectation that the proportion of visits received is directly proportional to floral abundance. In model 2, significance indicates that the presence of *I. glandulifera* significantly changes plant attractiveness, and in model 3 significance indicates that the relative abundance of *I. glandulifera* significantly changes plant attractiveness. Powers <1 indicate the plant is more attractive than expected and >1 less attractive than expected; the bigger the power, the less attractive. Power *a* is plant attractiveness according to its floral abundance. Power *b* is plant attractiveness according to floral abundance, in the presence of *I. glandulifera* (model 2), or at low *I. glandulifera* abundance (model 3). Powers *c* and *d* are plant attractiveness at medium and high *I. glandulifera* abundance respectively (model 3). Models were weighted by the number of bumblebees in the plot.

	N	**Model**	***a***	***b***	***c***	***d***	**AIC**	***p***	**R^2**
Epilobium hirsutum	10	1	1.918				-175.71	0.143	0.368
		2	10.247	1.291			-183.55	0.009	0.330
		3	10.247	1.289	1.291	5.207	-179.55	0.999	0.333
Purple flowers (18 spp.)	105	1	1.889				-25443.13	0.040	0.001
		2	0.425	3.438			-25446.13	0.004	0.026
		3	0.425	1.779	3.690	14.185	-25442.53	0.575	0.033
Lamiaceae (6 spp.)	53	1	5.179				-5718.27	<0.001	0.120
		2	1.578	6.859			-5722.37	0.006	0.274
		3	1.578	3.167	4.706	14.381	-5723.69	0.250	0.286

Table 3.7: Result of non-linear least squares (NLS) models based on the equation $V = F^a$ where V is the proportion of bumblebee visits received, and F is the proportion of floral units of plants grouped by height, which is raised to the power *a*, a measure of its attractiveness. Plant height was taken from 'PlantAtt' database (Hill et al. 2004). Short plants measured 0-99 cm, and medium plants 100-199 cm. Model 1 describes plant attractiveness according to floral abundance, and model 2 includes the additional variable of *Impatiens glandulifera* presence. Analysis of variance (ANOVA) was used to compare model 2 to model 1. In model 1, significance indicates deviation from the null expectation that the proportion of visits received is directly proportional to floral abundance. In model 2, significance indicates that the presence of *I. glandulifera* significantly changes plant attractiveness. Powers <1 indicate the plant is more attractive than expected and >1 less attractive than expected; the bigger the power, the less attractive. Power *a* is plant attractiveness according to floral abundance. Power *b* is plant attractiveness according to floral abundance in the presence of *I. glandulifera* (model 2). Models were weighted by the number of bumblebees in the plot.

	N	**Model**	***a***	***b***	**AIC**	***p***	**R^2**
Short	64	1	11.674		-9310.39	<0.001	0.069
		2	0.664	11.863	-9322.00	<0.001	0.313
Medium	144	1	1.172		-52581.68	0.209	0.382
		2	1.555	1.058	-52580.18	0.327	0.380

Short plants (defined using data from 'PlantAtt' database, Hill et al. 2004) were unattractive (Table 3.7, model 1), receiving significantly fewer visits than expected according to their floral abundance. In the presence of *I. glandulifera*, the measure of attractiveness was significantly 17.87 times smaller compared to when it was absent (Table 3.7, model 2 power *a* compared to power *b*, Figure 3.10). Visitation to medium plants did not differ significantly from the null expectation that the proportion of visits is directly proportional to floral abundance (Table 3.7, model 1). Their attractiveness did not significantly change in the presence of *I. glandulifera* (Table 3.7, model 2). Visitation to tall plants (excluding *I. glandulifera*) could not be explored as only three bumblebee visits to tall plant species were recorded, to *Campanula latifolia*, on a single sampling occasion. The relationship between plant height and the abundance of *I. glandulifera* (using model 3) could not be determined since the model couldn't fit the parameters to the data and no solution could be found. This may be solved with a larger data set.

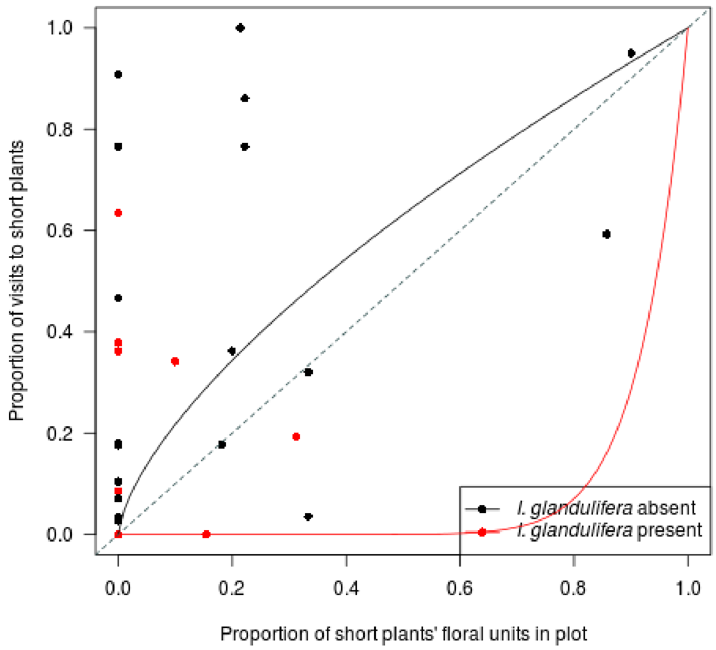


Figure 3.10: Proportion of bumblebees foraging on short plants (0-99 cm) in the absence and presence of *Impatiens glandulifera* in 16 experimental plots, sampled from 20 m transect walks in June, July and August 2010. Floral abundance was measured as a proportion of the total floral units present in the plot. The dashed line represents the null expectation that the proportion of visits is directly proportional to floral abundance. The black line is the proportion of visits received by short plants in the absence of *I. glandulifera*, and the red line is the proportion of visits received in the presence of *I. glandulifera*. Models were weighted according to the number of bees per plot, and fitted lines calculated using predicted values from the NLS model fitted to the data (Table 3.7).

3.5 Discussion

In this study we have demonstrated that the effect of *Impatiens glandulifera* on the pollinator community composition varies with both its abundance and with the spatial scale at which impact is assessed. Pollinator responses to *I. glandulifera* abundance were taxon specific, and highlights the importance of community-level studies in assessing the impact of invasive alien plants. *Impatiens glandulifera* was highly attractive to bumblebees: it received more visits than expected based on the proportion of floral units available; bumblebees responded more strongly to its presence than to its relative abundance; and *I. glandulifera* pollen dominated pollen samples. In the presence of *I. glandulifera*, visitation to co-flowering Lamiaceae and purple-flowered plants was reduced, although one purple-flowered species, *Epilobium hirsutum*, received more visits. Visitation to short plants was reduced whereas plants of medium stature were unaffected by invasion. This suggests that the traits of co-flowering plants used to assess the impact of invasive alien plants should be considered, as they may determine the outcome of the interaction.

3.5.1 Effect of flowering *I. glandulifera* on the under-storey pollinator community

The pollinator community composition at ground level varied with the abundance of flowering *I. glandulifera*. Pan traps could reflect the abundance of pollinators foraging within this aerial stratum. This is because some pollinators, such as honeybees and bumblebees, show a preference for foraging in a horizontal plane (Waddington 1979; Makino 2008) and so insects may only encounter pan traps at the same height as their preferred flowers (Cane et al. 2000). Theoretically, pan traps should capture insects where they are most active, and this is supported by field-based evidence. Tuell & Isaacs (2009) found that the abundance of bees was higher in pan traps at the height of preferred flowers compared to pan traps placed either on the ground or above the canopy, even though this represented a small vertical distance of between 0 and 1.8 m. In another study, pan traps at ground level caught few individuals of bees that mostly foraged at a different vertical strata (Cane et al. 2000). Vertically adjustable pan traps have demonstrated vertical stratification in the abundance of aphids and cicadellids in crops (Vega et al. 1990), and in tropical forests, insect diversity differed between pan traps positioned through the aerial stratum from ground level up to 30 m, reflecting the availability of floral resources (Nuttman et al. 2011). In another tropical forest study, some bee species demonstrated a preference for baited traps placed either at an average of 22 m or 5 m (Roubik 1993). However, other bee species did not show a preference, suggesting that some species forage opportunistically rather than specialising on flowers at a given stratum, or that there may be differences in species' tolerance of other environmental variables such as temperature and wind speed that differ with height (Roubik 1993). This suggests that some margin of error is likely in using pan traps to estimate vertical stratification. In this study, the vertical strata of the plant community varied over a comparatively small scale, reaching a maximum height of 3 metres for *I. glandulifera* (Clements et al. 2008). This exceeds the mean height of the majority of the other plant species recorded. There could be some vertical stratification in insect foraging at this scale, and insects collected at ground level could reflect visitation to flowers in the under-storey rather than to *I. glandulifera* flowers in the upper canopy. It is recommended that future studies sample the pollinator community in the upper canopy to confirm this hypothesis.

At both the plot and broad scale, hoverfly abundance strongly decreased with increasing *I. glandulifera* abundance. This could demonstrate that *I. glandulifera* is having a direct effect on visitation to co-flowering plants in the under-storey by outcompeting them for hoverfly visitation, which has negative implications for their pollination success. Hoverflies are generalist pollinators (Branquart & Hemptinne 2000) and have been recorded visiting *I. glandulifera* (Lopezaraiza–Mikel et al. 2007), although Nienhuis et al. (2009) found they did not favour *I. glandulifera*. *Impatiens glandulifera* flowers secrete nectar at a high rate (Raine & Chittka 2007a) and produce large volumes of pollen (Titze 2000). Nectar is held in a spur which exceeds the tongue length of most hoverflies (Gilbert 1981; Nienhuis & Stout 2009) thus apparently restricting nectar access. It appears more likely that hoverflies visit *I. glandulifera* for pollen, which is necessary for egg maturation (Haslett 1989). While some alien plants can increase butterfly populations through the provision of additional floral resources (Graves & Shapiro 2003), and mass flowering crops can increase early growth of bumblebee colonies (Westphal et al. 2009), it has yet to be established whether mass flowering *I. glandulifera* increases pollinator population sizes.

Rather than directly affecting visitation to co-flowering plants by competing for shared pollinators, *I. glandulifera* could indirectly affect visitation by changing the visitation patterns of pollinators that themselves did not visit the invader. If invasion reduces the local abundance of pollen, nectar or larval food plants, hoverflies could potentially move elsewhere. Hoverflies can use both olfactory and visual signals to select floral feeding sites (Sutherland et al. 1999, Laubertie et al. 2006). *Impatiens glandulifera* dominates the aerial environment due to its height and branching from the main stem (Beerling & Perrins 1993), and this could physically obscure low-growing co-flowering plants. *Impatiens glandulifera* is also strongly scented (Chittka & Schurkens 2001), which could interfere with scents of co-flowering species. An absence of hoverflies foraging in the plot offers an alternative hypothesis for the observed decrease in hoverfly abundance with increasing *I. glandulifera* abundance.

Social wasp abundance showed a small increase with increasing *I. glandulifera* abundance at both the plot and broad scales of invasion. Increased social wasp abundance could result in increased visitation to co-flowering plants, although this requires confirmation by measuring visitation. Lopezaraiza-Mikel et al. (2007) found that pollinator abundance, and also visitation to co-flowering plants, increased in the presence of *I. glandulifera*. However, as well as visiting flowers for nectar, social wasps also forage for animal protein and plant fibres (Spradbery 1973). Foraging for multiple resources could explain the weak relationship with *I. glandulifera* abundance. Increased social wasp abundance may not increase visitation to co-flowering plants as much as obligate foragers. However, social wasp colonies can be large (Spradbery 1963) and so could still exert a significant impact on local plant visitation.

The spatial scale at which pollinators respond to their environment depends on their dispersal ability and foraging range (Steffan-Dewenter et al. 2002). For example, hoverflies are highly mobile since they are not restricted by a nest (Jauker et al. 2009), and some species can migrate long distances (Svensson & Janzon 1984; Nielsen et al. 2010), meaning that they respond to resources at a landscape scale (Meyer et al. 2009). The relationship between pollinator abundance and *I. glandulifera* abundance at plot and broad scales were similar within taxa; hoverfly abundance decreased and social wasp abundance increased with *I. glandulifera* abundance at both plot and broad scales. By contrast, Jakobsson et al. (2009) found that the effect of invasive alien species varied with spatial scale, that is between local scales of metres and broad scales of hectares, in a study measuring the interaction between pollinator visitation and the presence invasive alien species. The broad scale in this study may not have been large enough to detect a difference in pollinator responses to *I. glandulifera* abundance at local and broad spatial scales.

Although not statistically significant, at a broad scale hoverfly abundance demonstrated a weak unimodal relationship with *I. glandulifera* abundance, peaking at low abundance. Rathcke's density-visitation model predicts that at low densities, co-flowering plants should facilitate each other's pollination, but compete for pollinators once floral density exceeds the number of pollinators that can visit them (Rathcke 1983). The weak unimodal response of hoverflies at the broad scale lends marginal support for this prediction, that is if the abundance at ground level reflects visitation to neighbouring flowers rather than an absence from the plot, and measuring invasion at a broader scale may have detected a statistically significant relationship. At a local scale, hoverfly abundance showed a strong exponential decrease with increasing *I. glandulifera* abundance, and social wasp abundance increased at both scales. This does not reflect the predictions of the density-visitation model. The relationship between *I. glandulifera* abundance and pollinator abundance instead differs between pollinator taxa. The decrease in hoverfly abundance with *I. glandulifera* abundance could reflect that *I. glandulifera* increasingly competes for hoverfly visitation, and the increase in social wasps could translate as increased visitation to co-flowering plants. However, whether changes in pollinator abundance does reflect visitation requires verification using field observations.

Shifts in the native plant community caused by invasion from alien plants are expected to have the greatest impact on pollinators that do not utilise the invader (Stout & Morales 2009). In another study, the number of oligolectic (specialist on a narrow range of plant species or families) butterfly species and their abundance was reduced in the presence of the invasive alien *Solidago canadensis*, which was considered likely to be the result of changes in the plant community caused by invasion (de Groot et al. 2007). Most sawfly larvae are highly specialised, feeding on a single host plant species or group of closely related plant species (references in Viitasaari 2002), and adult sawflies have not been recorded visiting *I. glandulifera*. However, we found no effect on sawfly abundance at any spatial scale. In our study, invasion may not have affected the plant community enough to impact on sawfly abundance. Alternatively, sawflies could be temporally isolated from the effects of *I. glandulifera* as few were collected in late August and September. This is because they are generally more abundant from spring until the end of June (Roller 2006), which is mostly before *I. glandulifera*'s flowering period.

There was no effect of *I. glandulifera* invasion on rarefied pollinator species richness, which concurs with the study by Bartomeus et al. (2010), but is contrary to Lopezaraiza et al. (2007) who found that pollinator species richness increased in invaded plots; and is contrary to Thijs et al. (2012) who found that pollinator species richness decreased in invaded plots. Hoverfly species richness can increase with increasing resource heterogeneity in the landscape (Meyer et al. 2009), and other pollinators such as solitary bees also benefit from heterogeneous habitats (Steffan-Dewenter et al. 2002). Differences between this and previous studies could reflect differences in the heterogeneity of the surrounding landscape, or the extent of invasion in the wider landscape, which should influence the local pool of pollinators. Studies examining plant-pollinator interactions have also highlighted that pollinator populations can be highly variable between years (Moragues & Traveset 2005; Larson et al. 2006), but due to time constraints, we were unable to repeat our study in consecutive years.

To measure the effects of the relative abundance of *I. glandulifera* on the pollinator community, we used existing invaded plots rather than manipulating its abundance due to the scale of the study. It is not possible therefore to separate out differences due to the level of invasion from differences in the plot that may cause this level of invasion. However, plots were selected to be as similar as possible in terms of their elevation and surrounding land cover. Observational studies have been criticised as they do not reveal the mechanisms involved in the invasion process (Levine et al. 2003). However, they do generate large data sets that are useful in detecting patterns and informing future research.

3.5.2 Effect of flowering *I. glandulifera* on bumblebee visitation

As in previous studies, we found that *I. glandulifera* was highly attractive to bumblebees and was well integrated in the plant-pollinator network (Lopezaraiza–Mikel et al. 2007; Nienhuis et al. 2009; Bartomeus et al. 2010). It is primarily pollinated by bumblebees (Nienhuis & Stout 2009), which mostly visit flowers for nectar (Titze 2000). *Impatiens glandulifera* received significantly more visits from foraging bumblebees than predicted by its floral abundance alone, although its attractiveness differed between bumblebee species. Long-tongued *Bombus hortorum* and *B. pascuorum*, and medium-tongued *B. lapidarius* showed a stronger preference for the invader compared to shorter-tongued *B. lucorum*, *B. terrestris* and *B. pratorum*. However, preference for *I. glandulifera* above that expected based on its floral abundance was only significant for short-tongued *B. lucorum* and *B. terrestris* and long-tongued *B. pascuorum*. This contrasts with Nienhuis et al. (2009) who found that short-tongued bumblebees did not frequently visit *I. glandulifera* but long- and medium-tongued species did. Competition for nectar among bumblebee species can be avoided through resource partitioning, where long-tongued species visit flowers with deep corollas, and short-tongued species visit flowers with shallow corollas (Heinrich 1976b; Stout et al. 1998). The overlap in tongue lengths of species using *I. glandulifera* in this study suggests that the amount of *I. glandulifera* was not sufficiently limiting to drive this partitioning. Alternatively, differences could be due to the size of *I. glandulifera* patches, as long-tongued bumblebees have been found to visit *I. glandulifera* more commonly when it is in smaller patches whereas short-tongued species visit more commonly when it is in larger patches (Sowig 1989). Differences between studies could also reflect the composition of the plant community; more bumblebee species would be expected to visit *I. glandulifera* if it is more rewarding than co-flowering species.

The abundance of bumblebees collected both at ground level and actively foraging was significantly higher in the presence of *I. glandulifera*. This is consistent with other studies (Lopezaraiza–Mikel et al. 2007; Bartomeus et al. 2010) but contrasts with Nienhuis et al. (2009) who did not find such an effect, possibly reflecting differences in the abundance of native plants. Unlike previous work, our study also examined the effect of the relative abundance of the invader. We found no evidence for a relationship between the abundance of *I. glandulifera* flowers and the abundance of bumblebees (either foraging or caught at ground level). This suggests that even small amounts of *I. glandulifera* flowers are sufficiently rewarding to increase local bumblebee abundance. Its nectar is replenished at a rate that is almost an order of magnitude higher than that recorded for any European bumblebee-pollinated plant species (Raine & Chittka 2007a), meaning that flowers could contain a reward even if they were recently depleted. Further work is required to distinguish between an increase in bumblebee abundance caused by the invader attracting individuals, and that caused by population increases through the provision of additional resources (Chapter six).

Previous studies have found that the presence of *I. glandulifera* increased visitation to co-flowering plants (Lopezaraiza–Mikel et al. 2007), had little or no effect (Nienhuis et al. 2009; Bartomeus et al. 2010), or decreased visitation (Thijs et al. 2012). These discrepancies may in part be a consequence of variation in *I. glandulifera*'s floral abundance. Based on the predictions of Rathcke's density-visitation model (1983), *I. glandulifera* should facilitate visitation to co-flowering plants at low floral abundance by attracting pollinators, but compete for visits at high abundance when pollinators are limiting. However, the results of this study did not support this model. The relationship between *I. glandulifera* floral abundance and visitation to co-flowering plants differed among plant species. There was no change in visitation to co-flowering *Chamerion angustifolium*, *Stachys sylvatica*, *Lamium album*, or *Symphytum x uplandicum* at any abundance of *I. glandulifera*. Also, all bumblebee species visited between four and nine plant species besides *I. glandulifera* and *Bombus hortorum*, *B. lapidarius* and *B. pratorum* did not show a significant preference for *I. glandulifera*. Other studies have also found that invasive alien species have no detectable effect on visitation to co-flowering native plants (Nienhuis et al. 2009; Bartomeus et al. 2010). Bumblebees deplete the most rewarding flowers before utilising lesser rewards (Heinrich 1976a), and so *I. glandulifera* should be highly visited. However, bumblebees tend to reduce the rewards available in different species to similar levels of profit (Heinrich 1976a). Therefore, co-flowering species may become as rewarding as *I. glandulifera* if the latter becomes depleted through high bumblebee visitation. Also, although individuals tend to remain constant to a particular plant species on a single foraging bout, they continue to sample different species in the community to enable them to track changes in floral resources (Heinrich 1979b). *Impatiens glandulifera* is thus unlikely to be visited to the exclusion of all other co-flowering species. Our result for *Stachys sylvatica* contrasts with Chittka and Schürkens (2001) who found reduced visitation to *Stachys palustris* in the presence of *I. glandulifera*, which is a similar species to *S. sylvatica*. Differences between studies could reflect variation in the abundance or spatial arrangement of *Stachys* or in the abundance of bumblebees and subsequently the amount of competition for resources.

Rathcke's density-visitation model predicts that *I. glandulifera* should facilitate visitation to co-flowering plants at low floral abundance (1983). While, in contrast to Munoz and Cavieres (2008), we did not find evidence for facilitation of visitation to co-flowering plants at low abundance of the invader, we did detect facilitation of visitation to co-flowering *Epilobium hirsutum* in the presence of *I. glandulifera*. We also found a small increase in bumblebee abundance in the pan traps at ground level in the presence of *I. glandulifera*, which could indicate facilitation if this reflects increased visitation to co-flowering plants in the under-story. Lopezaraiza-Mikel et al. (2007) also found that visitation to co-flowering plants increased in the presence of *I. glandulifera*. Particularly rewarding plant species can act as “magnet” species, whereby pollinators attracted to this species also visit less attractive co-occurring species (Laverty 1992, Johnson et al. 2003). *Impatiens glandulifera* has highly rewarding flowers (Chittka & Schürkens 2001), and our results support the theory that it may act as a magnet species (Lopezaraiza–Mikel et al. 2007), facilitating visitation to some co-flowering species, although not to others. Few studies have found invasive alien species to have facilitative effects however (Lopezaraiza–Mikel et al. 2007, Muñoz & Cavieres 2008), and fewer still report increased native plant seed set (Muñoz & Cavieres 2008). Movement between plant species can lead to substantial pollen loss, reducing seed set (Flanagan et al. 2010) and outcrossing rates (Bell et al. 2005). The receipt of alien pollen can also reduce seed set (Brown & Mitchell 2001; Matsumoto et al. 2010; but see Tscheulin et al. 2009). *Impatiens glandulifera* pollen dominated the pollen samples, regardless of the plant species on which the bumblebee was foraging. This suggests both heterospecific pollen transfer and conspecific pollen loss. *Impatiens glandulifera* pollen also dominated pollen networks in Lopezaraiza-Mikel et al.'s study (2007), who suggest that this could negate any increase in visitation caused by facilitation. However, the effect of *I. glandulifera* pollen on seed production in co-occuring plants has yet to be determined (but see Chapter four).

Most studies examining the abundance of invasive alien species do not support the predictions of Rathcke's model (1983) instead finding only competitive effects, which can be stronger at high abundance (Takakura et al. 2008; Flanagan et al. 2010, Dietzsch et al. 2011). Plants in the Lamiaceae family and purple-flowered plants (with the exception of *E. hirsutum*) experienced reduced visitation in the presence of *I. glandulifera*. Reduced visitation has important consequences for local plant populations, as it can reduce seed set (Campbell & Motten 1985), and in plants with mixed mating systems can increase selfing (Kalisz et al. 2004), which may make populations vulnerable to inbreeding depression through loss of genetic diversity (Ellstrand & Elam 1993). The relationship between floral density and visitation can depend on pollinator foraging constancy (Feldman 2008, Stout et al. 2008). The extent to which pollinators demonstrate floral constancy to the invader may therefore determine whether *I. glandulifera* exerts a competitive or facilitative effect on visitation to co-flowering species. Further studies that examine sequential pollinator visits could be used to measure floral constancy to *I. glandulifera*, and whether this changes with its abundance.

Pollinator sharing between invasive alien plants and co-flowering species can be predicted by the extent to which floral traits of symmetry and colour (in the visible spectrum) are shared (Gibson et al. 2012). This is because pollinators can use floral symmetry to distinguish between plant species (Neal et al. 1998), and determine floral rewards (Heinrich et al. 1977; Raine & Chittka 2007b) and so they are more likely to share pollinators if they share these floral traits (Memmott & Waser 2002). *Impatiens glandulifera* has pink or purple zygomorphic flowers (Beerling & Perrins 1993), sharing the same floral morphology with Lamiaceae flowers, and a similar colour with the purple-flowered plants. Thijs et al. (2012) found that the co-flowering purple-flowered species *Lythrum salicaria* which shared pollinators with *I. glandulifera* experienced reduced visitation and seed set, whereas no such effect was found in white-flowered *Alisma plantago-aquatica* and yellow-flowered *Oenothera biennis* which did not share pollinators with the invader. Plant traits may therefore be useful in predicting the impact of invasive alien plant species. *Epilobium hirsutum* exhibited a different response compared to other purple-flowered species however. It may be that a combination of factors, such as floral colour and shape, reward and plant height, contribute to the outcome of interactions.

