The Effect of Inflorescence Architecture on Pollinator Behaviour and Plant Mating

HARRAP, MICHAEL,JOHN,MUNRO

How to cite:

HARRAP, MICHAEL,JOHN,MUNRO (2014) *The Effect of Inflorescence Architecture on Pollinator Behaviour and Plant Mating*, Durham theses, Durham University. Available at Durham E-Theses Online: http://etheses.dur.ac.uk/10826/

The Effect of Inflorescence Architecture on Pollinator Behaviour and Plant Mating

Michael J.M. Harrap

Supervisors:
Dr Shane Richards
and
Dr Philip Stephens

Durham University
Department of Biological
and Biomedical Sciences

A Master's Thesis
Submitted 2014

The Effect of Inflorescence Architecture on Pollinator Behaviour and Plant Mating

Michael J.M. Harrap

Abstract:

The three-dimensional arrangement of flowers and sexual function within plant inflorescences is known as inflorescence architecture. Some plants possess an arrangement of male and female flowers on vertical inflorescences, such as racemes, where female(-phase) flowers are arranged below male(-phase) flowers. As pollinators often move upwards while foraging in racemes, Darwin's syndrome has been suggested to reduce inter-flower self-pollen transport. Reducing inter-flower pollen transport can improve plant fitness by reducing inbreeding and increasing pollen export. Despite these observations, the influences of the inflorescence and directional movement of pollinators have been, until recently, overlooked. In this thesis I investigate the effects of inflorescence architecture on directional foraging and pollen transfer.

Pollen transfer simulation models are used to assess the impacts of differing pollinator movement within plants. Plants where all flowers function bisexually and when flowers function as either males or females are investigated. These simulations reveal that consistent movement should increase outcrossing and pollen export for both inflorescence types, not just those where sexual function is separated over the inflorescence. These advantages were dependent on the consistency of pollinator foraging behaviour. Therefore, selection should favour traits that encourage directional foraging in both inflorescence types. However these pollen transfer advantages were much greater in plants showing Darwin's syndrome, suggesting selection for arrangements which encourage male flowers to be visited after female flowers.

Observation of wild pollinators revealed five bee species foraged differently on *C. angustifolium*, in manners that simulations suggested should alter geitonogamy and therefore plant fitness. Furthermore, upwards flight in the two most common visitors to *C. angustifolium* was observed to be less time consuming then downward movements, providing an energetic explanation for the largely unanswered question of why many pollinators show upward movement in vertical inflorescences. Together, these findings demonstrate the significant role of inflorescence architecture in modulating plant-pollinator interactions. **[299 words]**

Statement of Copyright

List of Contents

List of Figures

List of Tables

Acknowledgements

I would like to begin by thanking my supervisors Dr Shane Richards and Dr Philip Stephens, for giving me this opportunity and their valuable help over the course of this project. This project was funded by the Durham University Grevillea trust and the Durham Biosciences Postgraduate fund, to which I would like to express my gratitude, as I would not have been able to undertake this project had I not had their support.

I would like to thank the following members of Durham university staff for aiding me in this project. I would like to thank Miss A Graven and Ms Lucy Glasper for aiding me in all administrative issues associated with this project; Prof Brian Huntley and Dr Martin Lucas, for their advice and comments during thesis committee meetings; Dr Sean Twiss, for his advice on usage of video equipment and for lending me cameras; Dr Stephen Willis, for his useful advice on study sites about Durham; Dr David Baker, for his advice and all the Durham University Biosciences lab technicians especially Mr P Loftus, for their aid in acquiring equipment. I like to also thank Dr Geraldine Wright (Newcastle University) for her advice on use of microcapillaries.

I would also like to thank my friends and fellow postgrad students in the Durham BEER CEG, Seal and Fish labs for their aid, support, company and biscuits during lab meetings: J. Alison, A. Bishop, S.A. Brooker, M.L. Davis, T. Docherty, M. Forty, N. Gauld, M.R. Gibson, S. Hardman, B.J. Hindle, C. Howard, O.J Kinsey, N.K. O’Mahony, H.M. O’Neill, G. Palmer, E.R. Perrin, A.M. Rogers, T.H.E. Mason, A. Voskamp. With additional thanks to A. Bishop and J.C. Warrin for taking a lunch break off to come out to help with data collection. Finally, I’d like to thank Miss I. Richardson for being a great flatmate and moral support.

Chapter 1: Inflorescence architecture and pollen transfer

Introduction

Over 87.5% of the estimated 300,000 species of angiosperms are pollinated by animal vectors (biotic pollination), rather than by pollen dispersal processes such as wind or water (abiotic pollination) (Ollerton et al., 2011). As sexual reproduction in angiosperms depends of pollen transfer, pollinators have a strong influence on the mating success of biotically pollinated plants. Through its impacts on pollen transfer, biotic pollination promotes the widespread diversification of angiosperms as selection favours traits that ensure more effective pollen transfer (Chittka et al., 2001; Fenster et al., 2004; Castellanos et al., 2004, Bluthgen & Klein, 2011). Pollinator-mediated selection on plant traits can lead to exaggerated traits such as long spurred flowers, on *Lapeirousia anceps* (Iridaceae), which evolved due to selection for increased pollen transfer from the plant’s long probocid pollinators (Pauw et al., 2008; Johnson & Anderson, 2010). This example, and others reviewed by Harder & Johnson (2009) demonstrate that to understand angiosperm evolution and the adaptiveness of a floral trait, its influence on visiting pollinators and pollen transfer must be considered.