Plant height was estimated using the mean height for a given species according to a database (Hill et al. 2004) in lieu of field measurements due to the logistics associated with the large spatial scale of this study. The drawbacks of using species' mean height are clear, as plants are expected to show large inter-individual variation and should respond to local conditions. However, they were used as proxy for field measurements in this study because time constraints prevented direct measurement, and mean values were considered useful for detecting general trends between plant height and invasion. The advantage of employing plant traits when direct field measurements are not possible is illustrated by their widespread use in other studies (e.g. Keith et al. 2009). Short plants, that is plants less than 99 cm on average, were less attractive to bumblebees in the presence of *I. glandulifera*, whereas the attractiveness of plants of medium height (an average of 100-199 cm) was unaffected. This suggests that as well as floral characteristics, plant height may also be useful in predicting the impact of invasive alien plants. In another study, short plants were displaced by tall invaders, which was suggested to indicate competition for light (Thiele *et al.* 2010). Chapter two also found that the plot plant community was characterised by taller species with increasing *I. glandulifera* abundance. This may be because taller species are more able to compete for resources such as light (Pajunen, Oksanen, & Virtanen 2011), or alternatively could indicate pollinator-mediated effects. Low light levels can reduce pollinator visitation (McKinney & Goodell 2010), which may therefore reduce visitation to short plants growing beneath *I. glandulifera*. Also, as described above, pollinators can show a preference for foraging in a horizontal plane (Waddington 1979; Makino 2008). Foraging honeybees have been found to maintain flight at a given height, as this minimises energy expenditure per unit distance compared to vertical movement (Levin & Kerster 1973). *Impatiens glandulifera* can grow up to 3 metres (Clements et al. 2008) which suggests that bumblebees foraging on *I. glandulifera* are unlikely to encounter short plants if they forage in a horizontal plane, but may still encounter plants of medium height. This supports the hypothesis that insect abundance at ground level may reflect foraging behaviour at this height.

Visitation to Lamiaceae and purple-flowered plants decreased with increasing abundance of *I. glandulifera*, although this trend was not significantly different from visitation in its presence. Other studies have found that competition from invasive alien plants was strongest when the invader was at high abundance (Flanagan et al. 2010; Dietzsch et al. 2011). At high floral abundance, there should be fewer pollinators relative to the number of flowers in the community (Rathcke 1983), and so competitive effects of invasive species may be exacerbated at high abundance. That we found the strongest effect with presence rather than abundance of *I. glandulifera* suggests (as discussed above) that *I. glandulifera* is highly attractive to bumblebees. Alternatively, a stronger relationship between abundance and impact may be detected with a larger data set to reduce noise associated with co-varying factors. A drawback of the non-linear least squares models is that by using weighted least squares to fit the model, instances where plants received none of the bumblebee visits heavily influenced the fit. Further data may have increased the accuracy of the models by reducing instances of zero visits.

3.6 Conclusion

The impact of *I. glandulifera* on the pollinator community composition varied according to its abundance and the spatial scale at which impact was assessed. This demonstrates the importance of accounting for these factors to enable comparison between studies. The response of the pollinator community to invasion also varied with taxa, making generalisations difficult and emphasises the importance of using a community-level approach to examine the effects of invaders. Bumblebee visitation showed a stronger response to the presence of *I. glandulifera* rather than to its relative abundance, suggesting that it is highly attractive. We did not find evidence to support Rathcke's density-visitation model. Instead, in the presence of the invader, bumblebee visitation to co-flowering Lamiaceae and purple-flowered plants was reduced, although one species, *E. hirsutum*, received more visits. Visitation to short plants was reduced whereas plants of medium stature were unaffected by invasion. This suggests that the traits of co-flowering plants used to assess the impact of invasive alien plants should be considered, as they may determine the outcome of the interaction. Further research is needed to determine whether the changes observed in the pollinator community composition and in bumblebee visitation alter plant reproductive success. Bumblebees visit *I. glandulifera* for its copious nectar (Titze 2000) and pollen (Kleijn & Raemakers 2008). Future research could be directed towards determining whether this supports larger populations of pollinators, and in particular whether this facilitates visitation to plants that flower outside of the invader's flowering period.

3.7 Acknowledgements

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Chapter Four:

Interspecific pollen transfer: effects of pollen from *Impatiens glandulifera* on the reproductive success of a co-flowering native plant, *Lamium album* L. (white deadnettle; Lamiaceae)

4.1 Abstract

Pollinator movement between invasive alien and native plant species may be detrimental to native plant reproduction via interspecific pollen transfer (IPT). Despite growing concern, species-specific studies into the effects of pollen from invasive alien plants are still lacking. This is the first study to experimentally address the effects of pollen from the widespread invasive alien *Impatiens glandulifera* on native plant reproduction. Its effect on seed set and seed weight of the co-flowering native *Lamium album* was assessed by hand-pollinating flowers with one of five treatments: *untreated*, where no pollen was added to the stigma (negative control); *legitimate*, where 100 % *L. album* pollen was added (positive control, which should result in maximum seed set when pollen is not limited); *foreign*, where 100 % *I. glandulifera* pollen was added; *mixed*, where both *L. album* and *I. glandulifera* pollen were added; or left *open to pollinators*, where plants were exposed to pollinating insects. In each treatment, flowers were either emasculated or non-emasculated to separate the effects of self pollen from those of IPT. Overall, hand-pollination treatments resulted in low seed set, which limits the conclusions drawn from this study. However, *I. glandulifera* pollen did not appear to prevent seed set, or affect seed weight, suggesting that there is no competition due to pollen interference between these two species. *Lamium album* could be limited by the genetic diversity or amount of pollen received, as evidenced by low seed set in hand-pollination treatments compared to the *open to pollinators* treatment. Therefore, the quantity of pollen and the diversity of pollen donors received from pollinator visitation may have a greater effect on reproductive success than IPT. Competition between *I. glandulifera* and *L. album* for pollinators may therefore result in low seed set and increased self-fertilisation, but IPT is unlikely to be a contributing factor.

4.2 Introduction

Pollinator-mediated competition between co-flowering native plants has long been considered an important evolutionary force in structuring communities, as plant should be selected for traits to avoid competition, such flowering sequentially (Robertson, 1895 in Waser 1978a). Over shorter time spans, such competition may be important in structuring communities under invasion from alien plants. Competition for pollinators is predicted to reduce plant reproductive success (Levin & Anderson 1970; Waser 1978b) via changes in pollen *quantity* or pollen *quality* (Waser 1983). The quantity of pollen may be reduced through fewer pollinator visits, whereas the quality of the pollen, that is the purity of the pollen load (*sensu* Waser 1983) varies with the amount of conspecific versus interspecific pollen deposited onto the stigma (Waser 1978a; b). Pollen quality can also refer to its genetic properties, that is, the degree of relatedness between the pollen donor and recipient, and the diversity of and intrinsic genetic properties of donors (e.g. Price & Waser 1979; Janzen et al. 1980; Aigner 2004).

Mixed pollen loads resulting from pollinator visits to competing species causes interspecific pollen transfer (IPT; also known as heterospecific pollen transfer or improper pollen transfer), altering both the quantity and the quality of the pollen received and lowering the fitness of the male (the pollen donor) and the female (pollen recipient). Pollen is wasted as pollinators move between plant species and deposit pollen on foreign stigmas or other parts of the flower (Feinsinger et al. 1988; Rademaker et al. 1997; Brown & Mitchell 2001), which reduces the chance of pollen reaching a conspecific stigma and can ultimately reduce seed set (Campbell & Motten 1985; Flanagan et al. 2009). Deposition of mixed pollen loads can reduce seed production (Brown & Mitchell 2001; Kasagi & Kudo 2005; Matsumoto et al. 2010), decreasing female fitness through ovule wastage. This occurs via a range and combination of mechanisms, which include direct physical displacement of conspecific pollen grains (Thomson et al. 1981), stigma and stylar clogging (Randall & Hilu 1990), stigma closure (Waser & Fugate 1986), or pollen allelopathy (Sukhada & Jayachandra 1980).

For IPT to occur, plants need to be in the same location, overlap in their flowering period and share pollinator species (Morales & Traveset 2008). Pollinator sharing is common in plant communities (e.g. Campbell & Motten 1985; Waser et al. 1996; Bell et al. 2005). The amount of interspecific pollen reaching the stigma is highly variable, even between individuals of a single plant species (McLernon et al. 1996), and also varies temporally (Jennersten & Kwak 1991). The degree to which IPT occurs may be due in part to local variation in pollinator abundance, and how constant pollinators are to a particular plant species. Floral constancy, which refers to foraging selectively on one flower type despite alternative rewarding flowers being available (Wells & Wells 1983; Waser 1986), was first described more than 2,000 years ago by Aristotle (see

Darwin 1876; Bennett 1883). It varies according to many factors including the pollinator species (Bennett 1883; Christy 1883; Heinrich 1979; Goulson & Cory 1993), the individual within a species (Heinrich 1976), learning and handling time involved (Chittka 2002), and plant density and spatial arrangement (Rathcke 1983; Kunin 1997).

Although pollinator sharing is a common phenomenon, pollinator movement between native and invasive alien species may be particularly detrimental. This is because avoidance of IPT may be an important selective pressure in co-evolved communities (Waser 1978b), but a lack of co-evolved mechanisms for tolerance or avoidance of alien species (Callaway & Ridenour 2004) means that alien pollen may pose a particular threat to natives. Recently, studies have focused on competition between invasive alien and native species for pollinators, and many have found a decrease in native plant reproductive success (Chittka & Schürkens 2001; Brown & Mitchell 2001; Brown et al. 2002; Kasagi & Kudo 2005; Matsumoto et al. 2010), but not always (Grabas & Laverty 1999; Moragues & Traveset 2005; Bjerknes et al. 2007). IPT may be an important mechanism of native species exclusion by invasive aliens, but relatively few have isolated the effects of IPT from confounding factors such as visitation rates (Brown & Mitchell 2001; Moragues & Traveset 2005; Nielsen et al. 2008; Matsumoto et al. 2010). Determining which invasive alien species have high or low impacts is a fundamental question in invasive species biology. However, species-specific studies are lacking, even for widespread invasive aliens.

This study examines the effect of IPT from the invasive *Impatiens glandulifera* Royle (Himalayan balsam; Balsaminaceae) on the reproductive success of the co-flowering native *Lamium album* L. (white deadnettle; Lamiaceae). *Impatiens glandulifera* is a widespread invasive species which dominates European riparian habitats (Beerling & Perrins 1993). It poses a direct threat to native plant pollination since it produces numerous large and highly rewarding flowers, successfully competing for pollinator visits and reducing native plant seed set (Chittka & Schürkens 2001). Yet to be addressed however is the specific role of IPT in affecting native plant reproductive success. Given that *I. glandulifera* pollen has been found on native plant stigmas (Lopezaraiza–Mikel et al. 2007; Neinhuis 2009; Thijs et al. 2012), that it produces large quantities of pollen (Titze 2000), and that its bumblebee pollinators can have wide foraging ranges of up to 1.5 km (Osborne et al. 2008), there is potential for widespread impact on native plant communities. *Lamium album* was selected as a focal species to explore the effect of IPT because it is expected to receive *I. glandulifera* pollen under field conditions. This is because they were found to commonly co-occur in the same habitat (Chapter two), have a large overlap in their flowering period, share generalist bumblebee pollinators (Chapter three) and have similar floral morphologies. In this study, hand-pollinations were used to test the effects of *I. glandulifera* pollen on *L. album* seed set and seed weight. Seed set was used to measure plant reproductive success. Seed weight was also used to measure plant fitness, as it can influence germination and survival (Kalisz 1989; Delgado et al. 2008).

4.3 Methods

4.3.1 Study species

Lamium album is a hemicryptophyte plant, native across temperate Eurasia and widely naturalised (Preston et al. 2002). It is found along roadsides, hedgerows and river banks (Grime et al. 2007). It grows between 0.2 and 0.6 m tall (Rose 2006), has creeping rhizomes, and produces whorls of between 6 and 16 white flowers (Macukanovic & Blazencic 1998). There are multiple whorls on each stem. It flowers between May and December (Rose 2006) or January (Fitter & Fitter 2002). Flowers are 2 cm long (Rose 2006) and produce 467 µg of sugar per 24 hours (Raine & Chittka 2007a). *Lamium album* is homogamous (Denisow & Bozek 2008) that is, stamens and pistils mature simultaneously, and there is no mechanical barrier to within-flower self pollination. However, within-flower selfing has yet to be described. Each flower produces a maximum of four seeds, which have a large white elaiosome that may be used for myrmecochory (Daskalova 2007). *Impatiens glandulifera* is described in Chapter one.

4.3.2 Pollination experiment

A pilot study to assess pollen viability in both *L. album* and *I. glandulifera* and stigma receptivity in *L. album* is detailed in Appendix C. *Lamium album* pollen viability declined after 20 minutes and was non-viable after 140 minutes, whereas *I. glandulifera* remained viable for up to eight hours. *Lamium album* stigma receptivity could not be determined, despite extensive tests (Appendix C). *Lamium album* plants were collected from a single wild population in September 2010 and grown in a glasshouse in compost in four litre pots until the experiment in August 2011. Plants were cut back four weeks prior to the experiment to encourage flowering. An additional 26 plants were taken from a second wild population, 17 km away from the first, in May 2011 to be used as pollen donors. These were also kept in the glasshouse in compost in four litre pots. This was to ensure that the donor population was unrelated to the recipients. Thirty *I. glandulifera* plants were collected from a single wild population in July 2011, grown in compost in 10 litre pots and kept outside to be used as pollen donors.

Lamium album plants were each randomly assigned to one of five treatments (Figure 4.1, Table 4.1): *untreated*, where no pollen was added to the stigma (negative control); *legitimate*, where 100 % *L. album* pollen was added (positive control, which should result in maximum seed set when pollen is not limited); *foreign*, where 100 % *I. glandulifera* pollen was added; *mixed*, where both *L. album* and *I. glandulifera* pollen was added (such that this was double the total volume of pollen compared to *legitimate* and *foreign* treatments); or *open to pollinators* where flowers were exposed to pollinating insects (control to put results from the experiment into context). The order in which treatments were carried out was randomised, within four blocks (hereafter referred to as “treatment block”, used as an equivalent to treatment date). This was to avoid introducing bias associated with plant phenology over the duration of the experiment, and with improvement in hand-pollination technique. Each treatment block was made up of five random plants from each hand-pollination treatment described. To separate the effects of self pollen from the effects of IPT, in each treatment described flowers were either emasculated (anthers removed prior to dehiscence) or non-emasculated (anthers left intact) (Table 4.1). Flowers were emasculated by temporarily folding back the petals of mature buds, unfurling the stamens and stigma, and removing the undehisced anthers using fine forceps. Emasculation was done at this stage to remove anthers before dehiscence (Appendix C).

Figure 4.1: Schematic representation of experimental hand-pollination treatments on *Lamium album* plants; Untreated, Legitimate, Foreign, Mixed, and Open to pollinators. Filled squares represent *L. album* pollen, and open squares represent *Impatiens glandulifera* pollen. Plants were covered with mesh bags to exclude pollinators, apart from plants receiving the open to pollinators treatment.

Untreated	**Legitimate**	**Foreign**	**Mixed**	**Open to pollinators**
				
	■■■■■	□□□□□	■■■■■ □□□□□	
No pollen added to the stigma (negative control)	*Lamium album* pollen added to the stigma (positive control)	*Impatiens glandulifera* pollen added to the stigma	*Lamium album* and *I. glandulifera* pollen added in equal quantities	Flowers exposed to pollinating insects

Table 4.1: Final numbers of plants used in each hand-pollination treatment: *Untreated* = no pollen; *Legitimate* = 100 % *L. album* pollen; *Foreign* = 100 % *Impatiens glandulifera* pollen; *Mixed* = *L. album* and *I. glandulifera* pollen; and *Open to pollinators* = flowers exposed to pollinating insects. Flowers were either emasculated (anthers removed) or non-emasculated (anthers intact) to separate effects of self pollen from the effects of interspecific pollen transfer.

	Untreated	**Legitimate**	**Foreign**	**Mixed**	**Open to pollinators**
All experimental flowers on plant **emasculated**	20	19	20	20	19
All experimental flowers on plant **non-emasculated**	9	10	10	10	10
Total plants	29	29	30	30	29

A total of seven flowers were selected on each plant, which represents a small proportion of the total number of flowers produced per plant. On each plant, five flowers all received the same pollination treatment, and were all first either emasculated or left non-emasculated (Table 4.1). Thus, except for these control flowers, all experimental flowers received the same treatment on each plant. The remaining two flowers were within-plant controls – no pollen was added, but one was emasculated and the other was non-emasculated (essentially additional *untreated* flowers). Entire plants were covered with mesh bags to exclude pollinators (Figure 4.2 a), except for the *open to pollinators* treatment (Figure 4.2 b), and were kept in the glasshouse for the duration of the experiment. Doors to the glasshouse were left open to allow pollinators access to the *open to pollinators* treatment plants. Pollinating insects, largely bumblebees and hoverflies, were observed visiting the plants inside glasshouse. Plants were positioned on the glasshouse bench in a random order.

Hand-pollinations were carried out over four weeks beginning 1st August 2011. To ensure that all experimental flowers were at the same developmental stage, mature buds were selected on the treatment plant and colour marked on the sepals using entomology marking paint (Figure 4.2 c). Flowers were then emasculated (methodology described above). Buds were left for a day to open naturally before pollen was added to the stigma. In *legitimate* treatments, a pollen donor plant was randomly selected and two anthers from a mature bud were removed with fine forceps and put onto a petri dish (pollen was viable – see Appendix C for details). Pollen was removed using a wooden toothpick, mixed on the dish, and put onto the recipient stigma using x10 magnifying glasses. The volume of pollen was such that it thickly coated the entire stigma surface. The volume of *L. album* pollen was standardised by using pollen from two anthers for each treatment. The volume of *I. glandulifera* pollen was estimated to equal the volume of *L. album* pollen. It was not possible to weigh the pollen since time was constrained by the short period of viability of *L. album* pollen, and by the large number of hand-pollinations to carry out. In *foreign* treatments, anthers were collected from a mature *I. glandulifera* bud from each donor plant and put into glass vials for use within eight hours (pollen was viable – see Appendix C for details). The pollen donor plant was randomly selected, and pollen was applied to the recipient stigma using a wooden toothpick. In *mixed* treatments, equal volumes of legitimate and foreign pollen were mixed on a petri dish before application onto the stigma. Since *L. album* stigma receptivity could not be determined (Appendix C), application of pollen was repeated after 24 hours to increase the chance of successful pollination. Of the 1050 flowers in the experiment, 998 were recovered, with the remainder lost due to damage to the stems or growth of mould. The number of mature seeds per flower were recorded over a four week period. Seeds were collected when almost mature (when seeds changed from green to light brown) as they fall out from the sepals when fully mature; and were weighed when air dried (indicated by turning dark brown).

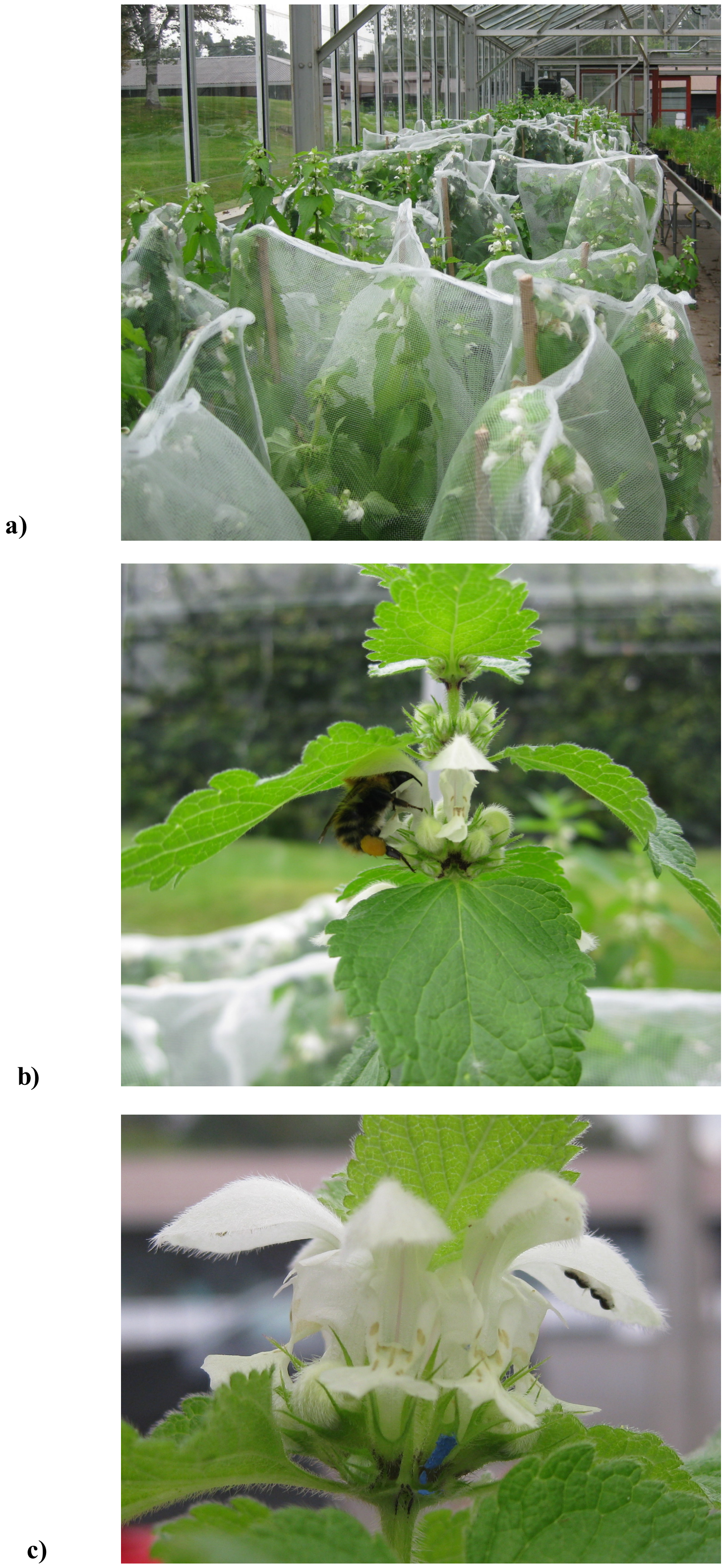

Figure 4.2: **a)** Experimental set up showing mesh bags to exclude pollinators; **b)** *Open to pollinators* treatment visited by *Bombus pascuorum*; **c)** Flowers receiving treatments were colour marked. Blue-marked flower shown has been emasculated.

4.3.3 Statistical analysis

Analysis was done in R v2.10.1 (R Development Core Team 2011). Variation in seed set and seed weight between treatments was tested using generalised linear mixed models (GLMMs) (lme4 package v0.999375-35, Bates et al. 2011). Each plant was treated as the unit of replication. A Binomial error distribution was used for seed number, since the number of seeds produced per flower is a maximum of four. Therefore, the model treated the number of seeds as the number of successes in four independent Bernoulli trials. A Gaussian error distribution was used for seed weight, and means were weighted by the number of seeds per plant. Treatment, emasculation, and their interaction were treated as fixed effects, and plant and treatment block (equivalent to treatment date) were treated as random effects. Minimum adequate models were obtained (Crawley 2002) using lowest AIC values found by removing non-significant terms in a backwards stepwise manner. Multiple comparisons between treatments were assessed using a post-hoc Tukey's HSD test on the model results (multcomp package, Hothorn et al. 2008).

4.4 Results

Final numbers of flowers used in the treatments are shown in Table 4.2. The effect of treatment block (equivalent to treatment date) was not significant overall (ANOVA, $F_{143} = 2.209$, $p = 0.112$), but treatment block 2 was significantly different from all other treatments ($p = 0.025$) (Figure 4.3) so was retained as a random effect in the model. This may reflect phenological differences. The final numbers of flowers in each treatment block were 328, 330, 167 and 173 in blocks 1-4 respectively.

Table 4.2: Number of *Lamium album* flowers recovered from hand-pollination treatments: *Untreated* = no pollen; *Legitimate* = 100 % *L. album* pollen; *Foreign* = 100 % *Impatiens glandulifera* pollen; *Mixed* = *L. album* and *I. glandulifera* pollen; and *Open to pollinators* = flowers exposed to pollinating insects. Flowers were either emasculated or non-emasculated. N = 998 flowers.

Treatment	**Number of flowers**	
	Emasculated	**Non-emasculated**
Untreated	206	156
Legitimate	95	45
Foreign	96	49
Mixed	100	48
Open to pollinators	124	79
Total	621	377

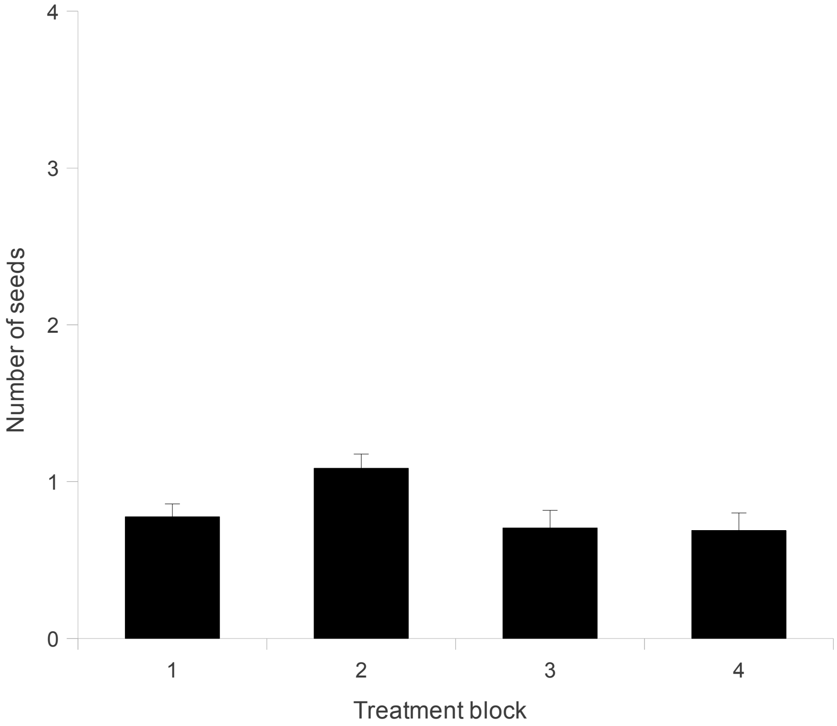

Figure 4.3: The effect of treatment blocks 1-4 (equivalent to treatment date), on the mean number of seeds produced by *Lamium album* flowers (maximum of 4 per flower) following hand-pollination treatments. Values are means + SE. N = 328, 330, 167, 173 flowers in treatment blocks 1-4 respectively.

4.4.1 Effect of foreign pollen on seed set

The number of plants that produced seed is shown in Table 4.4. Flowers that were non-emasculated had significantly higher seed set than emasculated flowers overall ($\chi^2_{(12, N=998)}$ = -835.34, $p < 0.001$ (Figure 4.4, Table 4.3). Flowers left *open to pollinators* had the highest seed set of all treatments (Figure 4.4, Table 4.3). Seed set in all other treatments was low, and in emasculated treatments, were not significantly different from each other (with the exception of the *open to pollinators* treatment). *Foreign* pollen resulted in the lowest seed set in emasculated treatments, although zero seed was expected in this control treatment. Of the non-emasculated treatments, the *untreated* flowers had the lowest seed set. The addition of foreign pollen, when both pure and mixed with legitimate pollen, resulted in similar numbers of seeds compared to the other treatments (except the *open to pollinators* treatment), and did not prevent seed set (Figure 4.4, Table 4.3).

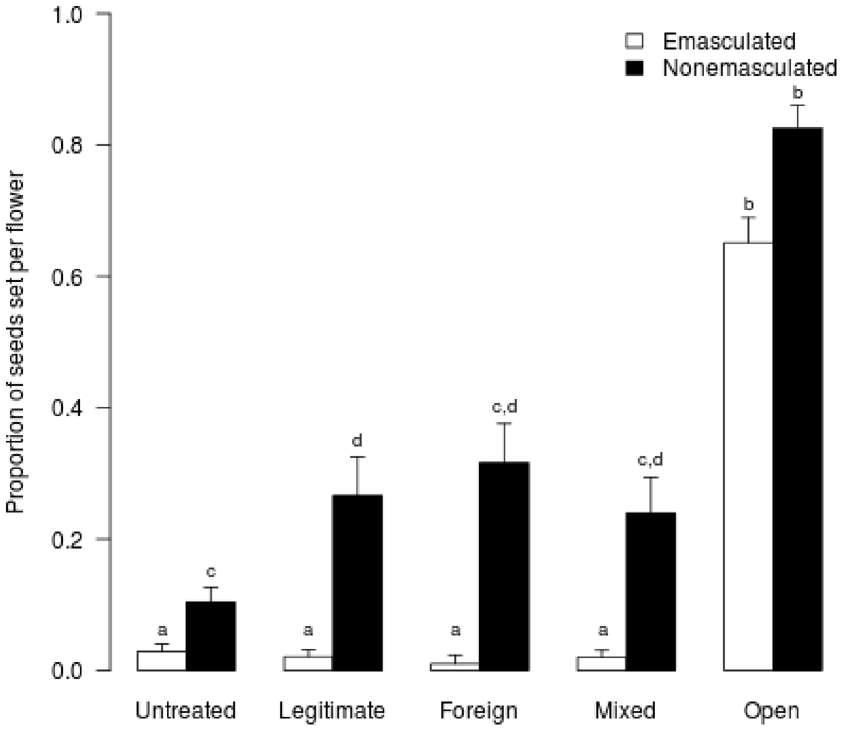

Figure 4.4: Proportion of seeds set (maximum of 4 seeds) per *Lamium album* flower in emasculated (N = 621) and non-emasculated (N = 377) flowers following hand-pollination treatments: *Untreated* = no pollen; *Legitimate* = 100 % *L. album* pollen; *Foreign* = 100 % *Impatiens glandulifera* pollen; *Mixed* = *L. album* and *I. glandulifera* pollen; *Open* = flowers exposed to pollinating insects. Values are means + SE. Significant differences between bars were determined from a post-hoc Tukey's HSD test. *Open to pollinators* treatments had the highest seed set, and non-emasculated treatments produced more seed than non-emasculated treatments. Hand-pollination treatments resulted in very low seed set.

Table 4.3: Analysis of number of seeds produced after *Lamium album* flowers received hand-pollination treatments: *Untreated* = no pollen; *Legitimate* = 100 % *L. album* pollen; *Foreign* = 100 % *Impatiens glandulifera* pollen; *Mixed* = *L. album* and *I. glandulifera* pollen; and *Open to pollinators* = flowers exposed to pollinating insects. Flowers were either emasculated (e) or non-emasculated (ne). Generalised linear mixed models were used according to a Binomial error distribution. Fixed effects were treatment and its interaction with emasculation. Treatment block and plant were treated as random effects. *P* values were derived from a post-hoc Tukey's HSD test. Non-significant comparisons are not reported.

Treatment 1	**Treatment 2**	**Estimate**	**SE**	***z***	***p***
Untreated e	Open to pollinators e	-5.416	0.445	-12.179	<0.001
Untreated e	Untreated ne	-1.372	0.293	-4.684	<0.001
Untreated e	Legitimate ne	-2.809	0.482	-5.827	<0.001
Untreated e	Foreign ne	-2.349	0.425	-5.520	<0.001
Untreated e	Mixed ne	-2.318	0.467	-4.969	<0.001
Untreated e	Open to pollinators ne	-6.132	0.467	-13.130	<0.001
Untreated ne	Legitimate e	-1.941	0.502	-3.867	0.004
Untreated ne	Foreign e	2.380	0.665	3.578	0.011
Untreated ne	Mixed e	2.598	0.480	5.416	0.001
Untreated ne	Open to pollinators e	-4.004	0.407	-9.940	<0.001
Untreated ne	Legitimate ne	-1.437	0.440	-3.264	0.031
Untreated ne	Open to pollinators ne	-4.760	0.431	-11.044	<0.001
Legitimate e	Open to pollinators e	-5.985	0.605	-9.887	<0.001
Legitimate e	Legitimate ne	-3.378	0.644	-5.246	<0.001
Legitimate e	Mixed ne	-2.887	0.639	-4.517	<0.001
Legitimate e	Open to pollinators ne	-6.701	0.622	-10.773	<0.001
Legitimate ne	Open to pollinators e	-2.607	0.545	-4.784	<0.001
Legitimate ne	Foreign ne	3.817	0.776	4.918	<0.001
Legitimate ne	Mixed ne	4.035	0.628	6.422	<0.001
Legitimate ne	Open to pollinators ne	-3.323	0.563	-5.903	<0.001
Foreign e	Open to pollinators e	-6.424	0.743	-8.650	<0.001
Foreign e	Foreign ne	-3.357	0.749	-4.483	<0.001
Foreign e	Mixed ne	-3.327	0.773	-4.306	<0.001
Foreign e	Open to pollinators ne	-7.140	0.756	-9.440	<0.001
Foreign ne	Open to pollinators e	-3.067	0.513	-5.980	<0.001
Foreign ne	Mixed ne	3.574	0.592	6.034	<0.001
Foreign ne	Open to pollinators ne	-3.783	0.532	-7.110	<0.001
Equal e	Open to pollinators e	-6.642	0.592	-11.220	<0.001
Equal e	Mixed ne	-3.544	0.623	-5.689	<0.001
Equal e	Open to pollinators ne	-7.357	0.609	-12.083	<0.001
Equal ne	Open to pollinators e	-3.098	0.542	-5.717	<0.001
Equal ne	Open to pollinators ne	-3.814	0.560	-6.811	<0.001

4.4.2 Effect of foreign pollen on seed weight

The number of seeds weighed from each treatment is shown in Table 4.4. The effect of treatment block was not significant (ANOVA, $F_{103} = 0.009$, $p = 0.923$) (Table 4.5, Figure 4.5), however, it was retained as a random effect in the model for consistency with models testing effects of treatment on seed number.

There was no difference overall in seed weight between emasculated and non-emasculated treatments (ANOVA, $F_1 = 1.114$, $p = 0.294$); that is pollination by self-pollen or outcross pollen did not impact on seed weight. Seed weight varied significantly with pollination treatment (ANOVA, $F_4 = 5.496$, $p = 0.001$) (Table 4.5, Figure 4.6), although the effects were small. In emasculated treatments, foreign pollen resulted in the lowest seed weight, although the sample size was very small (two plants, six seeds) (Table 4.4, Figure 4.6). The relationship between seed weight and germination success could not be tested because seeds became mouldy.

Table 4.4: Number of *Lamium album* plants that produced seeds, and the number of these seeds collected following pollination treatments: *Untreated* = no pollen; *Legitimate* = 100 % *L. album* pollen; *Foreign* = 100 % *Impatiens glandulifera* pollen; *Mixed* = *L. album* and *I. glandulifera* pollen; and *Open to pollinators* = flowers exposed to pollinating insects. Flowers were either emasculated or non-emasculated.

Treatment	**No. of plants that produced seeds**		**Total no. of seeds weighed**	
	Emasculated	**Non-emasculated**	**Emasculated**	**Non-emasculated**
Untreated	5	18	10	48
Legitimate	3	8	8	35
Foreign	2	8	6	53
Mixed	4	5	9	33
Open to pollinators	28	25	225	233
Total	38	64	258	402

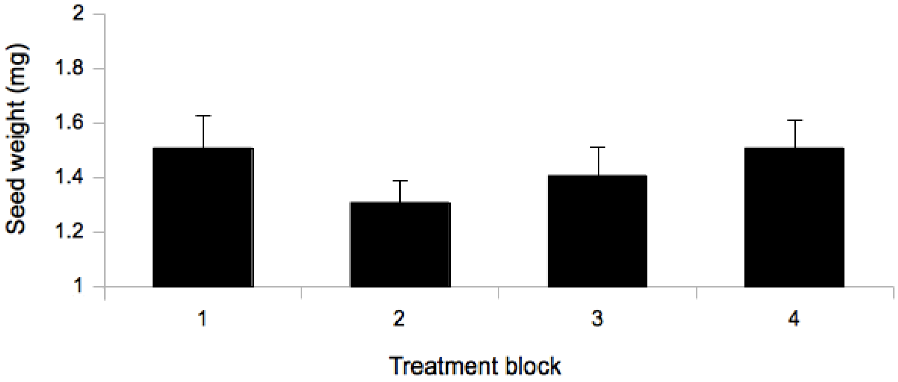

Figure 4.5: The effect of treatment blocks 1-4 (equivalent to treatment date), on seed weight (mg) produced by *Lamium album* flowers following hand-pollination treatments. Values are means + SE. N = 31, 46, 14, 14 seeds weighed in treatment blocks 1-4 respectively.

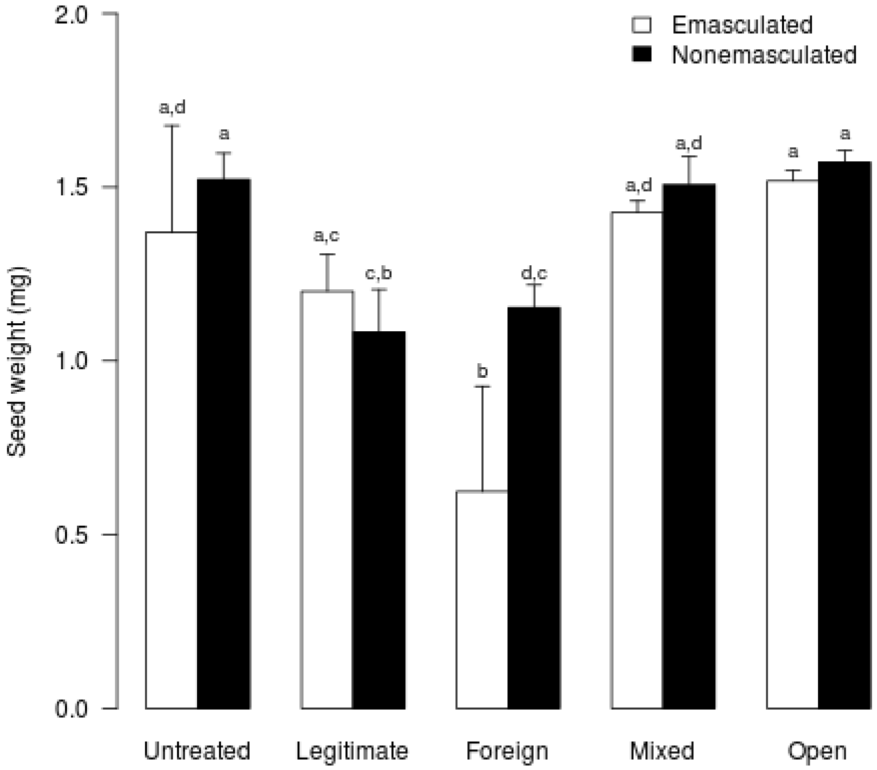

Figure 4.6: Seed weight (mg) produced by emasculated (N = 42) and non-emasculated (N = 64) *Lamium album* flowers following hand-pollination treatments: *Untreated* = no pollen; *Legitimate* = 100 % *L. album* pollen; *Foreign* = 100 % *Impatiens glandulifera* pollen; *Mixed* = *L. album* and *I. glandulifera* pollen; *Open* = flowers exposed to pollinating insects. Values are means + SE. Significant differences between bars were determined from a post-hoc Tukey's HSD test. There was little effect of treatment on seed weight, and there was no significant difference between emasculated and non-emasculated treatments.

Table 4.5: Analysis of seed weight produced after *Lamium album* flowers received hand-pollination treatments: *Untreated* = no pollen; *Legitimate* = 100 % *L. album* pollen; *Foreign* = 100 % *Impatiens glandulifera* pollen; *Mixed* = *L. album* and *I. glandulifera* pollen; and *Open to pollinators* = flowers exposed to pollinating insects. Flowers were either emasculated (e) or non-emasculated (ne). Generalised linear mixed effects models (GLMMs) were used according to a Guassian error distribution. Fixed effects were treatment and its interaction with emasculation. Treatment block and plant were treated as random factors. *P* values were derived from a post-hoc Tukey's HSD test. Non-significant comparisons are not reported.

Treatment 1	**Treatment 2**	**Estimate**	**SE**	***z***	***p***
Untreated e	Foreign e	1.134	0.215	5.280	<0.001
Untreated e	Legitimate ne	0.764	0.152	5.025	<0.001
Untreated ne	Foreign e	0.980	0.177	5.549	<0.001
Untreated ne	Legitimate ne	0.611	0.091	6.744	<0.001
Untreated ne	Foreign ne	0.270	0.082	3.315	0.024
Legitimate e	Foreign e	0.711	0.220	3.226	0.032
Legitimate ne	Mixed e	-0.641	0.155	-4.137	<0.01
Legitimate ne	Open to pollinators e	-0.647	0.075	-8.600	<0.001
Legitimate ne	Mixed ne	-0.580	0.099	5.869	<0.001
Legitimate ne	Open to pollinators ne	-0.762	0.074	-10.320	<0.001
Foreign e	Open to pollinators e	-1.017	0.168	-6.040	<0.001
Foreign e	Foreign ne	-0.710	0.176	-4.047	<0.001
Foreign e	Open to pollinators ne	-1.131	0.169	-6.712	<0.001
Foreign ne	Open to pollinators e	-0.306	0.064	-4.808	<0.001
Foreign ne	Open to pollinators ne	-0.421	0.062	6.763	<0.001
Mixed e	Foreign e	1.011	0.216	4.679	<0.001
Mixed ne	Foreign e	0.950	0.181	5.256	<0.001

4.5 Discussion

IPT from *Impatiens glandulifera* did not appear to prevent seed set in *Lamium album* and there was little effect on seed weight. This suggests that there is no interspecific competition due to pollen interference. Seed set was very low in hand-pollination treatments compared to flowers left open to pollinators, which limits the conclusions that can be drawn from this study. Low seed set may be due to a wide range of variables, such as mis-timed pollen applications (since stigma receptivity could not be determined), or a consequence of the amount of pollen received or its genetic diversity. Despite being self-compatible, seed set was higher when insect-pollinated and few seeds were produced via autonomous selfing. This dependence on pollinators for high seed set suggests that in the event of pollinator-mediated competition with *I. glandulifera*, changes in pollinator visitation frequency or behaviour may reduce seed set and increase selfing in *L. album*, which could lead to decreased fitness, but IPT is unlikely to have a substantial effect.

4.5.1 Effect of foreign pollen on *L. album* reproductive success

Foreign pollen, either when pure or mixed with legitimate pollen, did not prevent seed set in *L. album* and resulted in similar numbers of seeds as the other treatments (except for the *open to pollinators* treatments). In emasculated treatments, foreign pollen resulted in the lowest seed set and the lowest seed weight. Zero seed set was expected since this was a control treatment, receiving no *L. album* pollen. The few seeds produced in this treatment, and in the emasculated *untreated* treatment which was also a control, may have been via selfing within the bud prior to anther removal, or contamination from other flowers on the same plant. Where *L. album* pollen was present, either as self pollen from within the flower or as outcross pollen from a donor, seed set occurred despite addition of foreign pollen. This suggests that under field conditions, IPT from *I. glandulifera* is unlikely to lower reproductive success in *L. album*.

The effect of IPT on seed set varies between studies. IPT between co-flowering native plants can reduce seed set (Waser & Fugate 1986) or have no detectable effect (Armbruster & McGuire 1991; Kwak & Jennersten 1991). In particular, IPT from invasive alien plants can reduce seed set in native plants (Sukhada & Jayachandra 1980; Brown & Mitchell 2001; Nielsen et al. 2008; Matsumoto et al. 2010) or have no effect (Moragues & Traveset 2005; Tscheulin et al. 2009). Differences between studies may be due to the order in which conspecific and interspecific pollen is added. In one study, interspecific pollen applied prior to conspecific pollen reduced seed set more than simultaneous mixed pollination due to stigma closure when interspecific pollen was received (Waser & Fugate 1986). Future work could examine the effect of the order in which *I. glandulifera* and *L. album* pollen is applied to the stigma. There is also considerable variation between years of study (Moragues & Traveset 2005; Larson et al. 2006) and between populations (Moragues & Traveset 2005), making generalisations and predictions difficult. Differences may also be attributable to the plant species involved (Morales & Traveset 2008). Therefore studies such as this which target interactions between specific plant species are important in examining the highly variable impacts of invasive aliens.

This study determined that *L. album* is self compatible, as seed set occurred in the non-emasculated treatments in which only self pollen was available. Non-emasculated treatments produced significantly more seeds than emasculated treatments overall, showing the importance of within-flower self pollen as part of the species' reproductive strategy. In partially selfing species, selfing can be important for reproductive assurance when pollinators are lacking (Kalisz et al. 2004). In *L. album* such selfing produced low numbers of seeds however. It is unlikely that this reflects that conditions were not optimal in the glasshouse because the open to pollinators treatment (which experienced the same conditions) had high seed set. Instead, low seed set produced in the absence of pollinators could indicate that pollinators are necessary for high seed set. Some plants rely on pollinators for reproduction even when they are self compatible or self pollinated (Kwak & Jennersten 1991; Iponga 2010), and pollinators may be necessary for maximum seed set when plants are self compatible but have low autofertility (Karrenberg & Jensen 2000). Despite the benefits of reproductive assurance, selfing can reduce genotypic diversity (e.g. Lo et al. 2010) and can result in inbreeding depression (the reduction of fitness of inbred relative to outcrossed progeny) (Charlesworth & Charlesworth 1987). Multiple pollinator visits may be an adaptation for promoting outcrossing, since pollen deposited during different visits may originate from different donors (Kwak & Jennersten 1991). *Lamium album* may be vulnerable to inbreeding depression if competition with *I. glandulifera* reduces the frequency of pollinator visits. Although inbreeding depression is likely to be most severe for plants that are exclusively outcrossing, it is possible that substantial inbreeding depression occurs in species that are partially selfing (Charlesworth & Charlesworth 1987). In this study, selfing did not affect seed weight. Given that plant fitness can be assessed using seed weight as a predictor of germination and survival (Kalisz 1989), it is possible that selfing may not affect seed fitness in *L. album*, but this requires further investigation. The relationship between seed weight and germination success could not be measured since seeds became mouldy. In future investigations, it is recommended that seeds are germinated immediately after harvesting from the plant, or treated with a fungicide. Chapter five found that germination success was low for *L. album*, particularly in seeds produced in the summer compared to those in spring, although the factors determining this are unknown.

Low seed set in all treatments, apart from the *open to pollinators* treatment, limit our conclusions on the effects of *I. glandulifera* pollen on *L. album* reproduction. Low seed set could have been caused by mistimed pollen applications since it was not possible to determine the timing of the stigma's receptivity (Appendix C); or could indicate that the treatment itself was detrimental (Bierzychudek 1981) perhaps through damage to the stigma. The pollinator exclusion bags may also have contributed to the low seed set. *Lamium album* produces large quantities of nectar (Raine & Chittka 2007a), which remained in the calyx after flowers had withered when pollinators were excluded. The formation of mould as a result is likely to have hindered seed formation, and may have been exacerbated by the exclusion bags. This study would benefit from further work under field conditions, where pollinator visitation and subsequent seed set could be monitored in mixed patches of *L. album* and *I. glandulifera*. By using pollinators to transfer interspecific pollen rather than using hand applications, some difficulties incurred in this study may be avoided.

Low seed set in the hand-pollination treatments could alternatively reflect a low genetic diversity of pollen donors used in the study, which were collected from a single population. Pollen donors can differ in their vigour and fertilising ability (Stone Bookman 1984), and a low diversity of pollen can reduce reproductive success in hand-pollination experiments compared to natural pollination (Vander Kloet & Tosh 1984).

However, under field conditions, plants commonly receive pollen from near neighbours (Gonzales et al. 2006). They may therefore commonly receive pollen of low genetic diversity (depending on the relatedness of the neighbouring plants – see Chapter five), although long distance pollen dispersal events are also possible (see Chapter five).

Outbreeding depression, caused by mating between genetically dissimilar individuals, reduces progeny fitness but has received little attention compared to inbreeding depression (Edmands 2007). In some species, such as *Ipomopsis aggregata*, outbreeding depression has been detected over distances as little as 100 metres (Waser et al. 2000). Outbreeding depression has yet to be studied in *L. album*, and it is not known whether the low seed set in this study is attributable to genetic differentiation between donor and recipient populations. The extent of outbreeding depression in *L. album* is expected to depend on the spatial scale of gene dispersal, because this will determine local adaptation and the formation of genetically distinct populations. Chapter five demonstrated that beyond a scale of 3 metres, *L. album* showed no spatial genetic structuring and plants were no more related than if they were randomly distributed. This suggests that high levels of genetic mixing occur naturally within a population, and local adaptation is unlikely, so pollen from donors 17 km away may not result in outbreeding depression.

Low seed set could have been the result of selective abortion of seeds from treatment flowers. Seed abortion regulates the quality of offspring (e.g. Stephenson & Winsor 1986; Shi et al. 2005), and increases resources available for higher quality offspring (Darwin 1876). Selective seed abortion can be reduced by the addition of nutrients available to maternal plants (Shi et al. 2005), but not always (Melser & Klinkhamer 2001). Maternal resources were unlikely to be lacking in this study since plants were grown in compost and watered regularly. The large number of flowers on *L. album* plants may have enabled selective seed abortion however. According to the "wider choice model", plants with an "excess" of flowers are predicted to selectively mature seeds of high quality since there are more to choose from (Burd 1998). Thus, in future studies, removing non-treatment flowers could reduce seed abortion, provided that treatment flowers produce seed even if they are of low quality.

4.6 Conclusions

The true extent of the effect of *I. glandulifera* pollen on the reproductive success of the native *L. album* is difficult to determine from this study due to low seed set. However, IPT did not appear to prevent seed set in *L. album* suggesting that there is no competition due to pollen interference between these two species. Despite evidence that, in general, interspecific pollen is found on native plant stigmas (Bjerknes et al. 2007), if it does not affect reproductive success, it should not impact on plant fitness or its evolution. *Lamium album* could be limited by the amount or genetic diversity of pollen received. If there is competition between *I. glandulifera* and *L. album* for pollinator visitation, this may result in low seed set through reduced pollen quantity and genetic diversity, and increase self-fertilisation. This has implications for plant fitness as well as the mating system, population genetic diversity and evolutionary potential. *Impatiens glandulifera* has become widespread across Europe, and is predicted to continue its spread (Wadsworth et al. 2000). The influence of IPT from *I. glandulifera* may be species specific, and so it is recommended that other native plants should be targeted in future research.

4.7 Acknowledgements

Thanks to Julia Wilson for her help and advice in pollinating and growing *Lamium album*, and in providing the plants.

Chapter Five:

The effect of pollinator-mediated competition from *Impatiens glandulifera* on the mating system of *Lamium album*

5.1 Abstract

Competition between native and invasive alien plants for pollinators can be detrimental to native plant reproduction via changes in the quantity or purity of pollen received. Yet to be addressed however is whether invasive alien plants also influence the genetic quality of pollen received by native plants, by altering the foraging movements of shared pollinators. Here, we tested the hypothesis that the widespread invasive alien *Impatiens glandulifera* interrupts the mating system and reproductive success of the self-compatible co-flowering native *Lamium album*. In sympatric populations of *I. glandulifera* and *L. album*, we established two field plots. Prior to its flowering, the invader was removed from one plot in early summer. Progeny from *L. album* plants were sampled from each plot during the spring ("spring population", prior to the invader's flowering period - a within-plot control) and summer ("summer population", during the invader's flowering period). *Lamium album* progeny from each population were genotyped using six microsatellite markers to estimate the mating system parameters of multi-locus outcrossing rate, biparental inbreeding and correlated paternity. *Lamium album* plants co-flowering with *I. glandulifera* in summer showed a significant 38 % reduction in seed set compared to the spring population in the same plot. A trend towards altered mating system was evident in this summer population, with reduced outcrossing rates, an increase in the number of pollen donors that contributed to outcrossing events, and increased biparental inbreeding rates. Although not significant individually, together these effects indicate that *I. glandulifera* disrupts the mating system of *L. album*. This study demonstrates for the first time the potential for alien plants to disrupt native plant mating systems, with implications for individual plant fitness and local population genetic structure.