Moreover, many plants of economic importance are biotically pollinated (Aizen et al., 2008; Gallai et al., 2009). Klein et al. (2007) found that 70% of the world’s main crop species require pollinators for complete seed set. Due to the great importance of plant-pollinator interactions, the influences of floral traits on pollen transfer have been the focus of extensive research. Floral traits examined include: flower scent (Shuttleworth & Johnson, 2009); position of stamens and stigmas (Sinu & Shivanna, 2007); flower shape (Castellanos et al., 2004; Coombs & Peter, 2009) and flower colour (Stanton, 1987; Drumont et al., 2010). However, many plants produce multiple flowers as an inflorescence, so the fitness of a plant is the aggregate fitness contribution of each flower. Thus, a focus on individual flowers, a practice critically dubbed “floricentrism” (Harder et al., 2004), fails to consider the effects of the arrangement of flowers on plants (inflorescence architecture) on pollinator behaviour and pollen transfer, including pollen transfer within plants.

In this thesis I investigate the influence of inflorescence architecture on pollinator behaviour and how these changes in pollinator behaviour in turn influence pollen transport. In this chapter I largely review current knowledge of how inflorescence architecture affects pollinator behaviour and pollen transfer, highlighting poorly understood topics, which will be the focus of following chapter. However, I first discuss how pollen transfer and pollinator behaviour impact plant fitness.

Pollen transfer, high quality pollination and plant fitness

Most flowering plants are hermaphrodites, functioning as both males and females (Lloyd & Bawa, 1984; Schlessman, 1987), although a small number, c. 6% are dioecious and function as only a male or female (Renner & Ricklefs, 1995). The fitness of a hermaphrodite plant depends on the combined influences of both male and female reproductive success (de Jong, 2000). Male reproductive success in angiosperms is achieved through seeds sired (Irwin & Brody, 2000; Routley & Husband, 2003). Male fitness is often highest when seeds are sired on many mates (Brunet & Charlesworth, 1995; Maloof, 2001). High male fitness requires that a plant exports a large amount of its pollen on many pollinators (Harder, 1990), which depends on pollinators removing pollen from flowers. Thus, it is in a plant’s interest to encourage frequent pollinator visitation (Harder & Wilson, 1994). However, how a pollinator behaves after it removes pollen will also influence male fitness. For example, pollinators prone to grooming or losing pollen in transit will remove and subsequently lose large quantities of pollen

(Thomson 1986). This results in low dispersal to other plants and lower male fitness (Harder & Barrett, 1996; Rademaker et al., 1997; Castellanos et al., 2004; Richards et al., 2009).

From the female perspective, angiosperm fitness is maximised when most fertilised ovules survive to maturity, high pollen receipt promotes seed set (Ashman et al., 2004; Jersáková & Johnson, 2006). High female reproductive success requires sufficient pollen receipt to fertilise all the ovules a plant can develop (Engel & Irwin, 2003). However, not all pollen is of the same quality (Aizen & Harder, 2007). A plant can receive either outcross pollen (pollen from conspecific plants), or self-pollen (pollen from the plant's own anthers). The source of the pollen that sired each seed can impact the fitness of the resultant offspring (Husband & Schemske, 1995 and 1997; Marten-Rodriguez et al., 2012).

Self-pollination can occur between anthers and stigmas within the same flowers (autogamy) or among flowers of the same plant (geitonogamy). Autogamy can be facilitated when a pollinator visits flowers where both anthers and stigmas are active (Galloway et al., 2002; Routley & Husband, 2006) or occur autonomously (e.g., Liu et al., 2006; Zhang & Li, 2008). Geitonogamy requires individual pollinators to visit different flowers of the same plant, carrying self-pollen between them (Karron et al., 2004; de Jong et al., 2011). Consequently, whether a pollinator makes further flower visits within a plant, as opposed to departing, influences the incidence and intensity of geitonogamous self-pollination.

Angiosperm mating systems range from complete selfing, where all offspring are the result of self-fertilisation, to exclusive outcrossing, where self-fertilisation of ovules does not occur (Goodwillie et al. 2005). A selfed individual receives two copies of each allele from its single parent, whereas an outcross individual receives one copy from each of its two parents. Consequently, for every individual selfed offspring a parent transfers twice as many copies of its genes to the next generation than an outcrossing parent does to a single offspring (Fisher, 1941). If there are no fitness costs incurred by selfing, this transmission advantage favours selfing (Harder et al., 2007). However, transferring both copies of each allele from the same individual increases the chances of recessive deleterious alleles being expressed by offspring (Charlesworth & Charlesworth, 1999; Keller & Waller, 2002). Expression of poor quality alleles may lead to poor progeny survival at many stages of the plant’s life cycle, although many plants often show higher inbreeding depression during pre-seed dispersal stages of development (Husband & Schemske, 1995 and 1997; Marten-Rodriguez et al., 2012). These two conflicting influences will lead to a plant favouring outcrossing if selfed progeny survival is poor (Harder et al., 2007). Outcrossing is expected if inbreeding depression is high, especially after offspring gain independence (Lande & Schemske, 1985; Lloyd, 1992; Harder et al., 2007). Due to the high costs of inbreeding many plants have evolved self-incompatibility mechanisms to prevent self-fertilisation (Seavey & Bawa, 1986; Takayama & Isogai, 2005).

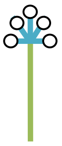
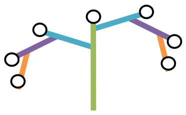
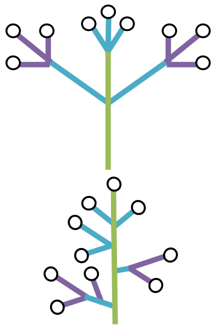
Even when self-fertilisation does not occur self-pollen deposition can impact plant fitness. For example, self-pollen can clog the stigma surface, reducing outcross pollen adherence and germination (Zhang et al., 2008), or self-pollen tubes may interfere with ovules impeding fertilisation by outcross pollen tubes (Sage et al., 1994), causing ovule discounting. Additionally, self-pollen deposition reduces the amount of a plant’s pollen available for export, which is referred to as pollen discounting (Harder & Wilson, 1995). A reduction in exportable pollen leads to a drop in male reproductive success. This cost suggests that selection should favour a reduction in self-pollen deposition in angiosperms if pollen discounting should limit male success (Harder et al., 2007), even when the costs to female reproductive success is low due to low inbreeding depressions or when self-incompatibility may prevent self-fertilisation (Harder & Barrett, 1996). It is thus possible that impacts on male fitness may favour reductions in self-pollen deposition. The costs of self-pollen deposition to male and female reproductive success should favour floral traits that reduce selfing and boost outcrossing (e.g. Adler & Irwin, 2005; Narbona et al., 2011; de Almeida et al., 2013).

Through the influence of self-pollen deposition and the associated pollen discounting on male and female success, pollinator foraging behaviour within plants has a major influence on the reproductive success of plants. Increases in the number of flowers each pollinator visits within inflorescences (foraging bout length) will result in greater opportunities for geitonogamy and therefore pollen discounting (Karron et al., 2004; Mitchell et al., 2004; Adler & Irwin, 2005; Albert et al., 2008). Increasing the number of visits made by each pollinator aggravates geitonogamy (Harder & Barrett, 1995; Routley & Husband, 2003), and thus, also lowers export (Klinkhamer et al., 1994; Harder & Barrett, 1995; Karron & Mitchell, 2012). However, increased flower visitation also increases outcross pollen receipt and pollen removal (Engel & Irwin, 2003). Due to these two balancing effects, there is a trade-off associated with bout length, between the benefits of visitation and the costs of geitonogamy (de Jong et al., 1992) and reduced export (Klinkhamer et al., 1994). Thus, plant traits that can increase bout length, like high levels of floral rewards (Johnson et al., 2004) and large inflorescences (Thomson, 1988; Galloway et al., 2002; Albert et al., 2008), or limit bout length such as rewardless-ness (Johnson et al., 2004; Jersáková et al., 2006), and the presence of secondary metabolites in nectar (Adler & Irwin, 2005; Irwin & Adler, 2008). By considering pollen transfer only at the flower level risks ignoring such effects and limits understanding of pollen transfer (Harder et al., 2004). Thus a consideration of inflorescence architecture and how pollinators forage through inflorescences is important.

Inflorescence architecture

Angiosperms show a wide range of diversity in inflorescence structure, as described by Troll (1969) (alse see Harder et al. 2004, Endress, 2010 and Harder & Prusinkiewicz, 2013). Table 1 displays the main inflorescence types by topological structure. Despite this diversity of inflorescence form, how architecture influences plant mating success has not been as widely investigated compared to floral characteristics (but see Galen & Plowright, 1985; Jordan & Harder, 2006; Ishii et al., 2008; Fenster et al., 2009; Iwata et al., 2012). Inflorescence architecture can be defined as the three-dimensional arrangements of flowers, and the sexual function of these flowers, within a plant. Architecture can be thought of as having four interacting components: topology, phenology, orientation and size (Harder et al., 2004). Topology represents the branching structure of the plant. Topology results in the basic inflorescence structures shown in Table 1.1. Phenology refers to flower development within the inflorescence. This includes timing and order of flower opening, in addition to the temporal separation of sexual function (dichogamy) expressed by flowers. Flower orientation describes the direction that flowers face away from the stem (Fulton & Hodges, 1999; Fenster et al., 2009). The size of the inflorescence display represents the fourth component of architecture. This last component has been studied more frequently than the others because of its strong association with the rate of flower visitation and bout length (Ohashi & Yahara, 2001; de Jong & Klinkhamer, 2005). Often, display size is considered an inflorescence trait rather than an aspect of architecture itself (e.g. Harder et al., 2004). Studies of display size normally focus on the number of open flowers in the inflorescence (Harder & Barrett, 1995; Galloway et al., 2002; Karron et al., 2004), but some work looks at the spatial volume occupied by the display (Ishii et al., 2008). As shown in table 1.1, inflorescence architecture encompasses a wide range of components that create the diversity in inflorescence form and could influence plant mating.