5.2 Introduction

Pollinator sharing is common in plant communities (e.g. Campbell & Motten 1985; Waser et al. 1996; Bell et al. 2005) but invasive alien plants can be particularly strong competitors, posing a threat to co-flowering native species. Although most introduced alien plants do not establish (Williamson 1996), some do establish and become invasive, occurring at high abundance in the community (Flanagan et al. 2010). Some invasive alien species occur at high abundance and have large floral displays (Bjerknes et al.

2007) which can make them highly attractive to pollinators, and they can disrupt native plant-pollinator interactions (Lopezaraiza–Mikel et al. 2007). Most studies to date have investigated pollinator-mediated effects of invasive alien species on native plant reproductive success by examining changes in the frequency of pollinator visits (Brown et al. 2002; Ghazoul 2004; Tscheulin et al. 2009); or changes in pollen quality (the purity of the pollen load, *sensu* Waser (1983)) by examining the effects of interspecific pollen transfer (Brown & Mitchell 2001; Matsumoto et al. 2010). How such competition may affect the genetic quality of pollen received, by altering patterns of insect-mediated pollen dispersal, is not well understood however. Plant outcrossing potential is predicted to decrease with pollinator sharing because of pollen wastage, i.e. pollen deposition on heterospecific flowers (Campbell 1985). Whilst little-studied to date, competition between native plants has been found to decrease outcrossing rates because pollen wastage reduced the diversity of pollen donors received (Bell et al. 2005). This effect has yet to be considered at all in addressing the impact of invasive alien plants on native plants.

Competition for pollinators may be a strong selective pressure favouring selfing in plants with mixed mating strategies (Fishman & Wyatt 1999). Most plants reproduce according to a 'mixed-mating' system (Vogler & Kalisz 2001) where a proportion of progeny are derived from self-fertilisation and the remainder from outcrossing. Selfing rates can increase as a direct response to the absence of pollinators (Kalisz et al. 2004). Some invasive alien species can be highly attractive to pollinators (Chittka & Schürkens 2001), which suggests they may exert a particularly strong selective pressure for selfing in competing native species. Selfing may also be important in providing reproductive assurance against the receipt of interspecific pollen in species where this leads to reduced seed set (Fishman & Wyatt 1999). This may be particularly relevant in the context of invasive alien species, as alien pollen can reduce seed set in some native species (Kasagi & Kudo 2005; Matsumoto et al. 2010). However, the loss of genotypic diversity caused by selfing (e.g. Lo *et al.* 2010) can result in inbreeding depression (the reduction of fitness of inbred relative to outcrossed progeny), because deleterious mutations are more likely to be expressed or because of heterozygote advantage (Charlesworth & Charlesworth 1987). Inbreeding depression can manifest as reduced fruit or seed set (Schemske 1983; Husband & Schemske 1997; Donohue 1998; Kruszewski & Galloway 2006), progeny growth (Breed et al. 2012a), biomass

(Holtsford & Ellstrand 1990), germination (Donohue 1998) and survival (Husband & Schemske 1997). Selfing poses a threat to the ability of species to adapt to environmental change, and for self-incompatible plants, reduces the availability of compatible mates (reviewed in Ellstrand & Elam 1993). Disruption to plant mating systems caused by invasive alien plants could in theory alter local plant population demographics and gene flow. This has implications for the persistence of local populations, as well as having evolutionary significance for mating systems (reviewed in Eckert *et al.* 2010).

Disruption to native plant-pollinator interactions by invasive alien species has the potential to alter the distance that native pollen is dispersed, with implications for the diversity of pollen donors and the relatedness of pollen received. This is because it is predicted that some invasive alien plants could attract pollinators with broad foraging ranges over wide distances (Lopezaraiza–Mikel et al. 2007). This could have positive effects of some long-distance outcrossing events for native species, if there is interspecific movement between invasive alien and native species. Pollen dispersal distance may vary according to pollinator species due to different levels of pollen carry-over (Campbell 1985) and their flight capabilities. Bumblebee-mediated pollen dispersal is predicted to occur over large distances (Chapman et al. 2003) because they have wide foraging ranges (Osborne et al. 2008; Carvell et al. 2012). However, although many pollinators have the potential to promote outcrossing over long distances, they often do not travel the full distance they are capable of and show local site fidelity (Heinrich 1976a; Waddington 1983). Pollen movement between populations can be highly restricted, with most pollen received from near neighbours (Gonzales et al. 2006). The genetic quality of pollen received will depend on the interaction between pollinator foraging patterns and the spatial genetic structure of the native plant population (Price & Waser 1979; Aigner 2004). Spatial genetic structuring, that is the nonrandom distribution of genotypes, depends on a combination of factors including plant mating system (whether outcrossing, mixed mating or selfing), life form (whether annual or perennial), seed dispersal mechanism (such as whether gravity or wind dispersed) (Hamrick & Godt 1989, 1996), and pollinator foraging behaviour. The latter can vary according to a wide range of factors such as the distribution of floral resources (Schulke & Waser 2001) and floral constancy (reviewed in Waser 1986).

To our knowledge, no other study has explored the effects of pollinator-mediated competition from invasive alien plants on native plant mating systems. In a novel approach, we measured the impact of the widespread invasive alien *Impatiens glandulifera* Royle (Himalayan balsam; Balsaminaceae) presence on the mating system of the self-compatible co-flowering native *Lamium album* L. (white deadnettle; Lamiaceae). *Impatiens glandulifera* poses a direct threat to native plant pollination since it produces numerous highly rewarding flowers, and successfully competes for pollinator visits (Chittka & Schürkens 2001). We expected that *I. glandulifera* would be highly attractive to pollinators which we predicted would disrupt the mating system of *L. album* by changing patterns of pollen dispersal. This was measured by genotyping progeny to determine the relative contribution of self and outcross pollen to pollination events, and elucidate changes in pollen dispersal patterns.

5.3 Methods

5.3.1 Study species

Lamium album L. (white deadnettle; Lamiaceae) is described in Chapter four. It spreads by clonal growth and by seed (Macukanovic & Blazencic 1998). There are multiple whorls of flowers on each stem which mostly bloom between May and December (Rose 2006) or January (Fitter & Fitter 2002). *Lamium album* is found in well-lit or partially shaded habitats (Hill et al. 2004). It is partially selfing (see Chapter four – Table 4.3, Figure 4.4) but to our knowledge nothing is known about its outcrossing rates. *Lamium album* was chosen for study as it co-occurs in the same habitat, overlaps considerably in its flowering period, and shares generalist bumblebee pollinators with *I. glandulifera*. Importantly, *I. glandulifera* flowers between July and October (Beerling & Perrins 1993), with peak flowering occurring between August and September, whereas *L. album* also flowers outside of this period in the spring (Rose 2006; Fitter & Fitter 2002). This enabled us to compare mating system parameters prior to the invader's flowering period with those during the invader's flowering, providing a temporal control to specifically address pollinator-mediated effects. *Impatiens glandulifera* Royle (Himalayan balsam; Balsaminaceae) is described in Chapter one.

5.3.2 *L. album* population genetic structure

The spatial scale of patch structure was determined to aid interpretation of patterns of outcrossing in *L. album*. Field counts of *L. album* stems do not reveal the distribution of individual plants since it grows by rhizomes and produces multiple stems per plant. Knowledge of patch structure, that is the spatial distribution of related plants, establishes the size of individual *L. album* plants. This enables determination of the distribution of self pollen, as well as the minimum distance over which a pollinator must travel to transfer outcross pollen. Knowledge of the spatial genetic structure informs the interpretation of the mating system estimates, because the pattern and spatial scale of pollinator movements can be inferred.

A single population was sampled in May 2011 (Figure 5.1 Plot 1). A leaf was collected from each of 155 plants, which were separated by a minimum of 1 m (to avoid potentially numerous samples from the same plant) and a maximum of 40 m (locations are in Appendix D, Figure D.1). Plants were genotyped using six microsatellite markers (the methodology is described in the primer note, Appendix E). Spatial autocorrelation in relatedness was determined using SPAGeDi software, version 1.3 (Spatial Pattern Analysis of Genetic Diversity) (Hardy & Vekemans 2002). Summary statistics of the genetic variation of the population were determined using GENAlEx software (Genetic Analysis in Excel) version 6.5 (Peakall & Smouse 2006, 2012). We assumed that spatial genetic structure from a May collection was a fair reflection of the structure throughout lifetime of the experiment, as little change was expected over the course of the season because *L. album* is a perennial.

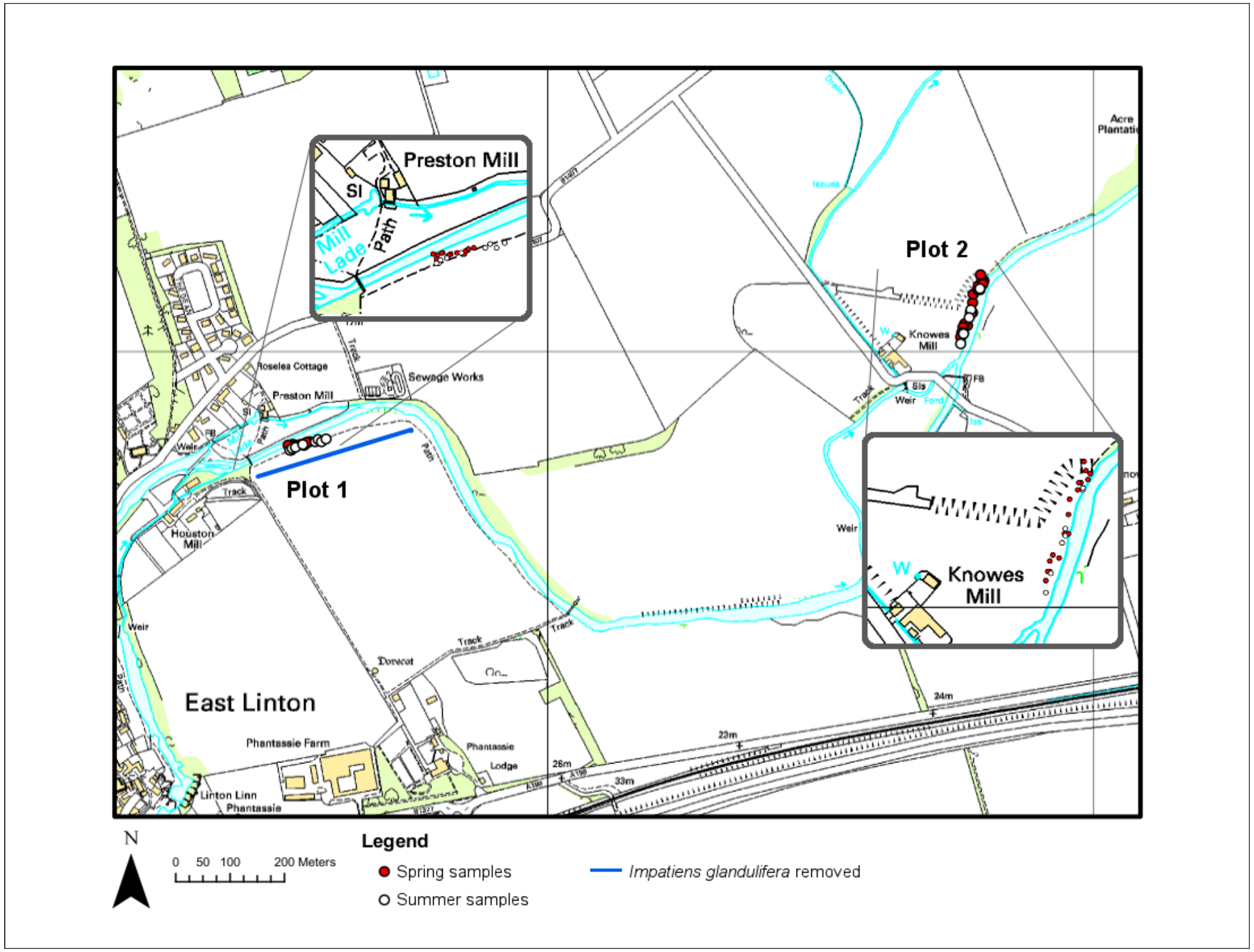

Figure 5.1: Field plots 1 and 2, in East Linton, central Scotland, UK in which *Lamium album* and *Impatiens glandulifera* occurred sympatrically. *Lamium album* samples were collected in spring (red circle) prior to *I. glandulifera*'s flowering season, and in summer (open circle) during *I. glandulifera*'s flowering season. *Impatiens glandulifera* plants were removed in early summer from a 500 m stretch of river bank surrounding plot 1.

5.3.3 *L. album* sample collection

In May 2011, two plots separated by 1 km were selected from a strip of riparian habitat surrounded by agricultural land, on the river Tyne, south-east Scotland (Figure 5.1) (NT 607 780; NT 595 778). Both plots were invaded by *Impatiens glandulifera*. The time since invasion was unknown. In plot one (Figure 5.1), *I. glandulifera* plants were removed by hand from a 500 m stretch of riverbank, from both sides of the river, in early July (the start of the flowering season). This plot was revisited every week until the end of *L. album* sample collection to remove any remaining *I. glandulifera* plants. *Impatiens glandulifera* plants were removed at this broad scale to reflect the potentially broad foraging range of its bumblebee pollinators (Carvell et al. 2012). In plot two (Figure 5.1), *I. glandulifera* plants were retained. The abundance of *I. glandulifera* was measured over a 500 m stretch of riverbank, from both sides of the river using the same methodology described in Chapter 2 (2.3.3) for measuring broad scale invasion, which was an average cover of 44 %.

To measure the effect of pollinator-mediated competition from *I. glandulifera* on *L. album* mating systems, samples were collected from both plots in May 2011 prior to *I. glandulifera*'s flowering season (a temporal control), and in August-September 2011 during *I. glandulifera*'s peak flowering season (samples hereafter are called spring and summer populations respectively). A single stem (with roots) from 20 maternal *L. album* plants were collected from each population. Samples were separated by a minimum of 5 m to avoid potentially collecting multiple stems of the same plant. To quantify the spatial context of these samples so that they could be considered in relation to the spatial scale of patch structure, the location of all patches of *L. album* in each plot were recorded to within a 1 m error using a GPS. Patches were defined as continuous areas of *L. album* stems, and patch boundaries were defined by the interruption of other plant species. The number of whorls of flowers on each stem in the patch were counted, and summed to find the total in each patch. *Lamium album* stems were planted in compost in 4 litre pots and kept in a glasshouse. Seeds were collected when almost mature (indicated by a change from green to light brown). Seeds could not be collected from stems in the field as they fall out from the sepals when fully mature, and mature at different rates. Seeds were fully formed at the time of removing the maternal plant and were close to maturity, so were unlikely to be affected by removing the plant. However, any effect incurred by transplanting the maternal plant from the field into the glasshouse would be the same across all four treatments. All flowers and buds were removed and discarded when the stem was collected to avoid collecting seeds from flowers that were not pollinated *in situ*. Seeds were counted, weighed when air dried (dark brown in colour), and stored in paper bags at room temperature.

During the first week of November 2011, once the summer population's seeds had matured, seeds from all populations were planted in compost in seed trays to germinate seeds for genotyping progeny. Seeds from spring populations were therefore older before being planted, but seeds were planted simultaneously for logistical reasons. Plants were grown in a heated glasshouse (where temperatures tracked the external temperature but were not allowed to fall below 10 ºC), over one year. This was to maximise the number of seedlings, since germination rates were low. Seeds were not chilled prior to planting as a trial planting showed germination was successful without chilling (results not shown). The number of progeny were counted and harvested when they had grown two leaves, each a minimum of 1 cm^2, which were dried and stored frozen for genotyping.

5.3.4 Genotyping progeny

Mating system parameters (multilocus outcrossing rate, t_m, biparental inbreeding, t_m- t_s, and correlated paternity, r_p) were determined by genotyping up to 20 randomly selected progeny from each maternal plant. The multilocus outcrossing rate, t_m, is the fraction of mating events not due to true selfing, as estimated using the full multilocus genotypes for all progeny; whereas the single locus outcrossing rate is estimated locus-by-locus across all progeny (Ritland 2002). Biparental inbreeding, t_m- t_s, is the contribution of mating between relatives to the overall level of inbreeding, and is quantified by the average relatedness between mates, excluding selfing events. Where biparental inbreeding occurs, the multilocus outcrossing rate should be higher than the single locus outcrossing rate and can be quantified by the difference between the two (Ritland 2002). Correlated paternity, r_p, refers to the fraction of siblings that share the same father (Ritland 2002).

Families of less than eight were not genotyped, since there is bias associated with low sample sizes when estimating outcrossing rate using MLTR (which is described below) using inferred maternal genotypes (Koelling, Monnahan, & Kelly 2012). An exception was made to include a single family of seven in order to maximise the number of families in the study, which was sometimes low due to poor germination. Variation in the number of families from each population resulted from differences in germination success, which could not be controlled. Individuals were genotyped using six microsatellite markers (methodology is described in the primer note, Appendix E).

5.3.5 Mating system estimation

Mating system parameters (multilocus outcrossing rate, t_m, biparental inbreeding, t_m- t_s, and correlated paternity, r_p) were estimated in MLTR (Ritland 2002), assuming equal female (ovule) and male (pollen) gene frequencies. Maternal genotypes were inferred using MLTR, since DNA did not consistently extract well from the maternal plant tissue collected. To calculate parameter variance for families, individuals within families were bootstrapped 1000 times. Some scoring errors occurred, that is, in some instances families had no common maternal genotype among progeny at a given locus. If one or two individuals could be identified as having been scored incorrectly (i.e. they did not share any alleles with the other progeny in the same family), they were omitted from the dataset at that locus, by being recorded as missing data values. If more than two individuals differed, the entire family was omitted from the dataset at that locus, since the problem individuals could not be identified with any certainty.

5.3.6 Statistical analysis

Analysis was done in R v2.10.1 (R Core Development Team 2011). Variation between treatments and the response variables of the number of seeds produced per plant, seed weight, the proportion of seeds that germinated, and mating system parameters of family-level outcrossing rates, biparental inbreeding and correlated paternity, were tested using generalised linear models (GLMs) (R Core Development Team 2011). The number of seeds produced per plant was tested as a function of the fixed effects of plot, with season as an interaction term, the number of whorls of flowers in each *L. album* patch in the plot (log), and floral connectivity (a measure of the distribution of whorls from other *L. album* plants in the plot, described below). GLMs were fitted to a quasi-Poisson error distribution to account for over-dispersion in the data. Models were repeated to examine mean seed weight per plant (according to a Gaussian error distribution, and weighted by number of seeds), germination (according to a quasi-Binomial error distribution), and family-level outcrossing rates, biparental inbreeding rates and correlated paternity (according to a Gaussian error distribution, weighted by the number of plants in the family). In Gaussian distributed models, minimum adequate models were obtained (Crawley 2002) using lowest AIC values found by removing non-significant terms in a backwards stepwise manner. In quasi- distributed models, the best fitting model was found by removing non-significant terms in a backwards stepwise manner, and qAIC values were found using general maximum likelihood estimations (bbmle package v 1.0.5.2 Bolker & R Development Core Team 2012).

The effect of neighbouring *L. album* patches on *L. album* mating system parameters were measured, because the local distribution of *L. album* pollen was considered to be potentially important in influencing pollination events. GLMs (described above) included both the number of whorls of flowers in each *L. album* patch in the plot and a measure of their spatial arrangement (referred to hereafter as floral connectivity) since accurate measurements of patch connectivity should include both parameters (Moilanen & Nieminen 2002). Neighbouring *L. album* patches were measured by counting the number of whorls on each stem, to estimate floral display. Floral connectivity was calculated by weighting *L. album* patches such that neighbouring patches had more influence than those further away, using a distance decay function:

$$\mathbf{d_i(x_m) = e^{-\lambda|x_i - x_m|}}$$

This equation is explained by: d_i takes a value between 0 and 1, being closer to 1 for neighbouring patches, and closer to 0 for distant ones. The location of the maternal patch *m*, is given by x_m and patch *i*, by x_i. d_i is the weighting of patch *i* according to the geographic distance from patch *m,* which was calculated using Pythagoras' theorem. The constant, λ, is the decay rate of the weighting with increasing x_i. Finally, a measure of the floral connectivity $\rho(x_m)$ of *L. album* surrounding the maternal patch was calculated:

$$\rho(\mathbf{x_m}) = \sum\nolimits_{\mathbf{i}} \mathbf{d_i}(\mathbf{x_m})\mathbf{n_i}$$

where the sum includes all the patches in the plot (excluding the maternal patch), each with n_i whorls of flowers, and is weighted according to *d*. Decay rates of $\lambda = 1/1$, 1/2, 1/5 and 1/10 per m were tested using each of the GLMs described above to find the most significant relationship. The decay rate selected was 0.2 m^{-1}, such that for every additional 5 m away from the maternal patch, the influence of any *L. album* flowers on *L. album* pollination is halved. This measure of *L. album* floral connectivity was used in the GLMs (as described above), to account for the effect of neighbouring *L. album* patches on *L. album* mating system parameters and seed set.

5.4 Results

5.4.1 *Lamium album* population structure

A total of 155 *Lamium album* individuals were genotyped at six polymorphic loci, from a single population. The total number of alleles detected for each locus ranged from 2 (LA35) to 13 (LA54) (Table 5.1). There were high levels of genetic diversity at all but one locus (LA35) (mean expected heterozygosity ranged from 0.490 to 0.842; 0.150 in LA35, Table 5.1).

Table 5.1: Summary of genetic variation at six microsatellite loci scored from a single *Lamium album* population of 155 individuals. N_A, number of alleles per locus; N_E, number of effective alleles per locus; H_O, observed heterozygosity; H_E, expected heterozygosity; and F, fixation index. Bold values indicate departures from Hardy-Weinberg equilibrium.

Microsatellite	N_A	N_E	H_O	H_E	F
LA5	3	2.135	**0.396**	**0.532**	**0.255**
LA25	3	1.961	0.474	0.490	0.033
LA33	6	2.262	**0.473**	**0.558**	**0.153**
LA35	2	1.177	0.164	0.150	-0.089
LA54	13	6.313	**0.763**	**0.842**	**0.093**
LA55	6	2.885	0.649	0.653	0.007
Multilocus estimate	5.5	2.789	0.486	0.537	0.075

The spatial scale of patch structure, within which individuals were more closely related than expected at random, reached to between 3 and 5 m (Figure 5.2 a), after which individuals were no more related than if they were randomly distributed. *Lamium album* reproduces by clonal growth and seed dispersal (Macukanovic & Blazencic 1998). Inclusion of clones in the estimate of patch structure caused an overestimation of relatedness, where the maximum pairwise kinship coefficient was 0.147 compared to 0.104 when one of each pair of clones were excluded (Figures 5.2 a and b respectively). Clonal plants accounted for a small number of samples in the population at 12 clonal pairs, out of 155 individuals. Spatial genetic structure of patches was thus mainly determined by related but different genotypes; hence reproduction by seed rather than clonal growth appeared to predominate. There was a strong patch structure up to 3 m when one representative of each pair of identified clones was excluded (Figure 5.2 b).

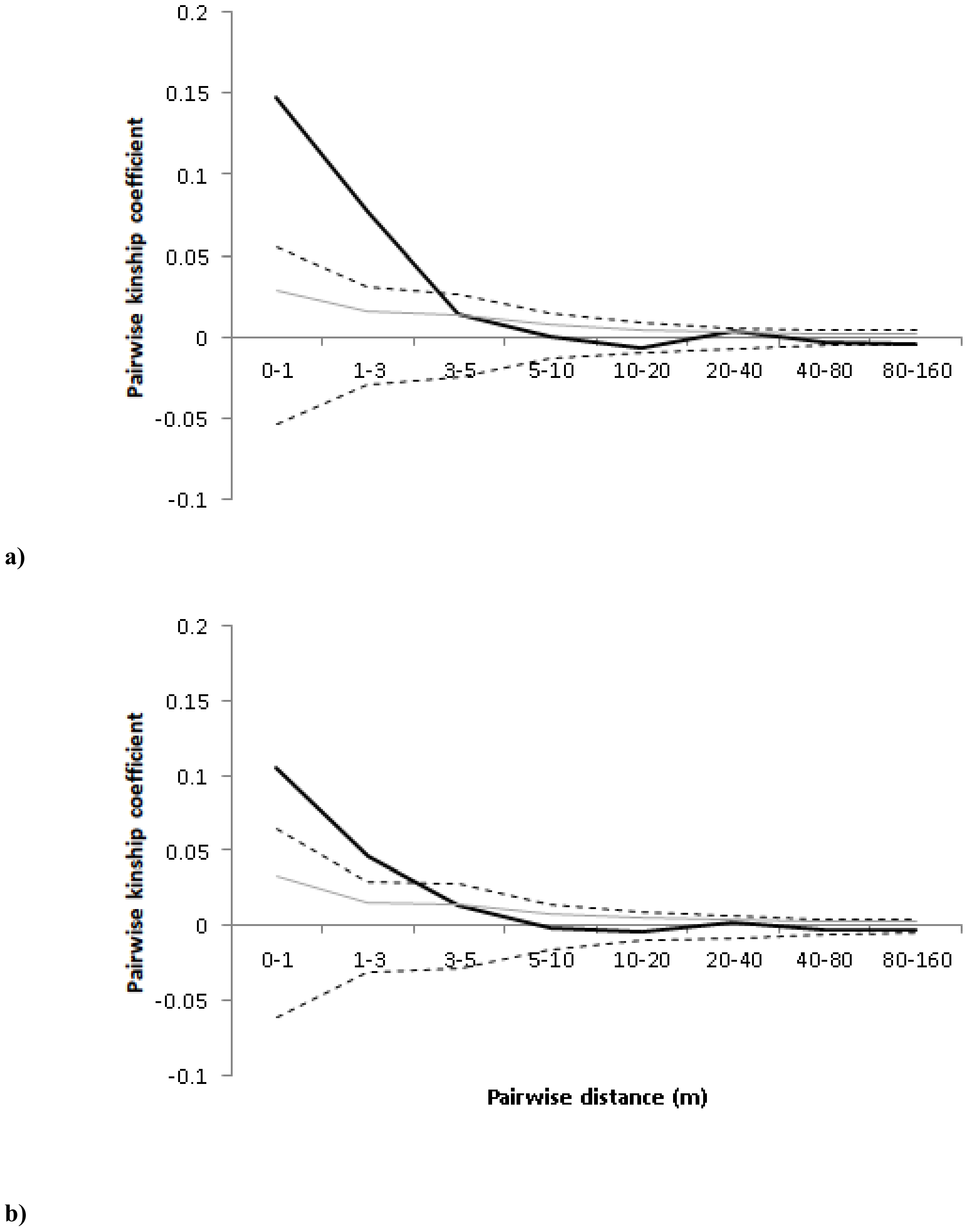

Figure 5.2: Spatial autocorrelation in relatedness (in metres) between *Lamium album* plants in a single population using six microsatellite markers. **a)** Relatedness of all genotyped individuals; and **b)** relatedness of individuals, excluding one individual from each pair of identified clones (total excluded = 6). Solid black line is pairwise kinship coefficient at all loci; dashed lines are upper and lower confidence intervals on the random expectation, the grey line, produced by bootstrapping (1000 permutations) using SPAGeDi software (Hardy & Vekemans 2002). N = 155, 149 plants in a) and b) respectively.