Table 1.1: The main inflorescence categories based on inflorescence topology. Also included are their classification criteria based on Troll (1969) and Endress (2010). As many of the classical inflorescence structures are included under racemose inflorescences in Troll's (1969) classification, the sub categories of racemose inflorescences are included. Categorization is based on branching axis number and elongation of branch axis. White circles represent flowers. Branching axes are coloured in the representations below: green, first axis; blue, second axis; purple, third axis; orange, fourth axis.

Inflorescence type	Definition	Diagram
Racemose Inflorescences	No limit on primary axis elongation but limited to only two inflorescence branching axes.	
Racemes	Extensive primary axis elongation. Second-order branching occurs throughout the primary axis. Longer levels of secondary axis branching.	
Spikes	Extensive primary axis elongation. Second-order branching occurs throughout the primary axis. No secondary axis branching.	
Heads	Limited primary axis elongation and little secondary axis elongation. Creates a cluster of flowers about the primary axis. Often creates a single flower-like structure known as a capitulum or pseudanthium.	
Umbels	No primary axis elongation and higher secondary axis elongation. Creates a flatter umbrella-like shape.	
Cymes	Little primary axis elongation and first axis branching but no limits on secondary axis elongation and levels subsequent branching axis number.	
Panicles	No limitation of branch axis and number of floral branches in each order. Branching tends to be greater at lower branches and higher at the top.	

Inflorescence architecture, pollinator movement and pollen transfer

Different inflorescence topologies influence the pattern and order of movements by visiting pollinators. Jordan & Harder (2006) observed bumblebee behaviour on artificial inflorescences representing different topologies (panicles, umbels and racemes). On racemes, bumblebees showed a strong directional tendency, generally beginning foraging on the bottom whorls of flowers (85% of arrivals), then moving upwards (71% of all movement between whorls of flowers) before departing from the upper whorls (58% of all departures). On panicles, bumblebees also tended to move upwards, although less strongly than on racemes. Bumblebees visited flowers on the lower branches first (98.2% of arrivals) and typically moved upward to depart from "distal flowers of upper branches" (55.5% of departures); however, bees occasionally departed from the end of a lower branch (10.2% of departures) (Jordan & Harder, 2006). In inflorescences with little vertical dimension, such as umbels and cymes, the pattern of movement is less consistent. Jordan & Harder (2006) reported that bees on umbels visited the outside flowers first, and then moved to central flowers. Bees in the centre of an umbel were as likely to move to outer flowers as to other central flowers. Bees on outer flowers were more likely to move inwards and more likely to leave. Similarly, de Jong et al. (2011) reported random foraging of bumblebees on cymes of *Echium vulgare* (Boraginaceae). These less predictable movement patterns by bumblebees in cymes and umbel suggest that the vertical arrangement created by racemes, and to a lesser degree panicles, results in the more predictable upwards foraging behaviours.

A strong tendency to move upwards through vertically arranged inflorescences is characteristic of bumblebees (Darwin, 1862; Waddington & Heinrich, 1979; Corbet et al. 1981; Harder et al., 2000; Routley & Husband, 2003; Fisogni et al., 2011; de Jong et al., 2011) and many other flower-visiting insects. Hawkmoths, such as *Basiothia schenki* (Jersáková & Johnson, 2007), and leaf-cutter bees (Iwata et al., 2012) like bumblebees show upward movement on racemes. In contrast, on *Dictamus albus* (Rutaceae) racemes, neither honey bees nor leaf-cutter bees (*Megachile sp.*), showed significant directional movement (Fisogni et al., 2011). Hummingbirds travel either downwards or less consistently upward through inflorescences, compared to bumblebees (Grant & Grant, 1968; Wolf & Hainsworth, 1986; Harder et al., 2004). *Dialictus* (Halictidae) bees travel laterally around racemes (McKone et al., 1995). Thus the directional movement by pollinators within inflorescences can depend on both the topology of the inflorescence and the species of the visitor. However, how pollinators differ in their foraging behaviours is poorly understood, as published observations often group species together when describing the general trend in pollinator movement. Additionally, many studies consider the movements of one, or a group of, species foraging on different plants (but see Harder et al., 2004 and Jordan & Harder, 2006). As plants can vary in inflorescence characteristics that may influence movement, more detailed analyses that focuses on the differences between species' foraging behaviours in racemes are required to understand species-specific differences in directional movements.

Despite widespread directional movement of pollinators on diverse plants, why pollinators behave in this manner is less well understood. Upward movement may convey an energetic advantage (Corbet et al., 1981; Lloyd & Webb, 1986), or insect may have better flight control when travelling upwards (Lloyd & Webb, 1986). Alternatively, pollinators may follow gradients in reward amounts through the inflorescence, starting where rewards are highest and departing after they drop too low (Fisogni et al., 2011). However, strong evidence exists to disprove this hypothesis

(Waddington & Heinrich 1979; de Jong et al., 2011). Thus, although pollinators commonly move directionally, there is debate as to why pollinators show these behaviours.