5.4.2 Seed production in *L. album*

The summer *L. album* population co-flowering with *I. glandulifera* experienced a significant 38 % reduction in the mean number of seeds produced, compared to the spring population (pre-*I. glandulifera* flowering season) at the same plot (Table 5.2, Figure 5.3). Seed weight did not differ either within or between plots, nor did the proportion of seeds that germinated (Table 5.2).

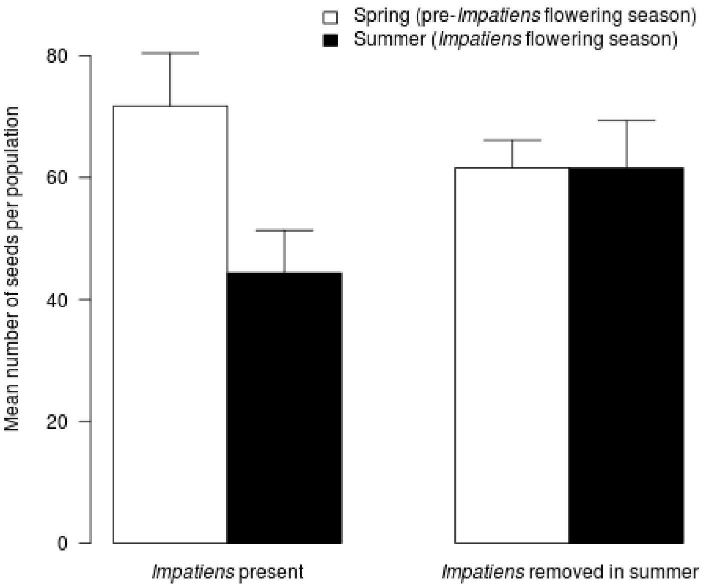

Figure 5.3: Number of seeds produced by *Lamium album* populations in spring (white bars; prior to *Impatiens glandulifera*'s flowering season) and summer (black bars; during *I. glandulifera*'s flowering season). Flowering *I. glandulifera* was present (left) and removed (right) in summer. Values are means + SE. N = 20, 20, 20, 22 plants for each bar (l-r).

Table 5.2: Analysis of the effect of presence of flowering *Impatiens glandulifera* on *Lamium album* seed number, seed germination and seed weight (mg). Generalised linear models (GLMs) were used according to a quasi-Poisson, quasi-Binomial and Gaussian error distribution for seed number, germination and weight respectively. Plot compares the plot where flowering *I. glandulifera* was removed in summer to the plot where it was present in summer; season compares summer populations to spring (that is, during and prior to *I. glandulifera* 's flowering season). The positive interaction term indicated a negative effect of *I. glandulifera* during its flowering season. The number of whorls of *L. album* flowers in each patch (log) and *L. album* floral connectivity (that is, the number of whorls in neighbouring patches weighted by a distance decay function) in each plot were removed to find the minimum adequate model.

Response variable		**Estimate**	**Std error**	***t***	***p***
Number of seeds	Intercept	4.237	0.108	39.454	
	Plot	-0.152	0.159	-0.956	0.342
	Season	-0.481	0.175	-2.743	0.007
	Plot x season	0.480	0.238	2.016	0.047
Proportion of germinated seeds	Intercept	-0.813	0.160	-5.078	
	Plot	-0.416	0.235	-1.770	0.081
	Season	-1.246	0.283	-4.409	<0.001
	Plot x season	0.370	0.398	0.930	0.355
Seed weight	Intercept	1.889	0.056	33.571	
	Plot	-0.132	0.081	1.638	0.105
	Season	-0.638	0.090	-7.115	<0.001
	Plot x season	0.193	0.120	1.606	0.112

5.4.3 Seasonal variation

There was a strong temporal effect on *L. album* seed weight and germination success. Seeds collected in the summer weighed significantly less than seeds collected in the spring (Table 5.2), decreasing from a mean of 1.79 mg (SE ± 0.035) to 1.24 mg (SE ± 0.053). This was mirrored in the proportion that germinated, decreasing from 26 % (SE ± 0.018) in the spring to 12 % (SE ± 0.019) in the summer. Seed weight and germination success were significantly positively correlated (Pearson's product-moment correlation, $b = 0.622$, $t = 7.109$, df = 80, p <0.001).

5.4.4 Effect of co-flowering *I. glandulifera* on *L. album* mating system

Progeny from mothers co-flowering with *I. glandulifera* showed a reduced outcrossing rate (t_m) relative to progeny from mothers grown where flowering *I. glandulifera* had been removed (Table 5.3, Figure 5.4). In the latter, and prior to *I. glandulifera* flowering (i.e. both spring populations), progeny were almost entirely outcrossed (Table 5.3, Figure 5.4). However, outcrossing rate was not significantly different between populations (Table 5.4). The biparental inbreeding rate (i.e. mating with relatives, t_m- t_s) was highest in progeny from mothers co-flowering with *I. glandulifera* (Table 5.3), although it was not significantly different between populations (Table 5.4).

Although outcrossing rate was reduced in the presence of flowering *I. glandulifera*, the correlated paternity (r_p) was very low, with r_p being two orders of magnitude smaller than any other population (Table 5.3). This means that the estimated number of fathers contributing to outcrossing events was high, but this was not significantly different between populations (Table 5.4).

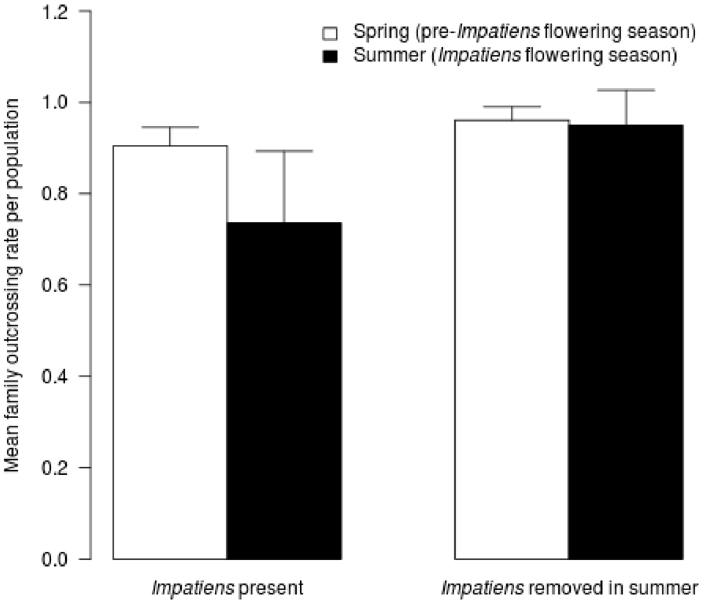

Figure 5.4: *Lamium album* outcrossing rate per population at the family-level in spring (white bars; prior to *Impatiens glandulifera*'s flowering season) and summer (black bars; during *I. glandulifera*'s flowering season). Flowering *I. glandulifera* was present (left) and removed (right) in summer. Values are means + SE. N = 18, 7, 17, 8 families for each bar (l-r).

Table 5.3: Mating system parameters for *Lamium album* populations, taken in spring and summer (prior and during *Impatiens glandulifera*'s flowering season respectively), and where in summer, flowering *Impatiens glandulifera* was present or had been removed. (^{N}family, total number of families genotyped per population (i.e. mother plants); ^{N}progeny, total number of progeny across families per population; t_m, multilocus outcrossing rate; t_m- t_s, biparental inbreeding estimate; r_p, multilocus correlated paternity; $r_{p(s)} – r_{p(m)}$, relatedness among fathers; standard errors in parentheses).

Population	**^{N}family**	**^{N}progeny**	**t_m**	**t_m- t_s**	**r_p**	**$r_{p(s)}$ - $r_{p(m)}$**
Spring (pre-*I. glandulifera* flowering season)						
I. glandulifera present	18	280	0.905 (0.040)	0.097 (0.043)	0.179 (0.051)	0.002 (0.041)
I. glandulifera present	17	227	0.961 (0.029)	0.017 (0.028)	0.116 (0.036)	0.007 (0.022)
Summer (*I. glandulifera* flowering season)						
I. glandulifera present	7	93	0.736 (0.157)	0.122 (0.034)	0.002 (0.051)	0.012 (0.055)
I. glandulifera removed	8	108	0.950 (0.077)	0.094 (0.042)	0.180 (0.041)	0.061 (0.062)

Table 5.4: Analysis of the effect of presence of flowering *Impatiens glandulifera* on family-level outcrossing rates, biparental inbreeding and correlated paternity in *Lamium album*. Generalised linear models (GLMs) were used according to a Gaussian error distribution. Plot compares the plot where flowering *I. glandulifera* was removed in summer to the plot where it was present; season compares summer populations to spring (that is, during and prior to *I. glandulifera*'s flowering season). The positive interaction term indicated a negative effect of *I. glandulifera* during its flowering season. The number of whorls of *L. album* flowers in each patch (log) and *L. album* floral connectivity (that is, the number of whorls in neighbouring patches weighted by a distance decay function) in each plot were removed in some models to find the minimum adequate model.

Response variable		**Estimate**	**Std error**	***t***	***p***
Outcrossing rate	Intercept	0.877	0.061	14.475	
	Plot	0.136	0.090	1.506	0.139
	Season	-0.263	0.121	-2.171	0.035
	Plot x season	0.191	0.169	1.132	0.263
Biparental inbreeding	Intercept	0.159	0.223	0.711	
	Plot	0.401	0.333	1.203	0.235
	Season	-0.052	0.446	-0.116	0.908
	Plot x season	-0.368	0.623	-0.590	0.558
Correlated paternity	Intercept	0.431	0.117	3.691	
	Plot	0.049	0.099	0.496	0.622
	Season	-0.188	0.130	-1.443	0.157
	Plot x season	0.158	0.187	0.847	0.402
	No. of whorls of *L. album* flowers (log)	-0.176	0.078	-2.255	0.030
	Floral connectivity	-0.004	0.002	-2.095	0.042

5.4.5 Effect of *L. album* floral cover

The number of whorls of *L. album* flowers within each patch in the plot and the floral connectivity (that is, the number of whorls in neighbouring patches weighted by a distance decay function) did not significantly affect *L. album* seed set (GLM, $t = 0.398$, -1.112; $p = 0.692$, 0.270 respectively) and were dropped from the final model (Table 5.2). These factors were also non-significant terms in models testing seed weight (GLM, $t = -0.468$, -0.891; $p = 0.641$, 0.376 for number of whorls and their connectivity respectively), and the proportion of seeds that germinated (GLM, $t = 0.914$, 0.775; $p = 0.364$, 0.441 for number of whorls and their connectivity respectively).

The number of fathers that contributed to mating was significantly higher with an increasing number of floral whorls and with greater floral connectivity, demonstrated by the negative correlation between the relatedness of fathers and these measures of floral cover (Table 5.4). *Lamium album* floral cover did not significantly affect *L. album* outcrossing rates (GLM, t = 1.354, -0.771, p = 0.183, 0.445 for number of whorls and their connectivity respectively) or biparental inbreeding (GLM, t = 0.552, 0.057, p = 0.584, 0.955 for number of whorls and their connectivity respectively), and were dropped from the final model (Table 5.4).

5.5 Discussion

In this study, we have demonstrated a novel approach for assessing the impact of invasive alien plants, adding to a growing number of studies that recognise they can change patterns of pollinator visitation (Lopezaraiza–Mikel et al. 2007; Vila et al. 2009) and pollen transport (Brown & Mitchell 2001; Matsumoto et al. 2010). This study is unique in using the parameters of the plant mating system to assess the effects of pollinator-mediated competition between alien and native species. *Lamium album* plants co-flowering with the invasive alien *Impatiens glandulifera* in summer showed a significant 38 % reduction in seed set compared to the spring population in the same plot (which flowered prior to *I. glandulifera*'s flowering). This summer population also showed a trend towards an altered plant mating system, showing reduced outcrossing rates, an increase in the number of pollen donors that contributed to outcrossing events, and increased biparental inbreeding rates. Although not statistically significant individually, together these effects indicate that *I. glandulifera* interrupts the mating system of *L. album*. The size of the experiment was restricted by logistics and poor germination, but a larger sample size is likely to have reached significance. Interspecific competition for pollinators between *L. album* and *I. glandulifera* may reduce seed set and alter plant mating systems due to a combination of reduced pollinator visitation and changes to the genetic quality of pollen received. This study demonstrates for the first time the potential for alien plants to disrupt native plant mating systems, with implications for individual plant fitness and local population genetic structure.

5.5.1 Effects of *I. glandulifera* on the mating system of *L. album*

Competition with alien plants for shared pollinators can reduce visitation rates to natives (Brown et al. 2002; Thijs et al. 2012, Chapter three). Reduced pollinator visitation may have contributed to the 38 % reduction in seed set observed in *L. album* plants co-flowering with *I. glandulifera*, if pollinators showed a preference for *I. glandulifera*. The invader was highly abundant in the experimental plot at a percentage cover of 44 %, and pollinators should favour abundant food sources (Levin & Anderson 1970) to maximise food intake per unit time spent foraging, according to optimal foraging theory (reviewed by Pyke 1984). *Impatiens glandulifera* flowers also offer a copious reward, secreting nectar at a rate of 11312 µg per 24 hours compared to 467 µg per 24 hours in *L. album* (Raine & Chittka 2007a). The top-ranking nectar source is most commonly used by bumblebees (Heinrich 1979a), which suggests that *I. glandulifera* will be preferred by bumblebee pollinators. Chittka and Schürkens (2001) found that *I. glandulifera* also successfully competed for pollinator visits and reduced seed set in the native *Stachys palustris* (but see Chapter three), which like *L. album* is in the Lamiaceae family, and its success was attributed to its superior floral reward. Also, visitation to *L. album* may be reduced on account of its height (Chapter three). *Impatiens glandulifera* can grow to 3 metres (Clements et al. 2008), whereas *L. album* can grow up to 0.6 metres (Rose 2006). Pollinators can prefer to forage in a horizontal plane (Waddington 1979; Makino 2008), to minimise energy expenditure per unit distance compared to vertical movement (Levin & Kerster 1973). Therefore, pollinators foraging on *I. glandulifera* are predicted to be unlikely to encounter *L. album* plants, if they maintain a horizontal plane. Low light levels can reduce pollinator visitation (McKinney & Goodell 2010). *Lamium album* is found in partially shaded habitats (Hill et al. 2004) but *L. album* plants growing beneath *I. glandulifera* may experience high levels of shading and reduced visitation as a consequence. The height of *I. glandulifera* could also isolate patches of *L. album* by obscuring them from searching pollinators. Spring populations of *L. album* had higher seed set and higher germination success compared to the summer populations. Spring populations may have experienced less shading by surrounding vegetation compared to summer populations where more species were in flower, but this requires further study. Low seed set in *L. album* plants co-flowering with *I. glandulifera* could alternatively be due to competition for other resources such as water, light or space. The effect of competition for pollinators could be separated from competition for these other resources by including an additional plot in future studies in which *I. glandulifera* flowers are removed but the plant is retained.

Reduced outcrossing rates observed in the population co-flowering with *I. glandulifera* compared to the populations grown in the absence of flowering *I. glandulifera*, could have been caused by reduced pollinator visitation to *L. album*. Plants commonly receive both self and outcross pollen (Darwin 1876), but outcrossing should be favoured as selfing can reduce genetic diversity (Lo et al. 2010) and reduce fitness through inbreeding depression (Charlesworth & Charlesworth 1987). Plants with mixed mating systems can passively select for outcross pollen via abortion of inferior fruits (Becerra & Lloyd 1992), and pollen tubes of outcross pollen can grow faster in some species (Mulcahy 1971; Weller & Ornduff 1989) which gives outcross pollen a siring advantage. Although there are fitness costs associated with self-pollination, it provides an opportunity for reproduction when outcrossing is not possible (Darwin 1876). It was shown experimentally in *Collinsia verna* that selfing rates increased due to autogamous pollination (within-flower selfing without a pollinator vector) as a direct response to the absence of pollinators (Kalisz et al. 2004). Due to the close proximity of the anthers and stigma in *L. album* flowers, there is no mechanical barrier preventing autogamous pollination in the event of reduced pollinator visitation. Despite providing *L. album* with reproductive assurance however, such changes in plant mating systems can alter the genetic structure of populations (reviewed in Ledig 1992), which has implications for the fitness and long-term persistence of local populations.

Pollinator visitation to focal plants is commonly measured using field observations during replicated short observation periods (e.g. Dietzsch et al. 2011), a method susceptible to short-term temporal variation. Rather than using direct pollinator observations, this study indirectly measured pollinator visitation using a retrospective analysis of progeny genotypes. This inferred pollinator foraging behaviour over a wide spatial and temporal scale, capturing pollination events that would have been missed using traditional field observation methods. Studies examining plant-pollinator interactions have also found variation between years, partly due to fluctuations in pollinator population sizes (Moragues & Traveset 2005; Larson et. al 2006). However, it was not possible to repeat this study due to costs and time constraints.

Despite the trend for reduced outcrossing in the population co-flowering with *I. glandulifera*, there was large variation between families within this population and it was not statistically significantly different from the other populations. Low germination success of summer compared to spring *L. album* populations limited the sample size. This meant that it was not possible to examine the effect of the distance to the nearest *I. glandulifera* plant on *L. album* reproduction, which may have been important in contributing to the variation in outcrossing rates observed. The study was also limited by a small sample size of four populations (two plots). However, this was dictated by the logistics of large-scale removal of *I. glandulifera*, which was necessary given the potentially large foraging ranges of bumblebees; and was due to the costs associated with genetic studies. However, the inclusion of a temporal control increased the robustness of the results. This four-way design was effective in enabling comparisons of before and during *I. glandulifera*'s flowering period. Ideally, the study would be replicated further, but the costs associated with genetics studies prevents this.

The observed selfing rates in *L. album* may be conservative estimates, since inbreeding depression can mean that seeds fail to germinate (Husband & Schemske 1996). This could be addressed in future studies by genotyping seeds. However, inbreeding depression can be expressed at different stages in the life cycle, and not necessarily at the germination stage (Husband & Schemske 1996). In this study, seedlings were genotyped rather than seeds due to the difficulties in extracting enough DNA from seeds.

Increased geitonogamous pollen transfer (pollen from another flower on the same plant) can reduce plant outcrossing (Karron et al. 2009), and may have contributed to the reduced outcrossing observed in *L. album* plants co-flowering with *I. glandulifera*. If pollinators showed a preference for *I. glandulifera* (discussed above, Chapter three) this should reduce competition for *L. album*, meaning that there is more reward available for individuals that do forage on it. Bumblebees contract their foraging range in rewarding patches (Heinrich 1979a), remaining for longer on an individual plant (Heinrich 1979a; Ishii et al. 2008). Therefore, bumblebees foraging on *L. album* may visit more flowers on the same plant, increasing geitonogamous pollen transfer. Since *L. album* can reproduce vegetatively, visitation between neighbouring clonal stems will also transfer geitonogamous pollen. As well as increasing selfing rates, which has implications for inbreeding depression (Charlesworth & Charlesworth 1987), geitonogamous pollen transfer also decreases the amount of pollen available for outcrossing, which can reduce the plant's siring success (Harder & Barrett 1995). Conversely, bumblebees are more likely to leave a plant if they encounter flowers that have been depleted of their reward (Pyke 1978), and fly further to the next plant which avoids near-neighbours that have a higher probability of being empty (Pleasants & Zimmerman 1979). This should promote outcrossing in *L. album* where it is not in competition with flowering *I. glandulifera*.

As well as receiving self pollen (true selfing), outcrossing rates may also be reduced by receipt of pollen from relatives (biparental inbreeding). Biparental inbreeding estimates were highest in the population co-flowering with the invader. Patches of *L. album* showed strong spatial genetic structure up to around 3 metres, after which plants were no more related than if they were randomly distributed. This suggests that in the presence of flowering *I. glandulifera* pollinators visited more related plants by contracting their foraging range to a few metres or less. However biparental inbreeding estimates were not significantly different from each other, and were low for all populations, indicating that true selfing predominated. This suggests that outcrossing rates decreased mostly via increased geitonogamous pollen transfer from foraging on the same plant, or by autogamous pollination in the absence of pollinators.

Although outcrossing rates were lowest in the *L. album* population co-flowering with the invader, the number of fathers contributing to these outcrossing events was high (however this was not significantly different from other populations). This relationship is most likely due to the effects of local *L. album* floral density, rather than the presence of *I. glandulifera*. This is because the number of pollen donors was significantly higher with more *L. album* flowers in the plot, and when *L. album* patches were more connected (that is, closer together). This suggests that pollinators transferred pollen between patches more. Alternatively, the receipt of pollen from more unrelated donors could be due to increased random movement between *L. album* plants. In rewarding areas, bees turn more in order to remain in the same patch but reduce the probability of revisiting flowers (Zimmerman 1979), which should disperse pollen from a wider range of neighbouring plants. The number of pollen donors could also be higher if pollinators carrying *L. album* pollen are attracted from long distances. In another study, *I. glandulifera* increased insect abundance, species richness and visitation to co-flowering native plants compared to a control plot where the invader was experimentally removed (Lopezaraiza–Mikel et al. 2007). It was hypothesised that these additional insects were attracted from further away due to *I. glandulifera*'s large floral display (Lopezaraiza–Mikel et al. 2007). This could result in some long-distance pollen dispersal events, but will depend on the amount of pollinator sharing between *I. glandulifera* and *L. album* and the amount of pollen carry-over. It was not possible to estimate the shape of the pollen dispersal curve from our data due to the low numbers of families genotyped, but it was possible to infer that typical dispersal distances were likely to have been above 3 metres given the low proportion of biparental inbreeding. Greater pollen diversity can increase progeny fitness by increasing heterozygosity and the acquisition of more “good genes” (Breed et al. 2012b). However, any potential benefits to *L. album* could be counter-balanced by possible inbreeding depression caused by the reduced outcrossing rates observed.

Reduced genetic quality of pollen may have contributed to the reduced seed set found in *L. album* co-flowering with *I. glandulifera*, although this effect cannot be determined from this study. However, inbreeding can reduce the number of seeds produced, most likely caused by expression of deleterious alleles due to homozygosity causing mortality at the embryo stage (Charlesworth & Charlesworth 1987). Compared to cross-pollination, selfing reduced seed set in 62 self-compatible plant species or populations by an average of 20 % (Husband & Schemske 1996). *Lamium album* populations were almost entirely outcrossed except when grown in the presence of flowering *I. glandulifera*, which suggests that it may be typically outcrossing. Given that the spatial genetic structure of *L. album* populations was estimated to be at around 3 metres, the distance to the nearest source of outcross pollen is small which should facilitate outcrossing. Populations with a long history of inbreeding show lower inbreeding depression compared to predominantly outcrossing species (Husband & Schemske 1996). This is predicted to be because in the former, the frequency of deleterious recessive alleles declines as they are purged by selection (Charlesworth & Charlesworth 1987; Barrett & Charlesworth 1991). In predominantly outcrossing species however, purging may take considerable time, and initial effects of selfing are decreased fitness due to homozygosity (Barrett & Charlesworth 1991). Therefore, if *L. album* is predominantly outcrossing, it may be particularly vulnerable to inbreeding depression. Similarly, other predominantly outcrossing species may also be vulnerable to the effects of pollinator-mediated competition with invasive alien species. However, typical outcrossing rates have yet to be established for many species.

Pollen wastage caused by pollinator movement between *I. glandulifera* and *L. album* flowers may have contributed to reduced outcrossing and seed set in *L. album*, although this mechanism was not tested in this study. Bell et al. (2005) found that outcrossing rates and seed set were lower in native *Mimulus ringens* when grown in experimental mixed arrays with a native competitor *Lobelia siphilitica*, compared to when it was grown without the competitor. This was due to pollen wastage caused by movement between the two species, which reduced the diversity of pollen donors received (Bell et al. 2005). Pollinator sharing between alien and native plants can also reduce seed set via the transfer of interspecific pollen (Brown & Mitchell 2001). *Impatiens glandulifera* pollen was found on native plant stigmas, and dominated pollen transport networks (Lopezaraiza–Mikel et al. 2007) which suggests pollinator sharing and could result in pollen wastage. The effect of the receipt of *I. glandulifera* pollen on native plant seed set has been little-studied (but see Chapter four).

5.6 Conclusion

Competition with flowering *I. glandulifera* reduced seed set and disrupted the mating system of *L. album*. This has implications for individual plant fitness, and for the genetic structure and persistence of local populations. Species whose floral phenologies overlap with invasive aliens are at risk from pollinator-mediated competition. However, plants can express phenological plasticity in response to a range of environmental variables (Rathcke & Lacey 1985; Inouye et al. 2002), and invasion by alien plants has been associated with shifts in timing of flowering in other native plant communities (Wilke & Irwin 2009). The first flowering date of *L. album* has become significantly earlier and it now routinely flowers in the winter, in relation to increased temperatures associated with climate warming (Fitter & Fitter 2002). This extended flowering offers more opportunities for matings and outcrossing, which means that *L. album* may be able to compensate for the negative effects of the competition with *I. glandulifera* that it experiences during summer. Ultimately this could lead to a shift in the flowering phenology of invaded populations. We have demonstrated that an invasive alien plant has the potential to disrupt native plant mating systems, using a common native species.

We recommend that future research is directed towards rarer species upon which the impact of disturbance from aliens may be of greater immediate conservation significance.

5.7 Acknowledgements

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Chapter Six:

General Discussion

6.1 Overview

This research aimed to examine pollinator-mediated effects of the invasive alien *Impatiens glandulifera* on native plant pollination. This was achieved using a wide range of approaches. In Chapters two and three, I measured the change in composition of plant and pollinator communities in response to increasing abundance of *I. glandulifera,* at multiple spatial scales of invasion; and changes in bumblebee-flower visitation patterns in response to the abundance of *I. glandulifera* at a plot scale. In Chapter four, I examined the effect of *I. glandulifera* pollen on native plant reproductive success, and finally, in Chapter five I explored the impact of pollinator-mediated competition on native plant mating systems.

Here, I provide an overview and general discussion of the main findings from each chapter, and recommendations for future research.

6.2 The relationship between impact and abundance

Whilst there are a number of studies that consider the impact of the presence of invasive alien plants on plant-pollinator interactions (e.g. Lopezaraiza–Mikel et al. 2007), few have considered the effect of their abundance (Chapter three; Dietzsch et al. 2011). In Chapters two and three, I found significant relationships between the abundance of *I. glandulifera* and the plant and pollinator community composition. The abundance of plant species varied and plant species richness decreased with increasing *I. glandulifera* abundance; and the proportion of bumblebee-pollinated plants increased at low abundance of *I. glandulifera*. Pan traps revealed that hoverfly abundance decreased and social wasp abundance increased with increasing *I. glandulifera* abundance, whereas bumblebee abundance increased in the presence of *I. glandulifera* but showed no relationship with abundance.

Since an observational approach was used to examine the composition of the plant and pollinator communities, it was not possible to differentiate between effects caused by the abundance of *I. glandulifera* itself and those due to habitat differences, such as soil nutrients, which may determine *I. glandulifera* abundance (or its absence). This was a limitation of the experimental design as space-for-time substitutions are inevitably correlative but reflects ethical constraints and the logistics of studying invasion; although these methods are commonly used in other invasive species studies (Hejda & Pyšek 2006). Despite these limitations, our results may be indicative of a real effect of invasion rather than reflecting other variables. This is because differences between plots were minimised by selecting those that shared similar surrounding land cover and elevation, and the abundance of *I. glandulifera* was unrelated to its position in the river catchment to avoid association with co-varying factors such as surrounding land use and river width. Also, plots appear to have been similar in their suitability for invasion since the abundance of *I. glandulifera* changed over the season and some plots in which *I. glandulifera* was initially absent were subsequently invaded. Here, changes in abundance could be due to late germination of seeds in the seed bank. These plants were characteristically short and were distinguishable from plants missed though observer error.

Chapter two generated a large data set in which a significant relationship between the abundance of *I. glandulifera* and the plant community composition was detected. This supports the hypothesis that differences between previous studies examining the impact of *I. glandulifera* may be partly due to the effects of its abundance (Chapters one, two and three). Hulme and Bremner (2006) found that plant species richness and diversity decreased in the presence of *I. glandulifera*, whereas Hejda & Pyšek (2006) and Hejda et al. (2009) found that the presence of *I. glandulifera* had a negligible effect on the plant community composition. Chapter two found that the proportion of bumblebee-pollinated plants was significantly higher at low *I. glandulifera* abundance, and showed a (non-significant) decrease with increasing *I. glandulifera* abundance. This non-linear relationship demonstrates support for Rathcke's density-visitation model. Chapter two also demonstrated that plant species richness significantly decreased with increasing abundance of *I. glandulifera*, and that there was a significant relationship between plant species abundance and *I. glandulifera* abundance. This suggests that there is a relationship between the abundance of *I. glandulifera* and its impact on the plant community composition.

More controlled studies, using common garden experiments for example, would separate the mechanisms involved from site-specific variation that is inherent within the study's design. For example, co-occurring plant species could be grown in containers with a gradient of *I. glandulifera* abundance, with standardised soil and the same watering regime to separate out the effects of invasion from other variables. However, despite its drawbacks, the present study is well-suited to examining the effect of invasion on the plant community composition. This is because by examining plant communities *in situ*, the responses of a large number of species can be incorporated into the study, and the community composition represents the long-term effects of invasion, being the outcome of multiple seasons. Also, the high abundance of *I. glandulifera* used in this study, particularly with respect to broad scale invasion, would not be achievable in common garden experiments due to the logistics of maintaining such large numbers of plants. An alternative approach could be to repeat the current study, but instead the abundance of *I. glandulifera* could be manipulated by removing different amounts from equally invaded plots, thus better controlling for site-specific variation. This would need to be carried out at a far smaller scale for logistical reasons, and would require a longer time scale to detect responses in the plant community. However, it could be useful in conjunction with the present work in untangling the effects of *I. glandulifera* from the effects of habitat.

Chapter three also demonstrated support for the hypothesis that differences between previous studies may be partly due to *I. glandulifera* abundance, since the pollinator community composition varied significantly with *I. glandulifera* abundance. Chittka and Schürkens (2001) found that pollinator visitation and seed set in the co-flowering native *Stachys palustris* decreased in the presence of *I. glandulifera*, whereas by contrast, Lopezaraiza–Mikel et al. (2007) found that visitation to the co-flowering plant community increased in the presence of the invader. Nienhuis et al. (2009) found that the presence of *I. glandulifera* had no effect on visitation to co-flowering plants. It was predicted that pollinator visitation would vary with *I. glandulifera* abundance according to Rathcke's density-visitation model (1983), but Chapter three demonstrated little support for this model. Instead, pollinator visitation varied mostly according to the presence of *I. glandulifera* (this is discussed in Section 6.4). However, the pollinator community composition did vary with the abundance of *I. glandulifera*, although responses were taxon-specific (Chapter three). This emphasises the necessity of community-level studies to capture the range of effects of invasive alien plants. Hoverfly abundance significantly decreased whereas social wasp abundance significantly increased with increasing abundance of *I. glandulifera*. At a broad scale, hoverfly abundance showed a weakly uni-modal response to *I. glandulifera* abundance. This curved relationship was non-significant, but the relationship lends marginal support for Rathcke's density-visitation model predictions. Further work is needed to determine the mechanism responsible for this correlation, which could due to direct responses to *I. glandulifera* or indirect effects if pollinators did not themselves visit *I. glandulifera*. Observational studies of foraging patterns may be useful in identifying these effects.

In contrast to the responses of hoverflies and social wasps, bumblebee abundance did not demonstrate a relationship with *I. glandulifera* abundance (Chapter three). Bumblebee abundance increased in the presence of *I. glandulifera* but further work is required to determine whether this was due to attracting individuals from the wider landscape, or due to increases in bumblebee populations through the provision of additional resources. As in previous studies, *I. glandulifera* was highly attractive to bumblebees (Lopezaraiza–Mikel et al. 2007; Bartomeus et al. 2010). Given widespread concern over the decline of pollinators (Chapter three; Biesmeijer et al. 2006; Cameron et al. 2011, but see Carvalheiro et al. 2013), the potential value of *I. glandulifera* as a food source is of particular note. Increased local abundance of bumblebees could facilitate neighbouring native plant pollination, which could impact on the plant community composition. This is supported by Chapter two, where the proportion of bumblebee-pollinated plants increased at low abundance of the invader, which could indicate facilitation (bumblebee-flower visitation is discussed in section 6.4). Chapter three shows the importance of using a range of pollinator taxa to examine the potential effects of invasive alien plants, since bumblebees, hoverflies and social wasps each demonstrated a different response to invasion.

6.3 The relationship between impact and spatial scale

There is currently a lack of knowledge regarding how the impact of invasive alien species varies with spatial scale (Bjerknes *et al.* 2007). However, it is important to determine the appropriate scale with which to measure impact in order to detect effects and to isolate the mechanisms driving the relationship. For example, studies examining impact at a local scale of 1 m^2 can perceive effects of competition between *I. glandulifera* and the native plant community for abiotic resources such as light

(Hulme & Bremner 2006), but may not detect pollinator-mediated effects that can occur at a broader scale of 100 m^2 (Chapter 3). In Chapters two and three, the effect of *I. glandulifera* abundance on plant and pollinator community composition varied with spatial scale. In Chapter two, the composition of the plant community was most strongly related to the abundance of *I. glandulifera* at a broad scale. In Chapter three, pan traps revealed that hoverfly abundance showed a strong exponential decrease with increasing *I. glandulifera* abundance at the plot scale, whereas it showed a weak (though non-significant) unimodal response at the broad scale, peaking at low *I. glandulifera* abundance. Social wasps showed a different response to hoverflies, increasing in abundance at both local and broad scales. Differences were expected between pollinator taxa, given that their perception of the environment depends on their dispersal ability and foraging range (Steffan-Dewenter *et al.* 2002). Other studies have found that plant-pollinator interactions vary with spatial scale (Bjerknes et al. 2007; Jakobsson et al. 2009), which together with these results confirms that multiple spatial scales should be used to assess the full extent of the impact of invasive aliens.

The response of the plant community to broad scale invasion could reflect pollinator-mediated effects, particularly since Chapter three demonstrated that pollinators responded to invasion at this scale; or could demonstrate broad scale variation in abiotic conditions that are responsible for both invasion and the plant community composition. Measures were taken to reduce variation in abiotic conditions (described above). However, further work is necessary to separate the effects of these different drivers on the plant community. This could be achieved with measurement of additional environmental variables such as soil composition, or the frequency of disturbance events such as flooding.

6.4 Using plant traits to predict the impact of invasion

In Chapter one, the choice of native plant species used to measure the effects of invasive alien plants was predicted to be an important explanatory factor where previous studies have found contrasting effects (Chittka & Schürkens 2001; Bartomeus et al. 2010; Thijs et al. 2012). Evidence to support this prediction was found in Chapter three, as co-flowering plant species showed contrasting responses to *I. glandulifera* invasion according to their traits. This is important because it may be possible to predict plant species responses to invasive alien species by their traits. The small increase in the abundance of bumblebee-pollinated plants in the presence of *I. glandulifera* (Chapter two) supports the suggestion that plants that share pollinators with the invader may experience an effect (Thijs et al. 2012). In Chapter three plants in the Lamiaceae family (whose flowers are zygomorphic, like *I. glandulifera*) and purple-flowered plants (i.e. a similar colour to *I. glandulifera*) experienced reduced bumblebee visitation when co-flowering with *I. glandulifera*. This agrees with Gibson et al. (2012) who found that plants sharing floral traits with alien species are more likely to experience an impact of alien plants, due to pollinator sharing. This effect may be positive, through facilitation, or negative via competition.

With increasing abundance of *I. glandulifera*, the plant community was characterised by species with high Ellenberg nitrogen and light indicator values, and that were taller (Chapter two). It is possible that plants with these traits are less likely to suffer negative effects under invasion, although further work, such as removal experiments, is necessary to separate correlation from causation as discussed above. However, I found further supporting evidence to suggest that impact may be predicted by plant height in subsequent chapters. In Chapter three, short plants received significantly fewer bumblebee visits in the presence of *I. glandulifera* than in its absence. Further work is needed to determine the mechanism behind this effect, which may be due to shade because *I. glandulifera* is the tallest annual in the UK (Beerling & Perrins 1993), and shading has been found to reduce pollinator visitation (McKinney & Goodell 2010). Alternatively, visitation may be reduced because pollinators can show a preference for foraging on a horizontal plane (Waddington 1979; Makino 2008), meaning that pollinators attracted to *I. glandulifera* are unlikely to encounter short plants. In Chapter three, pan traps were placed at ground level to examine the impact of *I. glandulifera* on pollinator visitation to the ground flora (the findings are described in section 6.2). This could reflect height-mediated competition for pollinators between *I. glandulifera* and co-flowering native species. To confirm this, the study should be repeated using pan traps raised on stakes to the height of *I. glandulifera* flowers. Also, potted native plants could be experimentally raised to different heights to examine the effect of height on pollinator visitation and seed set. Height-mediated competition for pollinators may also have contributed to *Lamium album*'s reduced reproductive success and the disruption to its mating system, found in Chapter five, as at a maximum height of 0.6 m (Rose 2006) it is considerably shorter than *I. glandulifera*. Altogether, these results suggest that the traits of co-flowering plants could be used to predict the outcome of their interaction with invasive alien species. To confirm this, it is recommended that future research is directed towards isolating the effects of plant traits by conducting controlled experiments that remove co-varying factors, such as those suggested for plant height. This could help to predict the impacts of invasive alien plants, and enable targeted conservation efforts.

6.5 The impact of invasion on native plant reproductive success

Previous studies have found contrasting effects of invasive alien species on the reproductive success of co-flowering native species (Chapters one, three and four; Chittka & Schürkens 2001; Nielsen et al. 2008). In Chapter five, competition for pollinators reduced *L. album* seed set which may have been the result of reduced pollinator visitation, reduced genetic quality of pollen, or a combination of both. Field observations of pollinator visitation patterns would add valuable insight into the relative importance of these mechanisms in determining seed set. Chapter five demonstrated a novel approach of using parameters of plant mating systems to measure the impact of invasive alien plants. There was a trend for reduced outcrossing rate, an increase in the number of pollen donors contributing to outcrossing events, and increased biparental inbreeding rates. Together, this indicates that *I. glandulifera* disrupts the mating system of *L. album*, although the effects were not significant individually. Such changes in plant mating systems can alter the genetic structure of populations (reviewed in Ledig 1992), which has implications for plant fitness due to the effects of inbreeding depression (Charlesworth & Charlesworth 1987), and for long-term persistence of local populations.

Chapter five was limited by small sample sizes however, and so it is recommended that the experiment is repeated with more samples, or using another plant species that has a higher germination rate. It would also be useful to measure individual fitness parameters such as growth rate, in the second generation of *L. album* to examine long term effects of disruption to plant mating systems. The bumblebee-flower visitation work in Chapter three could be extended by exploring whether the changes observed translate to changes in seed set, such as in Chittka & Schürkens study (2001). *Impatiens glandulifera* pollen did not appear to prevent seed set in *L. album* (Chapter four). However, the experiment was hampered by low seed set in hand-pollination treatments, which reflects the difficulties that can be associated with artificial pollination experiments (Petit 2011). The experiment could be repeated using a different native plant species that has been successfully used for artificial pollination in previous studies. The work could also be extended by using a field-based approach through manipulating mixed floral displays and monitoring visiting pollinators, interspecific pollen transfer and seed set. Despite limitations, Chapter four did highlight that *L. album* requires pollinators to achieve high seed set, which along with Chapter five, confirms that this species could be vulnerable to pollinator-mediated competition from *I. glandulifera*.

6.6 Further research

As part of Chapter three, the pollen loads of bumblebees foraging under different levels of invasion were collected to examine which native species were important sources of pollen, and to determine the extent to which *I. glandulifera* pollen was actively collected in the context of the availability of other plant species. However, time constraints prevented their examination. This work would be useful in identifying the preference for (and potential nutritional value of) *I. glandulifera* pollen, which has yet to be considered in any study to date, as well as in identifying native species most at risk from interspecific pollen transfer (IPT) if individuals move between *I. glandulifera* for nectar and co-flowering species for pollen. The nutritional value of *I. glandulifera* pollen would be useful to explore in terms of its impact on the reproductive success of bumblebee colonies, and in the egg maturation of hoverflies. It would also be important to examine in relation to the energy provided for migratory hoverflies, as it could influence the distance they can travel. The effects of time since invasion on the composition of plant and pollinator communities is worthy of further investigation. This could be achieved with long term studies or by comparing of plots that are known to have been invaded for substantially different time periods, such southern UK sites and more recently invaded northern UK sites.

6.7 Conclusion

The aim of this research was to examine pollinator-mediated interactions between *Impatiens glandulifera* and native plants, and address factors that may cause these interactions to vary. This study demonstrated a relationship between the abundance of *I. glandulifera* and the composition of plant and pollinator communities, which also varied with spatial scale. Accounting for both spatial scale and abundance is important therefore in enabling comparisons between other studies on *I. glandulifera* and in resolving apparent discrepancies, as well as for consideration in future research on other invasive alien plant species. By examining impact at the community level, it has been possible to demonstrate that co-occurring species respond to *I. glandulifera* invasion in different ways. *Impatiens glandulifera* was highly attractive to bumblebees, which reduced visitation to some co-flowering species, but caused increased visitation to a single native species, and had no detectable effect on others. Evidence was found to suggest that species responses to *I. glandulifera* may be predicted using floral traits, plant height, and using species Ellenberg light and nitrogen indicator values. This has wider significance for predicting the impacts of invasive alien plants, and could enable targeted identification of potentially vulnerable native species. This research also aimed to investigate previously unexplored effects of *I. glandulifera*. Interspecific pollen transfer did not appear to prevent seed set in a focal native species, but such effects are likely to be species-specific and the response of other natives could differ. *Impatiens glandulifera* was found to disrupt pollen dispersal patterns and native plant mating systems, demonstrating the need to address the more subtle effects of invasive alien plants. In conclusion, the direction and magnitude of the effects of invasion varied with the abundance of the invader, spatial scale, and the way in which impact was assessed. This demonstrates the need for a wide range of approaches in understanding the impact of invasive alien plants.

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Appendix A

Table A.1: *Impatiens glandulifera* abundance in 30 experimental plots tested as a function of land cover class from Centre for Ecology and Hydrology Land Cover Map 2000, in a 1 km area surrounding 30 experimental plots. Significance was tested using analysis of variance (ANOVA).

Land cover class	***F***	***P***
Arable	0.258	0.615
Grassland	1.006	0.324
Improved grassland	0.205	0.654
Urban	0.193	0.664
Woodland	0.013	0.911

Table A.2: Abbreviated and full species names of plants recorded in 30 experimental plots across central and south-east Scotland between May and September 2010, and their native status. Native status information from Hill et al. (2004) PlantAtt: Attributes of British and Irish plants. Raven Marketing Group, Cambridgeshire.

Abbreviated name	**Full name**	**Native status**
Aeg.pod	*Aegopodium podagraria*	Archeophyte
Aet.cyn	*Aethusa cynapium*	Native
Alc.vul	*Alchemilla vulgaris* agg	Native
All.pet	*Alliaria petiolata*	Native
All.urs	*Allium ursinum*	Native
Ant.syl	*Anthriscus sylvestris*	Native
Ast.	*Asteraceae* unknown species	
Bel.per	*Bellis perennis*	Native
Bra.1 – Bra.3	*Brassica* unknown species 1 - 3	
Bra.nap	*Brassica napus*	Neophyte
Cal.pal	*Caltha palustris*	Native
Cal.sep	*Calystegia sepium*	Native
Cam.lat	*Campanula latifolia*	Native
Car.ama	*Cardamine amara*	Native
Car.fle	*Cardamine flexuosa*	Native
Car.pra	*Cardamine pratensis*	Native
Cen.nig	*Centaurea nigra*	Native
Cer.fon	*Cerastium fontanum*	Native
Cha.ang	*Chamerion angustifolium*	Native

Abbreviated name	**Full name**	**Native status**
Chr.opp	*Chrysosplenium oppositifolium*	Native
Cir.lut	*Ciracaea lutetiana*	Native
Cir.arv	*Cirsium arvense*	Native
Cir.pal	*Cirsium palustre*	Native
Cir.vul	*Cirsium vulgare*	Native
Cla.sib	*Claytonia sibrica*	Neophyte
Con.arv	*Convolvulus arvensis*	Native
Cru.lae	*Cruciata laevipes*	Native
Dau.car	*Daucus carota*	Native
Dor.par	*Doronicum pardalianches*	Neophyte
Epi.hir	*Epilobium hirsutum*	Native
Epi.mon	*Epilobium montanum*	Native
Fal.jap	*Fallopia japonica*	Neophyte
Fil.ulm	*Filipendula ulmaria*	Native
Fra.ves	*Fragaria vesca*	Native
Gal.tet	*Galeopsis tetrahit*	Native
Gal.1, Gal. 2	*Galium* unknown species 1 & 2	
Gal.apa	*Galium aparine*	Native
Ger.mol	*Geranium molle*	Native
Ger.pra	*Geranium pratense*	Native
Ger.rob	*Geranium robertianum*	Native
Ger.san	*Geranium sanguineum*	Native
Geu.	*Geum* unknown species	Native
Geu.riv	*Geum rivale*	Native
Geu.urb	*Geum urbanum*	Native
Gle.hed	*Glechoma hederacea*	Native
Hed.hel	*Hedera helix*	Native
Her.man	*Heracleum mantegazzianum*	Neophyte
Her.sph	*Heracleum sphondylium*	Native
Hes.mat	*Hesperis matronalis*	Neophyte
Hie.	*Hieracium* unknown species	Native
Hya.non	*Hyacinthoides non-scripta*	Native
Hyp.per	*Hypericum perforatum*	Native
Ile.aqu	*Ilex aquifolium*	Native
Lam.alb	*Lamium album*	Archeophyte
Lap.com	*Lapsana communis*	Native
Lat.pra	*Lathyrus pratensis*	Native
Lys.vul	*Lysimachia vulgaris*	Native
Men.aqu	*Mentha aquatica*	Native

Abbreviated name	Full name	Native status
Men.spi	*Mentha spicata*	Archeophyte
Mer.per	*Mercurialis perennis*	Native
Myo.sco	*Myosotis scorpioides*	Native
Myr.odo	*Myrrhis odorata*	Neophyte
Ori.vul	*Origanum vulgare*	Native
Pap.rho	*Papaver rhoeas*	Archeophyte
Per.mac	*Persicaria maculosa*	Native
Pet.hyb	*Petasites hybridus*	Native
Pla.lan	*Plantago lanceolata*	Native
Pot.ans	*Potentilla anserina*	Native
Pru.vul	*Prunella vulgaris*	Native
Ran.acr	*Ranunculus acris*	Native
Ran.fic	*Ranunculus ficaria*	Native
Ran.rep	*Ranunculus repens*	Native
Rub.fru	*Rubus fruticosus*	Native
Rub.ida	*Rubus idaeus*	Native
Scr.nod	*Scrophularia nodosa*	Native
Sen.jac	*Senecio jacobaea*	Native
Sil.dio	*Silene dioica*	Native
Son.asp	*Sonchus asper*	Native
Sta.pal	*Stachys palustris*	Native
Sta.syl	*Stachys sylvatica*	Native
Ste.gra	*Stellaria graminea*	Native
Ste.hol	*Stellaria holostea*	Native
Ste.nem	*Stellaria nemorum*	Native
Sym.off	*Symphytum officinale*	Native
Sym.x.upl	*Symphytum x uplandicum*	Neophyte
Tan.vul	*Tanacetum vulgare*	Native
Tar.	*Taraxacum* agg.	Native
Tri.	*Trifolium* unknown species	
Urt.dio	*Urtica dioica*	Native
Ver.cha	*Veronica chamaedrys*	Native
Ver.per	*Veronica persica*	Neophyte
Vic.cra	*Vicia cracca*	Native
Vic.sep	*Vicia sepium*	Native
Vio.riv	*Viola riviniana*	Native
Unk.1 -Unk.7	Unknown species 1 - 7	

Table A.3: Plant species richness in 30 experimental plots sampled multiple times between May and September 2010, as a function of *Impatiens glandulifera* sbundance. GLMMs were used, according to a Poisson error distribution. Fixed effects were *I. glandulifera* abundance, date, number of trees (log), and bare ground (log). Plot and river were treated as random effects. A quadratic term, and an interaction term between *I. glandulifera* abundance and date were removed to find the minimum adequate model. Models contained non-significant terms as the lowest AIC value was used to find the best-fitting model.

***I. glandulifera* abundance**	**Other fixed effects**		**Estimate**	**Std error**	***z***	***p***
Quadrat scale (log)			0.035	0.079	0.445	0.656
	(Intercept)		2.323	0.135	17.170	
	Date	June	-0.136	0.124	-1.096	0.273
		July	-0.029	0.092	-0.319	0.749
		August	-0.089	0.120	-0.739	0.460
		September	-0.027	0.097	-0.278	0.781
	Number of trees (log)		0.116	0.184	0.629	0.529
	Bare ground (log)		-0.079	0.098	-0.804	0.421
Plot scale (log)			-0.113	0.089	-1.276	0.202
	(Intercept)		2.433	0.143	17.009	
	Date	June	-0.125	0.125	-0.998	0.318
		July	-0.012	0.093	-0.127	0.899
		August	-0.047	0.123	-0.385	0.700
		September	0.019	0.103	0.183	0.855
	Number of trees (log)		0.146	0.184	0.796	0.426
	Bare ground (log)		-0.094	0.099	-0.949	0.343
Plot scale: Rank max. abundance						
Low			0.073	0.173	0.424	0.672
Medium			0.041	0.170	0.241	0.810
High			-0.246	0.193	-1.271	0.204
	(Intercept)		2.329	0.174	13.377	
	Date	June	-0.129	0.125	-1.037	0.300
		July	-0.029	0.092	-0.315	0.753
		August	-0.081	0.120	-0.674	0.500
		September	-0.022	0.097	-0.227	0.820
	Number of trees (log)		0.231	0.195	1.189	0.234
	Bare ground (log)		-0.080	0.098	-0.818	0.413
Broad scale			-0.010	0.004	-2.320	0.020
	(Intercept)		2.533	0.160	15.807	
	Date	June	-0.107	0.126	-0.852	0.395
		July	-0.008	0.096	-0.086	0.932
		August	-0.053	0.121	-0.442	0.659
		September	0.015	0.010	0.148	0.882
	Number of trees (log)		0.031	0.180	0.174	0.862
	Bare ground (log)		-0.065	0.101	-0.640	0.522

Table A.4: The abundance of bumblebee-pollinated plant species in 30 experimental plots sampled multiple times between May and September 2010, as a function of *Impatiens glandulifera* abundance. GLMMs were used, according to a Poisson error distribution. Fixed effects were *I. glandulifera* abundance, date, number of trees (log), and bare ground (log). Plot and river were treated as random effects. A quadratic term, and an interaction term between *I. glandulifera* abundance and date were removed to find the minimum adequate model. Models contained non-significant terms as the lowest AIC value was used to find the best-fitting model.