The order in which a pollinator visits flowers during a plant visit, the visit sequence, influences the amount of self and outcross pollen that each individual flower receives from a pollinator. The flower visited first can receive self-pollen only by autogamous transfer (Routley & Husband, 2006). However, flowers visited subsequently can receive self-pollen through autogamy and geitonogamy (Rademaker et al., 1997). Flowers of an inflorescence visited later in a pollinators' visit sequence will receive greater quantities of self-pollen, because the pollinator accumulates more the plant's own pollen on its body with every flower visit it makes (de Jong et al., 1992; Rademaker et al., 1997; Karron et al., 2004). Outcross pollen deposition is expected to follow the opposite trend, as pollinators visit more flowers, more donor pollen would be deposited and lost (de Jong et al., 1992; de Jong, 2000). Additionally, as any pollen removed from a plant early in the visit sequence will be more likely to be lost or deposited by geitonogamy, flowers visited early in the visit sequence will export less of their pollen. Consequentially, the quantity and quality of pollen a flower receives form each pollinator and the amount of pollen each flower exports on each pollinator will depend on its place in a pollinator's visit sequence, as determined by the pollinator's movement through the inflorescence. The influence of pollinator movement on geitonogamy, and therefore pollen receipt and discounting experienced by the whole plant and individual flowers, shows that the impact of inflorescence architecture on pollinator movement must be considered to understand how architecture influences reproductive success (Harder et al., 2004).

Directional movement within inflorescences can have several consequences for mating outcomes in hermaphrodite plants in which all the flowers of a plant function as males and females simultaneously (adichogamy). Topologies that encourage movements that involve many revisits and more within-inflorescence movement can lead to increased geitonogamous self-pollination (Jordan and Harder 2006). Additionally, topologies that promote a more consistent directionality may cause differential selfing between flowers in different positions. For example, a raceme encourages upwards movement by bumblebees, promoting geitonogamous pollen deposition of topmost flowers (Harder et al., 2000; Jordan & Harder, 2006). In this way topology is expected to alter the mating outcomes of different flowers within inflorescences. While we know much about how directional movement may lead to changes in deposition according to a flower's place in the visit sequence we do not currently have a great understanding of how these predictable pollinator movements can affect the fitness hermaphroditic plants with adichogamous, bisexual flowers (but see Harder & Barrett, 1996 and Harder et al. 2000).

Non-random floral arrangements

Many plants show separation of sexual function among flowers. This can be achieved by having temporally distinct sex phases within flowers, a trait known as dichogamy, or by having flowers that function only as males or females, a trait known as monoecy. Dichogamy occurs in two main forms depending on the order of male and female phases. Protandry involves an initial staminate ('male') phase, during which pollen is presented, followed by a pistillate ('female') phase, during which receptive stigmas are presented. The reverse pattern is known as protogyny. These categories are further refined based on other criteria discussed in table 1.2.

Table 1.2: The main sub-categorizations of dichogamy as reviewed in Lloyd and Webb (1986) and Renner (2001)

Criteria	**Subdivisions**
Order of presentation: Which sexual function is presented first?	PROTANDRY**:** A staminate (male) phase is presented first followed by a pistillate (female) phase. PROTOGYNY: A pistillate (female) phase is presented first followed by a staminate (male) phase.
Number of floral morphs involved: Do all individual plants have the same order of sex presentation?	DICHOGAMY: a single morph showing either protogyny or protandry. HETERODICHOGAMY: two floral morphs, one protogynous the other protandrous, function in the opposing sex phase to the other morph at one time.
Extent of within-plant synchrony: Are sex phases between different flowers on the plant in sync.	SYNCHRONOUS DICHOGAMY: flower sex phases are in sync. ASYNCHRONOUS DICHOGAMY: flower sex phases are out of sync.

Dichogamy, prevents autogamy (Shetler, 1979; Bertin, 1993; Ramirez & Seres, 1994) and can reduce geitonogamy, depending on the extent that pollinators visit female-phase flowers before male-phase flowers (Harder et al. 2000). Complete synchronous dichogamy (see table 1.2) prevents the occurrence of autogamous and geitonogamous selfing (Bhardwaj & Eckert, 2001; Narbona et al., 2011), because receptive stigmas and anthers are never present at the same time on a plant (Harder & Aizen, 2004). When plants show complete asynchronous dichogamy (see table 1.2) autogamy remains impossible, as no flower has active stigmas and anthers simultaneously. Geitonogamy can occur in plants that show asynchronous dichogamy, as pollen can be carried from male-phase flowers to female-phase flowers of the same plant (Lloyd & Webb, 1986; Harder et al., 2000; Harder & Aizen, 2004). However when flower development is ordered within an inflorescence a plant creates a non-random arrangement of male- and female-phase flowers by having flowers of different stages of development, or flowers of different sexes, at different positions (Darwin, 1862; McKone et al., 1995; Narbona et al., 2011).

When pollinators move predictably, non-random floral arrangements of male and female flowers allow plants to bias the direction of pollen movement by the pollinator through the inflorescence. For example, if female-phase flowers are presented where pollinators normally arrive and male-phase flowers are presented where pollinators normally depart a plant, then female-phase flowers would tend to be visited before male-phase flowers. If pollinators tend to visit female-phase flowers before male-phase flowers of the same inflorescences then the likelihood of geitonogamous self-pollination would be lessened (Darwin, 1862; Harder et al. 2000). However, the effectiveness of these non-random arrangements at reducing geitonogamy depends on the extent of directional movement (Jordan & Harder, 2006). It is currently unclear how consistent this directional tendency needs to be to grant these pollen transfer benefits to the plant. A pollinator species with inconsistent directional tendencies, such as hummingbirds, or an inflorescence topology that does not encourage upward movement, such as umbels, should increase the frequency of female-phase flowers being visited after male-phase flowers, leading to geitonogamous selfing (Harder et al. 2000; Harder & Aizen, 2004; Jordan & Harder, 2006).