***I. glandulifera* abundance**	**Other fixed effects**		**Estimate**	**Std error**	***z***	***p***
Quadrat scale (log)	Quadratic term		-0.323	0.216	-1.499	0.134
	Linear term		0.605	0.381	1.590	0.112
	(Intercept)		0.794	0.252	3.155	
	Date	June	-0.201	0.268	-0.751	0.453
		July	0.136	0.209	0.650	0.516
		August	0.261	0.275	0.946	0.344
		September	0.044	0.227	0.194	0.846
	Number of trees (log)		-0.124	0.262	-0.471	0.638
	Bare ground (log)		-0.124	0.209	-0.594	0.552
Plot scale (log)	Quadratic term		-0.932	0.310	-3.005	0.003
	Linear term		1.554	0.527	2.948	0.003
	(Intercept)		0.973	0.300	3.242	
	Date	June	-0.140	0.266	-0.525	0.599
		July	0.156	0.205	0.760	0.447
		August	0.385	0.273	0.412	0.233
		September	0.267	0.224	1.192	0.233
	Number of trees (log)		0.029	0.264	0.108	0.914
	Bare ground (log)		-0.184	0.209	-0.882	0.378
Plot scale: Rank max abundance						
	Low		0.695	0.233	2.990	0.003
	Medium		0.332	0.229	1.453	0.146
	High		0.161	0.245	0.660	0.509
	(Intercept)		0.480	0.282	1.699	0.089
	Date	June	-0.125	0.266	-0.469	0.639
		July	0.185	0.203	0.914	0.361
		August	0.351	0.269	1.302	0.193
		September	0.146	0.215	0.682	0.495
	Number of trees (log)		0.030	0.260	0.117	0.907
	Bare ground (log)		-0.085	0.206	-0.414	0.679
Broad scale	Linear term		0.009	0.006	1.531	0.126
	(Intercept)		0.530	0.241	2.198	
	Date	June	-0.191	0.261	-0.729	0.466
		July	0.194	0.207	0.937	0.349
		August	0.314	0.265	1.183	0.237
		September	0.191	0.218	0.873	0.383
	Number of trees (log)		0.047	0.236	0.198	0.843
	Bare ground (log)		-0.242	0.208	-1.163	0.245

Table A.5: Community weighted average of plant abundance according to their Ellenberg light tolerance values, in 30 experimental plots sampled multiple times between May and September 2010. Generalised linear mixed models were used, according to a Gaussian error distribution. Fixed effects were *I. glandulifera* abundance, at the quadrat plot and landscape scales, percentage cover of bare ground in the plot (log) and the number of trees in the plot (log). River and plot were treated as random factors. Date was removed to find the minimum adequate model. Significance values were calculated by permutation tests. Models were weighted by the number of sampling occasions. Models contained non-significant terms as the lowest AIC value was used to find the best-fitting model.

***I. glandulifera* abundance**	**Other fixed effects**	**Estimate**	**Std error**	***t***	***p***
Quadrat scale (log)		0.200	0.065	3.09	0.001
	(Intercept)	6.133	0.096	63.65	
	Bare ground (log)	-0.200	0.058	-3.42	0.058
	No. of trees (log)	-0.547	0.166	-3.30	<0.001
Plot scale (log)		0.078	0.076	1.03	0.250
	(Intercept)	6.238	0.097	64.20	
	Bare ground (log)	-0.195	0.062	-3.16	0.068
	No. of trees (log)	-0.581	0.161	-3.62	<0.001
Broad scale		0.007	0.004	1.68	0.023
	(Intercept)	6.081	0.144	42.17	
	Bare ground (log)	-0.235	0.064	-3.68	0.037
	No. of trees (log)	-0.415	0.178	-2.33	0.004

Table A.6: Community weighted average of plant abundance according to their Ellenberg nitrogen tolerance values, in 30 experimental plots sampled multiple times between May and September 2010. Generalised linear mixed models were used, according to a Gaussian error distribution. Fixed effects were *I. glandulifera* abundance, at the quadrat plot and landscape scales, percentage cover of bare ground in the plot (log) and the number of trees in the plot (log). River and plot were treated as random effects. Date was removed to find the minimum adequate model. Significance values were calculated by permutation tests. Models were weighted by the number of sampling occasions. Models contained non-significant terms as the lowest AIC value was used to find the best-fitting model.

***I. glandulifera* abundance**	**Other fixed effects**	**Estimate**	**Std error**	***t***	***p***
Quadrat scale (log)		-0.097	0.010	-0.97	0.173
	(Intercept)	6.860	0.131	52.21	
	Bare ground (log)	-0.017	0.094	-0.18	0.887
	No. of trees (log)	-0.368	0.239	-1.54	0.011
Plot scale (log)		-0.010	0.114	-0.08	0.902
	(Intercept)	6.789	0.146	46.62	
	Bare ground (log)	-0.017	0.097	-0.17	0.883
	No. of trees (log)	-0.371	0.238	-1.56	0.011
Broad scale		0.009	0.006	1.63	0.011
	(Intercept)	6.597	0.186	35.53	
	Bare ground (log)	-0.015	0.101	-0.15	0.904
	No. of trees (log)	-0.332	0.229	-1.45	0.039

Table A.7: Community weighted average of plant abundance according to their height, in 30 experimental plots sampled multiple times between May and September 2010. Generalised linear mixed models were used, according to a Gaussian error distribution. Fixed effects were *I. glandulifera* abundance, at the quadrat plot and landscape scales, percentage cover of bare ground in the plot (log) and the number of trees in the plot (log). River and plot were treated as random effects. Date was removed to find the minimum adequate model. Significance values were calculated by permutation tests. Models were weighted by the number of sampling occasions. Models contained non-significant terms as the lowest AIC value was used to find the best-fitting model.

***I. glandulifera* abundance**	**Other fixed effects**	**Estimate**	**Std error**	***t***	***p***
Quadrat scale (log)		-0.012	0.020	-0.62	0.429
	(Intercept)	2.021	0.027	73.55	
	Bare ground (log)	-0.043	0.018	-2.45	0.092
	No. of trees (log)	-0.111	0.051	-2.17	<0.001
Plot scale (log)		0.051	0.021	2.41	0.002
	(Intercept)	1.969	0.031	64.48	
	Bare ground (log)	-0.034	0.018	-1.86	0.191
	No. of trees (log)	-0.126	0.047	-2.66	<0.001
Broad scale		0.004	0.001	3.55	<0.001
	(Intercept)	1.920	0.039	49.17	
	Bare ground (log)	-0.042	0.019	-2.22	0.130
	No. of trees (log)	-0.084	0.048	-1.75	0.018

Appendix B

Table B.1: Species list and conservation status of all insects captured by pan trapping between May and September 2010 in 30 plots across central and south-east Scotland. Conservation status information was gathered from the Hoverfly Recording Scheme website, www.hoverfly.org.uk; and the Bees Wasps and Ants Recording Scheme website, www.bwars.com, accessed September 2012. Species information for sawflies is not available.

Taxonomic grouping	Species	Conservation status
Hymenoptera		
Vespidae: Eumeninae	*Ancistrocerus oviventris*	Widespread, but recent decline in central and eastern England
Vespidae: Vespinae	*Dolichovespula sylvestris*	Widespread and common
	Vespula austriaca	Widespread
	Vespula germanica	Widespread and common
	Vespula rufa	Widespread
	Vespula vulgaris	Widespread and common
Crabronidae: Larrinae	*Trypoxylon attenuatum*	Widespread
	Trypoxylon calvicerum	Widespread
Crabronidae: Crabroninae	*Crossocerus elongatulus*	Widespread
	Crossocerus pusillis	Widespread
	Ectemnius cavifrons	Widespread. In Scotland, only in the south-east
Crabronidae: Mellininae	*Mellinus arvensis*	Widespread and common
Apidae: Andreninae	*Andrena bicolor*	Widespread but mainly coastal in Scotland
	Andrena fucata	Widespread
	Andrena haemorrhoa	Widespread
	Andrena nigroaenea	Widespread
	Andrena scotica	Widespread
	Andrena subopaca	Widespread
Apidae: Halictinae	*Halictus rubicundus*	Widespread
	Halictus tumulorum	Widespread
	Lasioglossum albipes	Widespread
	Lasioglossum calceatum	Widespread
Apidae: Megachilinae	*Megachile centuncularis*	Widespread
	Osmia bicornis	Widespread and common
Apidae: Anthophorinae	*Anthophora furcata*	Widespread
	Nomada ruficornis	Widespread

Taxonomic grouping	Species	Conservation status
Apidae: Apinae	*Apis mellifera*	Widespread and common
	Bombus barbutellus	Widespread, rarely common
	Bombus bohemicus	Widespread, more common in the north
	Bombus hortorum	Widespread and common
	Bombus lapidarius	Widespread and common
	Bombus lucorum/terrestris	Widespread and common
	Bombus pascuorum	Widespread and common
	Bombus pratorum	Widespread and common
Diptera Syrphidae	*Baccha elongata*	Widespread where woodland occurs
	Eoseristalis arbustorum	Widespread and common, migrant species
	Eoseristalis horticola	Widespread and frequent, more abundant in Scotland
	Eoseristalis interruptus	Widespread and common
	Eoseristalis pertinax	Widespread and common
	Episyrphus balteatus	Abundant and widespread
	Eristalis abusivus	Widely distributed, more frequent near the coast
	Eristalis tenax	Widespread and very common
	Eumerus strigatus	Widespread
	Eupeodes corollae	Common
	Eupeodes latifasciatus	Widespread but scarce. May be migrant species
	Eupeodes luniger	Widespread and common
	Eupeodes nielseni	Notable (Falk 1991). Associated with conifers
	Heliophilus hybridus	Widespread but local
	Heliophilus pendulus	Widespread and common
	Melangyna labiatarum/compositarum	
	Melanostoma mellinum	Abundant and widespread
	Melanostoma scalare	Widespread and common
	Merodon equestris	Widespread and common
	Neoascia obliqua	Notable (Falk 1991). Widely distributed but generally scarce

Taxonomic grouping	Species	Conservation status
Diptera Syrphidae	*Neoascia podagrica*	Widespread and very common
	Platycheirus albimanus	Widespread and common
	Platycheirus clypeatus	Widespread and common
	Platycheirus granditarsus	Widespread and common
	Platycheirus manicatus	Widespread and common
	Platycheirus nielseni	Widespread in north and west
	Platycheirus peltatus	Widespread and common
	Platycheirus scutatus	Widespread and common
	Platycheirus tarsalis	Widespread and abundant in woodland; scattered records in the north
	Portevinia maculata	Widespread, particularly abundant where larval food plant *Allium ursinum* occurs.
	Rhingia campestris	Widespread and very common
	Ripponensia splendens	Widespread and common, but scarcer in the north
	Scaeva pyrastri	Widespread, migrant species
	Sericomyia silentis	Widespread and abundant
	Syritta pipiens	Widespread and common
	Syrphus ribesii	Widespread and abundant
	Syrphus vitripennis/rectus	Widespread and common
	Volucella pellucens	Widespread and common
	Xylota segnis	Widespread and common
	Xylota sylvarum	Widespread and common, local in Scotland
Symphyta	*Aglaostigma fulvipes*	
	Athalia lineolata	
	Athalia rosae	
	Dicrodolerus vestigialis	
	Empria pallimacula	
	Eutomostethus ephippium	
	Macrophya albicincta	
	Macrophya albipuncta	
	Monophadnoides geniculata	
	Monophadnes pallescens	
	Monostegia abdominalis	

Taxonomic grouping	Species	Conservation status
Symphyta	*Pachynematus obductus*	
	Pachyprotasis rapae	
	Phriophorus brullei	
	Poodolerus fumosus	
	Pristiphora species	
	Protoemphytus carpini	
	Protoemphytus pallipes	
	Tenthredo arcuata complex	
	Tenthredo colon	
	Tenthredo D group	
	Tenthredo livida	
	Tenthredo mesomelas	
	Tenthredo notha	
	Tenthredo velox	
	Tenthredopsis nassata	

Table B.2: Abundance of hoverflies in 30 experimental plots sampled multiple times between May and September 2010, as a function of *Impatiens glandulifera* abundance. GLMMs were used, according to a Poisson error distribution. Fixed effects were *I. glandulifera* abundance, number of trees (log), date, bare ground (log), estimated plant species richness (log) and estimated number of open flowers (log). Plot and river were treated as random effects, and individual level random effects were included to account for overdispersion in the data. In some models, bare ground and plant species richness were removed to find the minimum adequate model. Models contained non-significant terms as the lowest AIC value was used to find the best-fitting model.

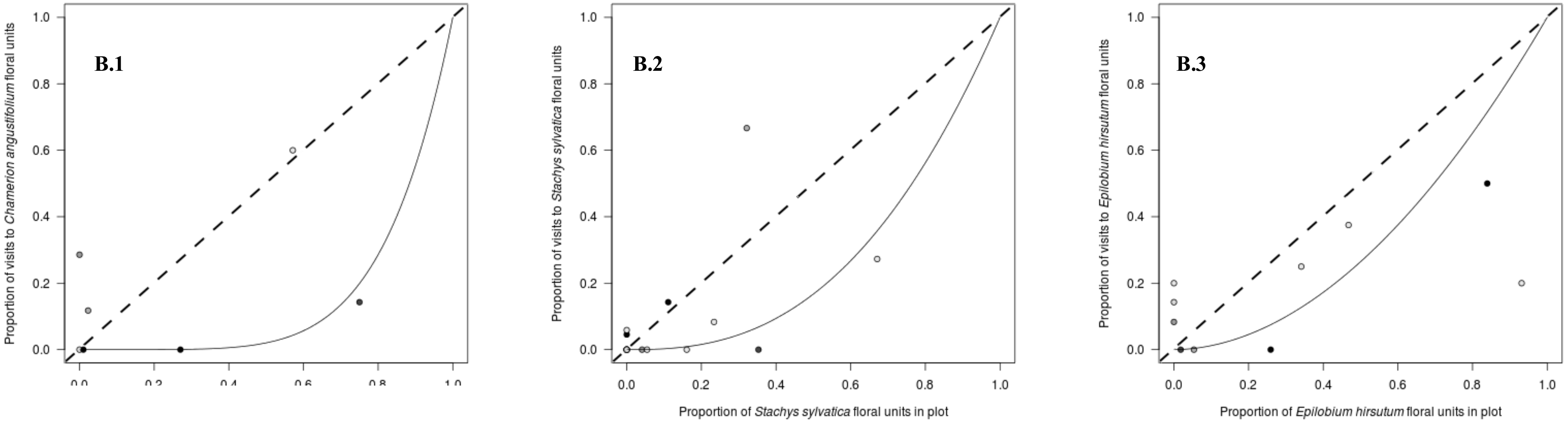
***I. glandulifera* abundance**	**Other fixed effects**		**Estimate**	**Std error**	***z***	***p***
Plot scale (log)			-0.821	0.093	-8.778	<0.001
	(Intercept)		2.557	0.235	10.891	
	No. of trees (log)		-0.613	0.218	-2.805	0.005
	Date	August	0.335	0.067	5.062	<0.001
		September	0.508	0.082	6.220	<0.001
	Bare ground (log)		-0.350	0.177	-1.975	0.005
	No. of flowers (log)		0.755	0.098	7.684	<0.001
Broad scale			-0.034	0.007	-4.780	<0.001
	(Intercept)		2.425	0.600	4.042	
	No. of trees (log)		-1.446	0.293	-4.930	<0.001
	Date	August	0.099	0.071	1.402	0.161
		September	0.186	0.087	2.140	0.032
	Plant species richness (log)		0.844	0.446	1.895	0.058
	Bare ground (log)		-0.353	0.219	-1.609	0.108
	No. of flowers (log)		0.618	0.110	5.604	<0.001

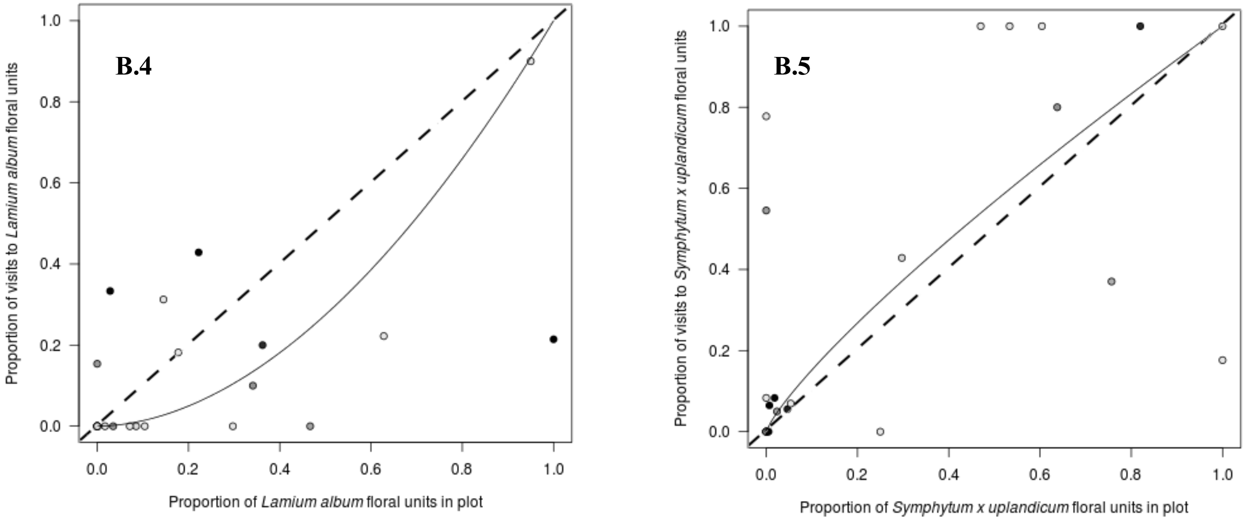
Table B.3: Abundance of social wasps in 30 experimental plots sampled multiple times between May and September 2010, as a function of Impatiens glandulifera abundance. GLMMs were used, according to a Poisson error distribution. Fixed effects were *I. glandulifera* abundance, number of trees (log), date, bare ground (log), estimated plant species richness (log) and estimated number of open flowers (log). Plot and river were treated as random effects, and individual level random effects were included to account for overdispersion in the data. In some models, bare ground and plant species richness were removed to find the minimum adequate model. Models contained non-significant terms as the lowest AIC value was used to find the best-fitting model.

***I. glandulifera* abundance**	**Other fixed effects**	**Estimate**	**Std error**	***z***	***p***
Plot scale (log)		0.663	0.304	2.180	0.029
	(Intercept)	-1.366	1.478	-0.924	0.356
	No. of trees (log)	0.310	0.688	0.450	0.653
	No. of flowers (log)	0.426	0.235	1.815	0.069
	Plant species richness (log)	0.511	1.110	0.460	0.645
Broad scale		0.041	0.020	2.047	0.041
	(Intercept)	-2.221	1.729	-1.285	0.199
	No. of trees (log)	1.085	0.875	1.240	0.215
	No. of flowers (log)	0.431	0.251	1.717	0.086
	Plant species richness (log)	0.696	1.212	0.574	0.566

Table B.4: Abundance of bumblebees in 30 experimental plots sampled multiple times between May and September 2010, as a function of *Impatiens glandulifera* abundance. GLMMs were used, according to a Poisson error distribution. Fixed effects were *I. glandulifera* abundance, number of trees (log), date, bare ground (log), estimated plant species richness (log) and estimated number of open flowers (log). Plot and river were treated as random effects, and individual level random effects were included to account for overdispersion in the data. In some models, bare ground and plant species richness were removed to find the minimum adequate model. Models contained non-significant terms as the lowest AIC value was used to find the best-fitting model.

***I. glandulifera* abundance**	**Other fixed effects**		**Estimate**	**Std error**	***z***	***p***
Plot scale (log)			0.240	0.236	1.016	0.309
	(Intercept)					
	No. of trees (log)		2.051	0.582	-3.526	0.0004
	Date	August	0.465	0.142	3.269	0.001
		September	-0.222	0.194	-1.146	0.252
	No. of flowers (log)		0.657	0.255	2.580	0.010
	Bare ground (log)		-0.356	0.431	-0.825	0.409
	Plant species richness (log)		2.463	0.919	2.681	0.007
Broad scale			-0.003	0.014	-0.195	0.845
	(Intercept)		-2.592	1.292	-2.007	0.045
	No. of trees (log)		-2.456	0.446	-1.194	0.233
	Date	August	0.157	0.158	0.996	0.319
		September	-0.566	0.211	-2.680	0.007
	No. of flowers (log)		0.588	0.290	2.026	0.043
	Plant species richness (log)		3.402	0.999	3.404	0.001





Figures B.1 - B.5: Proportion of bumblebees foraging on **B.1)** *Chamerion angustifolium* **B.2)** *Stachys sylvatica* **B.3)** *Epilobium hirsutum* **B.4)** *Lamium album* and **B.5)** *Symphytum x uplandicum* in 16 experimental plots, sampled from 20 m transect walks in June, July and August 2010. The dashed line represents the null expectation that the proportion of visits is equal to the proportion of floral units.

Floral units were measured as a proportion of total floral units present of a given species, at the plot scale, in five 1m^2 quadrats. The abundance of *Impatiens glandulifera* is represented by a grey scale where the darker the point, the higher its abundance (measured as the proportion of *I. glandulifera* flowers in the plot). The fitted line was calculated from NLS models (Table 3.8) and weighted according to the number of bumblebees per plot.

Appendix C

A series of experiments were conducted to determine 1) whether *Lamium album* flowers are self-compatible, 2) when *L. album* stigmata are receptive and anthers dehisce, 3) when *L. album* pollen is viable, and 4) when *Impatiens glandulifera* pollen is viable.

1. Within-flower self-compatibility in *Lamium album*

Methods

In May 2011, 15 plants were randomly assigned to one of three treatments: *closed*, where flowers were unmanipulated; *selfed*, where flowers were pollinated with pollen from the same flower; or left *open to pollinators*. Entire plants were covered with mesh bags to exclude pollinators, except for the open to pollinators treatment, and were kept in the greenhouse for the duration of the experiment. Doors to the greenhouse were left open to allow pollinators access to the *open to pollinators* treatment plants. Plants were positioned on the glasshouse bench in a random order.

Newly opened flowers were selected and the lip of the corolla was cut to identify them. All flowers from three whorls on each plant received the treatment. In selfed treatments, pollen was transferred from the anthers to the stigma of the same flower using a wooden toothpick. This was repeated after 24 hours. Plants were left for 3 weeks, then seed was counted as it matured.

Results and Discussion

The number of flowers used in the study and seeds produced are shown in Table C.1. Flowers left open to pollinators had the highest seed set (Figure C.1). Selfed and closed flowers had low seed set.

Table C.1: Number of *Lamium album* flowers used in hand-pollination treatments: *Closed* = no pollen; *Selfed* = *L. album* pollen; and *Open to pollinators*. Total number of seeds produced by each treatment, and the maximum seed number possible, given that each flower can produce 4 seeds.

Treatment	Number of flowers	Number of seeds	Max. number of seeds
Closed	186	13	744
Selfed	139	41	556
Open to pollinators	190	349	760

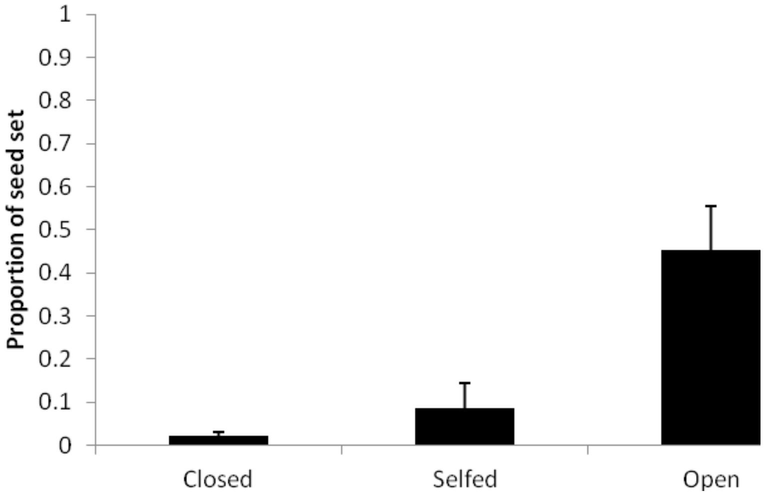


Figure C.1: Proportion of seed set (maximum of 4 seeds) per flower following treatments: *Closed* = unmanipulated; *Selfed* = within-flower *Lamium album* pollen; *Open* = open to pollinators. Values are means per treatment + SE.

Seed was produced in the closed and selfed treatments, indicating that *L. album* is self compatible. Seed set was highest when open to pollinators, suggesting that facilitated selfing or outcross pollen could result in higher seed set.