Plants with racemous inflorescences often also exhibit protandry and acropetalous flower development (lower flowers develop before higher flowers: Lloyd & Webb, 1986; Bertin & Newman, 1993). By combining these architectural traits, a plant creates a non-random arrangement with younger, male-phase flowers above older, female-phase flowers. This non-random arrangement of male and female flowers was discussed by Darwin (1862) with respect to the orchid *Spiranthes spiralis*, and is often referred to as 'Darwin's Syndrome' (McKone et al., 1995; de Jong et al., 2011). In addition to orchids (Jersáková & Johnson, 2007; Li et al., 2011; Iwata et al., 2012), Darwin's Syndrome occurs in many angiosperm families, including: Onagraceae

(Routley & Husband, 2003); Rutaceae (Fisogni et al., 2011); Lamiaceae (Leshem et al., 2011); Ranunculaceae (Zhao et al., 2008; Ishii & Harder, 2012) and Plantaginaceae (de Jong et al., 2011). Plants showing Darwin's syndrome have been the main focus of studies examining the benefits of non-random floral arrangements of male- and female-phase flowers (Galen & Plowright, 1985; Routley & Husband, 2003; de Jong et al., 2011). Similar non-random arrangements of male- and female-phase flowers, with female-phase flowers located at pollinator start positions and male-phase flowers at departure positions also occur widely, such as the many protandrous Asteraceae capitula (heads, see table 1.1) with younger male-phase flowers at the centre and older female-phase flowers about the periphery of the inflorescence (Burtt, 1977; Webb, 1981). As bumblebees and perhaps other pollinators show a strong tendency to travel upwards in racemes, Darwin (1862) predicted this arrangement was adaptive, because it reduced geitonogamous selfing. As reduced within plant pollen transfer also reduces pollen discounting Darwin syndrome has also been linked to an increase in pollen export (Harder at al. 2000; Jordan & Harder 2006, Jersáková & Johnson, 2007). However, the various studies attempting to demonstrate these dual benefits from Darwin's syndrome have found inconsistent results, finding either both predicted advantages (Harder et al., 2000) or only an export advantage (Routley & Husband, 2003; Jersáková & Johnson, 2007).

Above I have discussed the varied forms of inflorescence architecture in nature and how they may influence pollinator foraging and its consequences for pollen transfer. In the following chapter I shall begin by investigating what effects on pollen transfer that pollinator movement in inflorescences has on adichogamous plants (see Chapter 2). This may reveal insight into why racemose architecture and other plant characteristics associated with directional movement exist in adichogamous plants.

This shall be done by use of a model which simulates pollinator bouts of variable consistency of pollinator movement. I will then utilise this model to address how consistent pollinator movement alters pollen transfer in inflorescences that show non-random arrangements of male(-phase) and female(-phase) flowers (Chapter 3). Incorporating non-random arrangements of flower sex function into this model allows investigation of whether variable movement behaviour explains the inconsistency of results concerning the pollen transfer benefits of Darwin's syndrome. Having established what impacts variable foraging in inflorescences should have on plant fitness I shall investigate how related pollinator species vary in upward foraging and attempt to address why many pollinators show upward foraging within raceme inflorescences (Chapter 4). These latter points will be carried out by direct observation of pollinators of racemose *Charmerion angustifolium*. The overall goal of this thesis will be to further our understanding of how inflorescence architecture impacts pollinator foraging behaviour and subsequently how pollinator foraging behaviour impacts pollen transfer and angiosperm fitness.

Chapter 2: Predictable pollinator foraging in plants with adichogamous flowers

Introduction

When visiting plants with one or more inflorescences, pollinators tend to visit multiple flowers per plant (Ohashi & Yahara, 2001). An increase in the number of flowers that each pollinator visits per inflorescence, longer bout length, promotes pollen receipt by increasing the opportunities for pollinators to deposit pollen. Longer foraging bouts also allow increased pollen removal, potentially increasing pollen export (Adler & Irwin, 2005; Aizen & Harder, 2007). However, within plant pollinator movements transport self-pollen between a plant's flowers, a process known as geitonogamy. Increased self-pollination, through geitonogamy, can reduce female reproductive success, especially in plants which have no self-incompatibility mechanisms (chapter 1). Additionally, increased self-pollen deposition decreases the amount of pollen available for export (pollen discounting) (Harder & Wilson, 1995; Harder & Barrett, 1995; Karron & Mitchell, 2012), having an impact on male success that is independent of whether self-fertilization or inbreeding depression actually takes place (chapter 1). Thus, it is expected that traits associated with increased bout length represent a balance between the benefits of increased outcross pollen receipt and increased pollen export with the costs of increased geitonogamy and associated pollen discounting (Klinkhamer et al., 1994; Harder & Barrett, 1996; de Jong & Klinkhamer, 2005, discussed in chapter 1).

Although much is understood about the effect of foraging bout length on the fate of plant's pollen, less is understood about the consequences of the order of visits made by pollinators. A pollinator's visit sequence affects pollen deposition on each flower (chapter 1). Flowers visited late in the visit sequence tend to receive less outcross pollen from a pollinator and more self-pollen (Harder & Barrett, 1996; Harder et al., 2000; de Jong et al., 2011; chapter 1). Predictable pollinator movement, whereby all pollinators move through the inflorescence in the same order, should result in different amounts of pollen deposition among flowers, based on the flower's place in the visit sequence. Therefore the pattern of pollinators' movement within an inflorescence should influence the quantity and quality of pollen received by each flower. So the impact of pollinator foraging on the reproductive success of angiosperms depends not only how frequently pollinators move within inflorescences, but also on visit order (Harder & Barrett, 1996; Harder et al., 2004). In this study I use a simulation model to investigate the effect of predictable pollinator movement on pollen transfer within hermaphrodite plants with flowers which function simultaneously as males and females, adichogmaous plants.

Hermaphroditic plants show traits that encourage directional movement of pollinators. Vertical racemes, such as those in the in Fumariaceae (Kudo et al., 2001; Zeng et al., 2009), Liliaceae (Ishii & Sakai, 2001), Pontederiaceae (Harder et al., 2000) and Asparagaceae (Cao et al., 2011) promote upwards movement of foraging pollinators (chapter 1, but see Ishii & Sakai, 2001). The impact of predictable directional pollinator movement on overall plant success has been primarily investigated within plants in which flowering order and dichogamy combine to create non-random arrangements of male- and female-phase flowers (chapter 1). Although a few of these studies considered the advantage of predictable directional movement in dichogamous plants relative to adichogamous plants (Harder et al., 2000; Routley & Husband, 2003; Jordan & Harder 2006), the impact of predictable foraging through inflorescences on overall fitness of plants with adichogamous flowers has received little attention. Jordan & Harder (2006) found that the less predictable movement of bumblebees within umbels lead to a slightly longer bout length then in other inflorescence topologies. Simulation of the expected pollen transfer in these plants revealed increased geitonogamy in umbels, compared to racemes and panicles, but also increased export when pollinator visitation was not sufficient to remove all of a plants pollen, as more pollen was removed in additional flower visits then discounted. Thus, whether consistent directional movement is beneficial to such plants independently of influences of pollinators' bout length remains unclear.

Understanding how predictability of pollinator foraging behaviour influences plant mating success informs several questions about angiosperm evolution. Most notably how traits that influence pollinator foraging bout length, such as inflorescence size length (Ohashi & Yahara, 2001; de Jong & Klinkhamer, 2005) or floral reward levels (Johnson et al., 2004; Jersáková et al., 2006), and predictability of pollinator movement within the inflorescence, such as inflorescence topology (Jordan & Harder, 2006; Ishii et al., 2008), mediate pollen transfer and plant success? Thus answering the question of why these traits evolved? Movement behaviours that change bout length, as discussed, would impact geitonogamous and outcross pollen receipt as well as pollen export (Engel & Irwin, 2003; Albert et al., 2008). When bout length is unchanged, movement that increases flower revisits would reduce pollen export, owing to pollinators removing less pollen from flowers they have already visited due to depletion of pollen in those flowers. In this way predictable movement could confer a pollen-transfer advantage in adichogmaous plants.

The impact of directional pollinator movement in adichogmaous plants on the pattern of pollen deposition each flower receives has received limited theoretical (Harder & Barrett, 1996; Jordan & Harder, 2006) and empirical analysis (Harder et al., 2000; Zeng et al., 2009). These studies predict increased geitonogamy and reduced outcross pollen receipt and export in flowers typically visited later in the visit sequence (chapter 1). Except for Jordan & Harder (2006), these studies focussed only on a strong directional tendency's impact on pollen transfer (highly predictable movement behaviour) and did not address how less consistent pollinator movements influence this deposition pattern. This uncertainty of movement is of importance as the strength of the directional movement has been seen to vary depending on the plant topology and pollinator species (McKone et al., 1995; Harder et al., 2004; Fisogni et al., 2011).

How pollinator movements within inflorescences affect pollen deposition across a plant may also influence patterns of expression of sexual function in inflorescences. When the quality of received pollen differs with flower position, as expected from directional pollinator movement in adichogamous plants, flower reproductive success should differ (Brunet & Charlesworth, 1995). This positional variation in success within inflorescences may favour differential expression of sexual function, with flowers expressing the sex role that they perform best (Lee, 1988; Thomson, 1989). Such a pattern of expression would prevent waste of resources on pollen or ovules that would otherwise be discounted (Charlesworth & Charlesworth, 1999). Differential expression of male and female function with flower position in the inflorescence has been seen in many plants (Diggle, 2003; Herrera, 2009), most notably in racemes showing Darwin's syndrome (discussed in chapter 1). Thus, how predictable pollinator foraging influences the pattern of deposition across adichogamous plants may shape the evolution of non-random arrangements of male and female flowers that we see as a result of dichogamy. Additionally, as pollinator directional movement is quite variable (Harder et al., 2004; Fisogni et al., 2011), understanding how consistent pollinator movement has to be to result in differing patterns of deposition will allow identification of the conditions under which these patterns of allocation are favoured.