2. *Lamium album* stigma receptivity and anther dehiscence

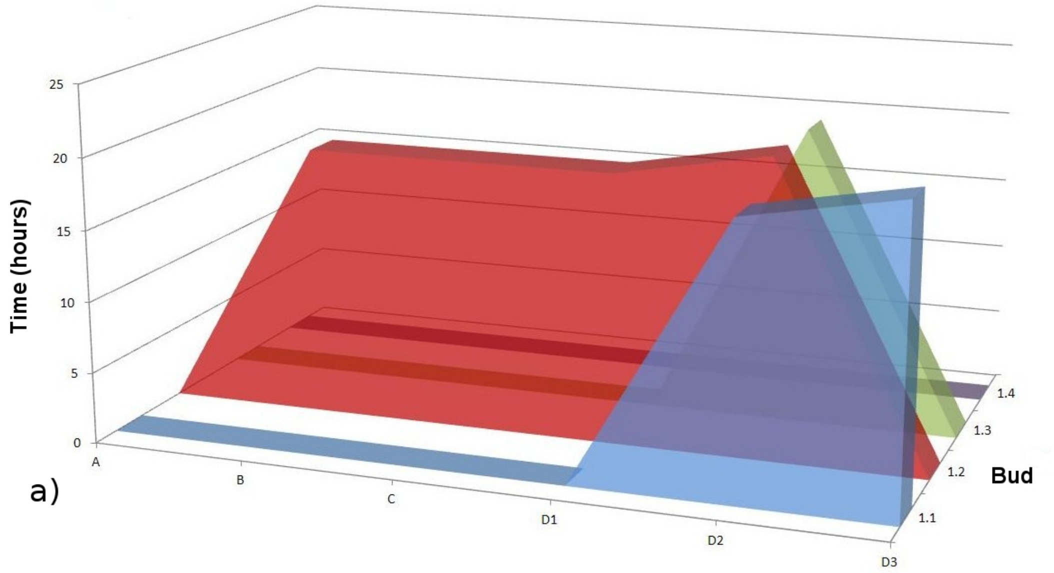
a) Events in the single flower

Methods

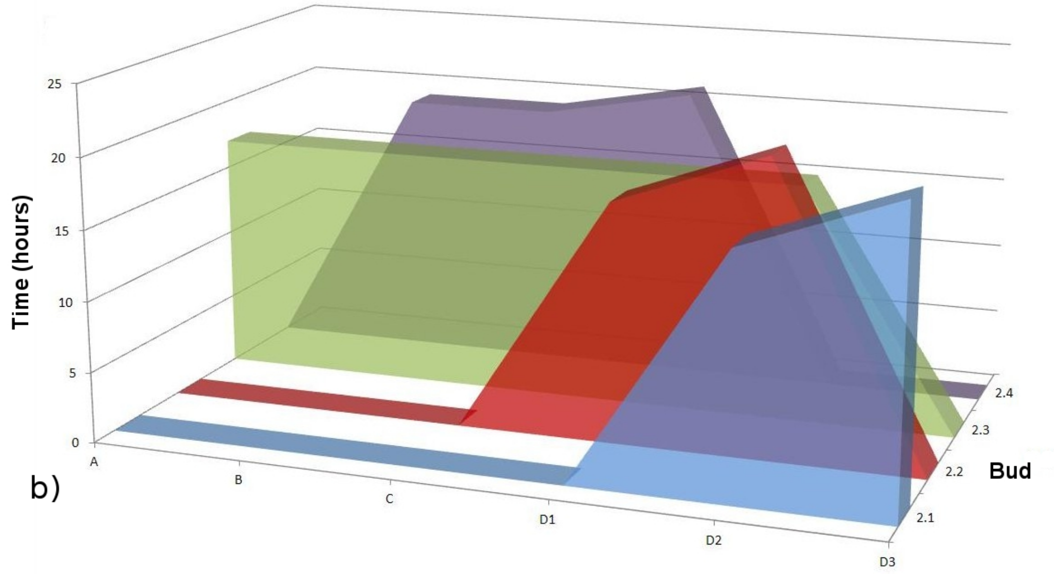
Methods were adapted from Dafni et al (2005). In June 2011, between two and five flowers from five plants were monitored every 2 - 4 hours in the daytime, from bud stage to wilting, between 17.00 on day one until 14.00 on day two. Flowers were noted as being a small bud, large bud, opening, in full bloom or wilting. At the full bloom stage, anthers were recorded as pre-pollen exposure, exposing pollen, or post-pollen exposure. At the wilting stage, the order in which the floral organs wilted was recorded. Throughout the study, any changes in the stigma position, colour or shape were recorded.

Results and Discussion

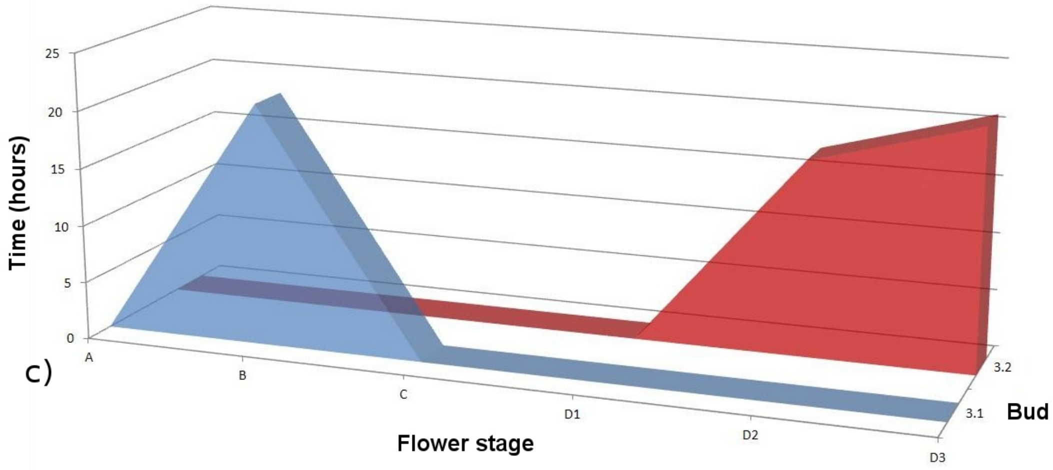
The time taken for buds to open showed large variation, from two hours to not opening in the duration of the experiment (Figure C.2). Anthers dehisced before flowers were in full bloom, indicating that pollen is released while the flower is still in bud. There were no morphological changes to the stigma to indicate its receptivity.


Time (hours)
25
20
15
10
5
0
A
B
C
D1
D2
D3
1.1
1.2
1.3
1.4
Bud

a)


Time (hours)
25
20
15
10
5
0
A
B
C
D1
D2
D3
2.1
2.2
2.3
2.4
Bud

b)


Time (hours)
25
20
15
10
5
0
A
B
C
D1
D2
D3
Flower stage
3.1
3.2
Bud

c)

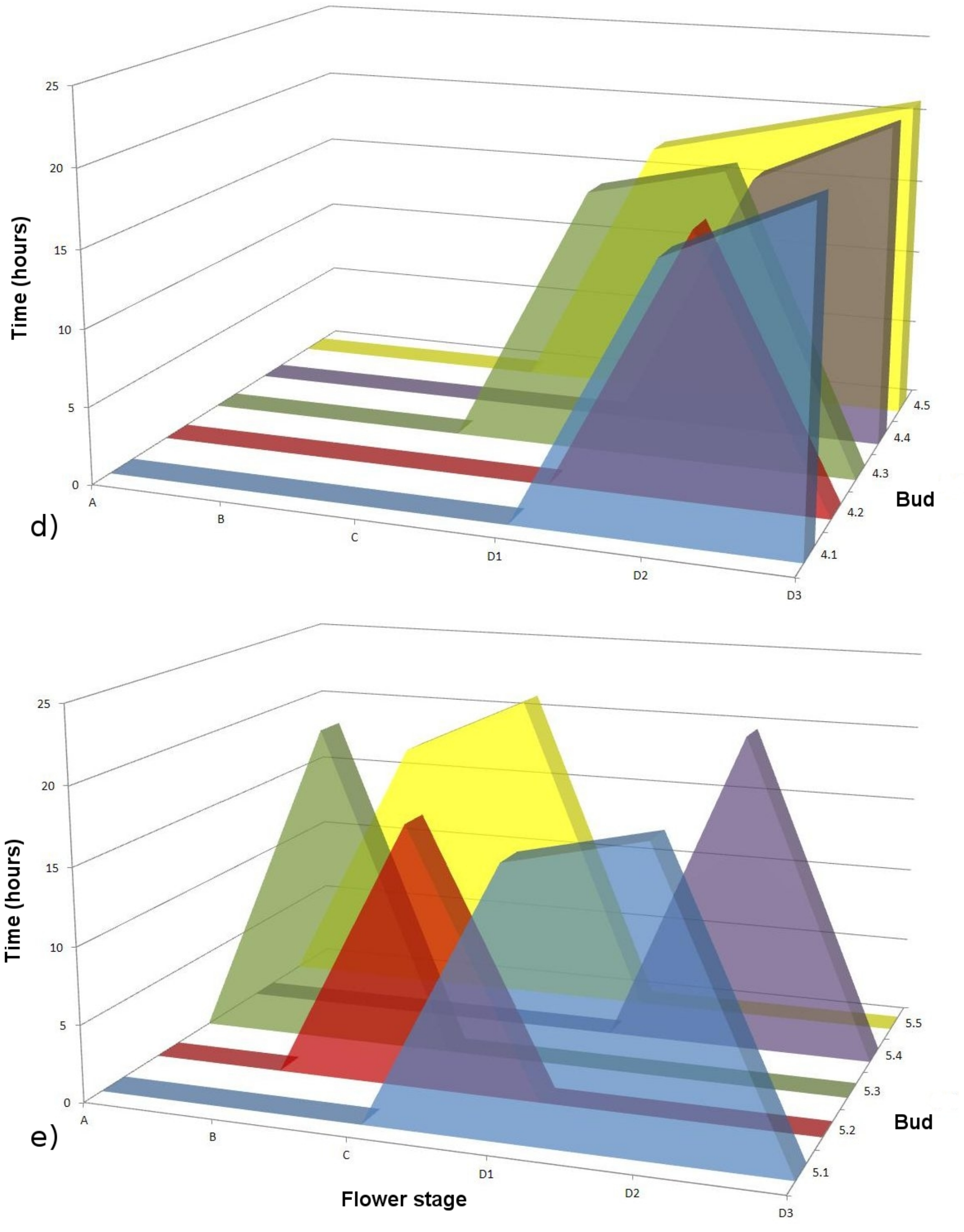


Figure C.2: The life span of *Lamium album* flowers. Time taken (hours) for buds of *L. album* flowers to open, bloom and wilt. Floral stages were categorised as **A** small bud, **B** large bud, **C** opening, **D1** in full bloom but anthers not fully dehiscing, **D2** in full bloom with anthers fully dehiscing, or **D3** wilting. Five *L. album* plants (Figures **a** to **e**) were selected and up to five buds were monitored on each plant. Methods adapted from Dafni et al (2005), Practical Pollination Biology, Enviroquest Publishing.

b) Stigma receptivity

Peroxtesmo KO test paper (Macherey-Nagael, GmbH, Duren, Germany) and Baker's test (Dafni 1992) were tried on *L. album* stigmas but showed no response. Instead, pollen tube growth was measured from hand-pollinated stigmas to determine receptivity.

Methods

Pollen tube growth was measured on stigmas at different stages of development; inside the bud, open flowers when anthers were fully dehiscing, and when anthers had shed all pollen (Dafni *et al.* 2005). This was repeated at 0, 2, 4, 6 and 24 hours after hand-pollinations. Two flowers from each stage and from five plants were used for each treatment.

Results and Discussion

Pollen and tubes were not visible as they stained the same colour as the stigma. Pollen tubes were visible on one stigma however, pollinated at the bud stage, which may have been due to differences in the way in which the stigma was cut when preparing the slides. Methods were adjusted by varying timings in the protocol, but no further pollen tubes were visible. Stigmas appeared receptive during the bud stage therefore, but since stigma receptivity could not be satisfactorily determined, it was decided that flowers should be pollinated twice in the experiment, with 24 hours between pollinations.

3. Pollen viability in *L. album*

Methods

Non-dehiscing anthers were removed from eight mature *L. album* buds and placed onto a microscope slide. Using a dissecting needle, pollen was removed and added to a drop of Brewbaker-Kwack medium (Brewbaker & Kwack 1963) on a microscope slide. The slide was placed on damp filter paper in a closed petri dish and left to germinate in the dark for 24 hours, at room temperature. This process was repeated with pollen taken from the anther after having been left on the slide for 10 minutes, then after 20 minutes, and at 20 minute intervals until 140 minutes. A drop of Analine blue stain was added to the slide prior to viewing under a dissecting microscope at x10 magnification. The number of pollen grains with and without tubes were counted from 5 randomly chosen fields of view.

Results and Discussion

Pollen was viable from unopened anthers. Viability quickly decreased over time, and started to decrease after 10 minutes, and was no longer viable at 140 minutes (Figure C.3). Therefore, pollen should not be stored during the experiment, and will be used fresh from unopened anthers.

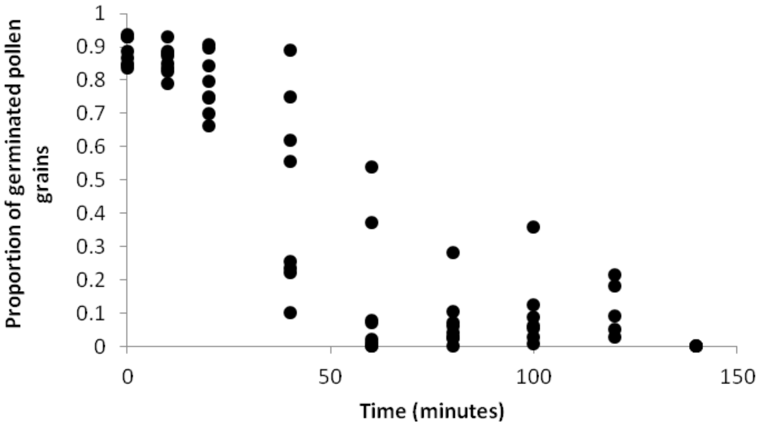


Figure C.3: Proportion of germinated (viable) pollen grains from 8 *Lamium album* flowers over time (minutes).

4. Pollen viability in *I. glandulifera*

Methods

Non-dehiscing anthers were removed from six mature, unopened *I. glandulifera* buds. The same methods as above were used. Additionally, anthers from six flowers were put into glass tubes to test whether pollen can be stored for use in the experiment. Pollen was sampled at 0, 30, 60, 120, 240 and 480 minutes.

Results and Discussion

Pollen was viable from unopened anthers and remained viable for up to 140 minutes (Figure C.4). Pollen remained viable when stored in a glass tube for up to 480 minutes (Figure C.5). *Impatiens glandulifera* pollen will therefore be stored in tubes during the experiment, and discarded after 8 hours.

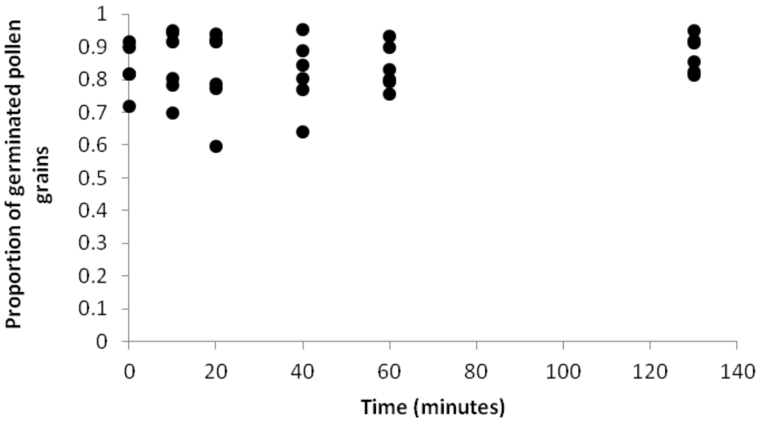


Figure C.4: Percentage of germinated (viable) pollen grains from 6 *Impatiens glandulifera* flowers over time (minutes).

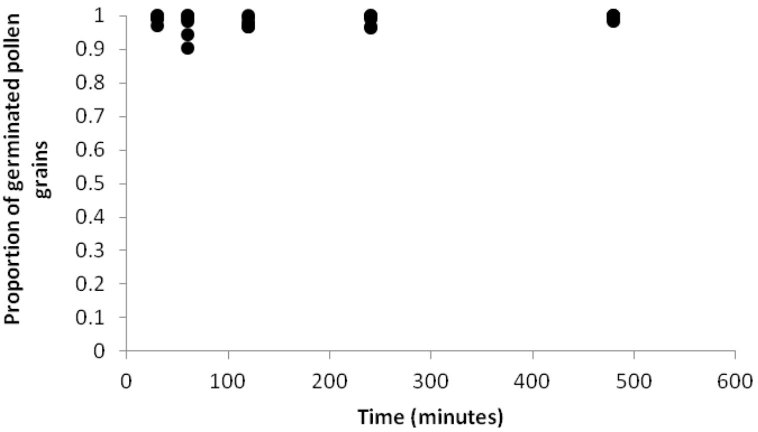


Figure C.5: Percentage of germinated (viable) pollen grains from 6 *Impatiens glandulifera* flowers over time (minutes), when anthers are stored in glass tubes.

References

Brewbaker, J.L. & Kwack, B.H. (1963) The essential role of calcium ion in pollen germination and pollen tube growth. *American Journal of Botany*, **50**, 747–858.

Dafni, A. (1992) *Pollination Ecology: a Practical Approach*. Oxford University Press.

Dafni, A., Kevan, P.G. & Husband, B.C. (2005) *Practical Pollination Biology*. Enviroquest.

Appendix D

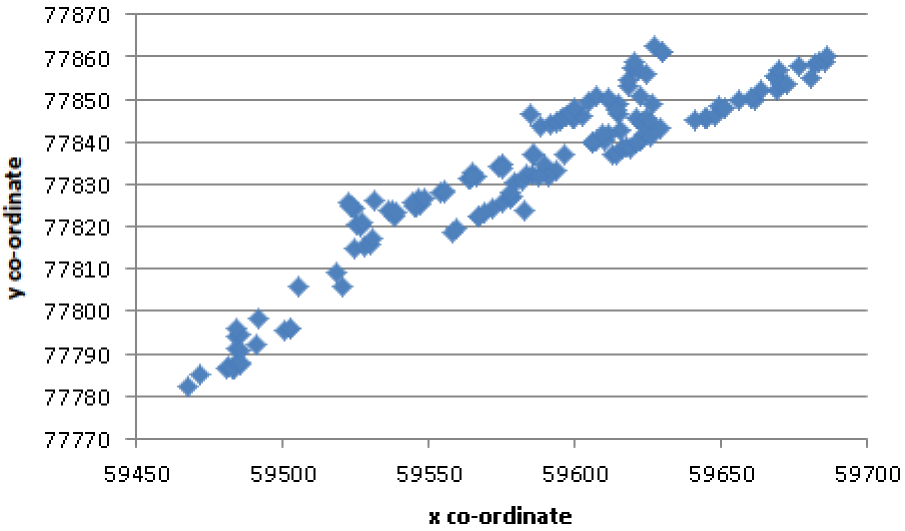


Figure D.1: Location of 155 *Lamium album* leaf samples from plants growing in riparian habitat in south-east Scotland, May 2011, collected to estimate the genetic structure of the population. Co-ordinates are British National Grid, NT, and measured to within a 1 m error with a GPS.

Appendix E

This Appendix was submitted as a primer note to Molecular Ecology Resources. Co-authored by S. Cavers and A. Telford.

Isolation and characterization of microsatellite markers for white deadnettle, Lamium album

Abstract

11 polymorphic microsatellite primers were isolated and characterized for *Lamium album*, a plant species that is native across temperate Eurasia. Six of these markers are recommended for use in genotyping. Markers were tested in 38 individuals from one location within the species range, and the number of alleles ranged from 3 to 11. All loci conformed to Hardy-Weinberg proportions, and observed heterozygosity levels ranged from 0.2 to 0.838. No gametic disequilibria were detected.

Introduction

White deadnettle, *Lamium album* (Lamiaceae) is an insect-pollinated hemicryptophyte plant. It is native across temperate Eurasia and is widely naturalised (Preston et al, 2003). Studying population structure and gene flow in this species will provide an insight into patterns of pollen dispersal within and between populations.

We describe the isolation and characterization of eight trinucleotide and three tetranucleotide microsatellite loci from *Lamium album*. DNA was extracted from leaf tissue taken from individuals on the river Tyne in East Linton, south-east Scotland (NT 607 780) (*n* = 40) using a DNeasy Plant mini kit (QIAGEN). Approximately 5m bp of genomic DNA sequence was obtained from one individual by 454 sequencing. The sequence was searched for 2, 3 and 4 bp repeat sequences using msatcommander (Rozen & Skaletsky, 2000; Faircloth, 2008) and primers were designed for potential marker loci, with preference for loci in multiplex combinations. Potential markers were tested for polymerase chain reaction (PCR) amplification in 8 individuals from the sample set and those showing consistent amplification, polymorphic and easily interpretable banding patterns were amplified in the whole sample set.

Methods

PCR conditions were optimized using eight individuals; subsequently all 40 individuals in the population were genotyped. Two individuals amplified poorly and were discarded. All microsatellites were amplified using 10 µl PCR reactions, each comprising 1µl of genomic DNA, 1µl PCR buffer, 0.2 µl primers, 0.2 µl dNTPs, and 0.08 µl of *Taq* DNA polymerase (New England Biolabs). Reactions were run on a Hybaid MBS thermocycler using the following protocol for all loci: 3 min at 94 °C, then 40 cycles of 1 min at 94 °C, 1 min at 60 °C, 1 min at 72 °C, followed by 1 cycle of 10 min at 72 °C. In all cases the forward primer was labelled with either IRD 700 or 800 fluorescent label (MWG Biotech). PCR products were then separated on 6% denaturing polyacrylamide gel (25 cm), and visualised using a LI-COR 4200 IR2 automated genotyper. PCR products were run out alongside a microSTEP DNA size standard (Microzone Limited) and fragment sizes were scored using SAGA™ software. Numbers of alleles, allelic richness, observed and expected heterozygosities and tests for Hardy-Weinberg (HW) proportions were calculated (GenAlEx v6, Peakall & Smouse, 2006). Null allele rates and gametic disequilibrium were estimated (Genepop v4.0, Rousset 2008).

Results and Discussion

On the basis of the population at East Linton, six microsatellite markers are recommended for use in genotyping. The numbers of alleles per locus ranged from 2 to 11, with levels of observed heterozygosity among loci ranging from 0.2 to 0.838. All loci conformed to HW proportions. The possible presence of null alleles was detected at some loci, but no gametic disequilibria between loci ($P > 0.05$) were observed. These loci should be highly useful for studies of diversity, gene flow and population structure in *L. album*. A further five microsatellite markers were developed, but a high probability of the presence of null alleles was detected, and all but one did not conform to HW proportions. This may be due to ascertainment bias, and there may be fewer problems in other populations. The number of alleles per locus ranged between 3 and 6, with observed heterozygosity among loci ranging from 0.258 and 0.417.

References

Faircloth, B.C., 2006. MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Molecular Ecology Resources*, **8**: 92-94.

Peakall, R. and Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288-295.

Preston, C.D, Pearman, D.A. and Dines, T.D. 2003. New Atlas of the British and Irish Flora, Oxford University Press.

Rousset, F., 2008. Genepop '007: a complete re-implementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103-106.

Rozen, S. and Skaletsky, H.J., 2000. Primer3 on the WWW for general users and for biologist programmers. Bioinformatics Methods and Protocols: Methods in Molecular Biology (eds. Krawetz, S. and Misener, S.), Humana Press, Totowa, NJ.

Table E.1: Primer sequences, characterisation and basic descriptive statistics of 11 microsatellite markers isolated from Lamium album. Bold loci are recommended for use in genotyping. *Indicates fluorescently labelled primer. Abbreviations: Number of alleles (N_A), Expected heterozygosity (H_E), Observed Heterozygosity (H_O), HW – deviation from Hardy-Weinburg proportions (* P<0.05, ** P<0.01, *** P<0.001).

Locus\ Gen Bank Accession	Primer Sequence 5'-3'	Repeat Motif	Population	Allele size	N_A	H_E	H_O	HW	Null Allele Frequency
LA5 KC621919	**F:* TGCCAAACGGCCCATATTC** **R: ACTGAATTTGCACAGTGATCTTG**	$(AAT)_7$	East Linton	266-272	3	0.359	0.286	NS	0.2
LA33 KC621920	**F: * ACCAGGGAAAGTGTCTCCAC** **R: GCAGCAGACTTGGCTGTTC**	$(ACT)_6$	East Linton	200-218	6	0.633	0.625	NS	0.0342
LA25 KC621921	**F:* GGAAGGGATGTCAGTCAGGG** **R: GTTGGCTCCTGTAAGATGCAC**	$(AATT)_4$	East Linton	180-195	3	0.537	0.583	NS	0.0412
LA54 KC621922	**F: * CAACTGGTGAAGACCATCGC** **R: GACAATTCTCGCTCCAACCG**	$(ACAT)_{15}$	East Linton	252-314	11	0.818	0.838	NS	0.0198
LA35 KC621923	**F: * TCTCCACTCGTTAATCGCAC** **R: ATTACATGATGGGATTAGGACAAC**	$(AATC)_4$	East Linton	212-216	2	0.180	0.200	NS	0.0
LA55 KC621924	**F: * TCCAGAGCTTCCCGATACC** **R: ACTATGGCGCTCAGCAAATG**	$(ACAT)_7$	East Linton	218-244	7	0.711	0.737	NS	0.0803
LA7 KC621925	F: * GAAGCCTAGTGAGGCGGTG R: CTCCCTAAGTCGTTTCTCGTG	$(AAG)_6$	East Linton	143-152	4	0.589	0.417	*	0.2613
LA34 KC621926	F: * CGTACGCTACAGGCAGAAC R: AGACACAATGCTAGCCATCC	$(ATT)_6$	East Linton	233-239	3	0.570	0.258	**	0.4577
LA16 KC621927	F: * AGTCACATGGAACTGATGGAAG R: CTGTACGGCGCAGATTTCG	$(AAT)_8$	East Linton	327-345	3	0.423	0.324	**	0.3077
LA63 KC621928	F: * AGCCTCGAACACTGACTCC R: CACTCACTCTGCCAATAGCC	$(ATT)_8$	East Linton	219-246	6	0.672	0.371	*	0.5652
LA58 KC621929	F: * TCATCACAAGAAATGGTCGACAG R: CCTGCGAGTCGTTGTTTCC	$(AGC)_6$	East Linton	190-196	3	0.431	0.405	NS	0.7392