In this study, I develop a simulation model of pollen transfer and pollinator foraging within adichogamous plants to identify how non-random pollinator movements alter pollen transfer. Pollen fates are monitored to assess male success when pollinator foraging behaviour differs. Pollen receipt under changing pollinator foraging behaviour is investigated in two ways. First, a plant's overall pollen receipt is used as a measure of female reproductive success. Second, pollen receipt of each flower is monitored to assess inter-floral variation, allowing evaluation of whether observed patterns of sex phase expressions would be favourable when pollinators show directional pollinator movement.

Methods

Simulation of pollen movement within inflorescences

The following simulations were implemented using Visual Basic for Applications (within Excel, Microsoft Office 2011). Consider a plant with a single raceme of n adichogamous flowers arranged in a single column. Flowers within this raceme are numbered starting from the bottom upwards. This inflorescence topology resembles that of *Spiranthes* species that possess single spirals of flowers such as *S. sinensis* (Iwata et al., 2011).

The model depicts both pollinator movement and the associated pollen transfer. Pollinators move according to one of two rules: either random movement whereby a pollinator moves randomly among a plant’s flowers, vertically biased movement. As pollinators visit flowers they remove and deposit pollen, so the consequences of these rules for pollen transfer can be compared.

Pollinator movement model

Pollinator movement is represented by probabilities of different foraging actions. q_j is the probability that a pollinator arriving at the inflorescence visits flower j first. Following a visit to flower j a pollinator departs the inflorescence with probability d_j, or it moves to flower i with probability $p_{j,i}$. This movement model allows pollinators to revisit flowers, including revisits to the flower just visited ($p_{j,j}$). These movement probabilities are depicted in figure 2.1.

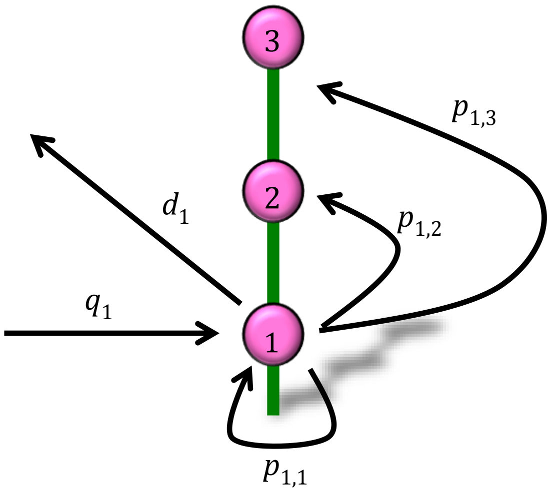


Figure 2.1: The options for pollinator movement relating to the lowest flower (flower 1) of a three flowered raceme. Arrows represent possible movements. q_1 is the arrival probability at flower 1, d_1 is the probability of departure from the inflorescence, and $p_{1,i}$ is the probability of from flower 1 to flower number *i* given that the pollinator does not depart. Circles depict flowers.

Movement rules

Movement probabilities between flowers were generated using two movement rules. With random movement

$$q_{j,i} = \frac{1}{n}, \tag{2.1}$$

where *n* is the number of flowers on the inflorescence. Biased movement requires a more complicated formulation. Let *i* be the total number of flowers to which a pollinator could move after visiting flower *j*. If the destination flower lies outside the inflorescence, then the movement is considered a departure; otherwise the pollinator moves to another flower on the plant. In the simulations below, *i* always includes flower *j* and the 2 flowers above and below flower *j*, therefore $i = 5$. With probability *c* a pollinator moves randomly between these *i* flowers, otherwise it moves to the flowers directly above or below flower *j*. Let *x* denote the probability the pollinator moves to the flower immediately above *j* as opposed to the flower immediately below *j*, given that it does not move randomly within the *i* flowers. *x* describes the strength of the pollinator’s upwards-directional tendency and c describes the uncertainty in this tendency. Therefore, the movement probability to flower j+1 is

$$p_{j,j+1} = c\frac{1}{i} + x(1-c), \tag{2.2}$$

and to flower j-1 is

$$p_{j,j-1} = c\frac{1}{i} + (1-x)(1-c)\,. \tag{2.3}$$

The probability of random movement from j to a specific flower among the i possible is

$$p_{j,i} = c\frac{1}{i}\,. \tag{2.4}$$

When c=0 pollinators can only visit flowers j+1 and j-1 with probabilities x and 1-x, respectively. When uncertainty, c, is large pollinators move less predictably.

Pollen transfer model

Pollen transfer is modelled as an exponential decay process (Bateman, 1947; de Jong et al., 1992; Klinkhamer et al., 1994). My version corresponds closely with that of Rademaker et al. (1997 & 1999). Simulated pollinators carry pollen from two general sources: focal plant pollen (i.e. pollen picked up from the current inflorescence) and non-focal plant pollen (i.e. pollen picked up from conspecific plants). Let Ls_p and Lo_p denote the numbers of focal and non-focal plant pollen grains, respectively, carried on the body of pollinator p that are available for pollen transfer. The current number of pollen grains on the anthers of flower f is A_f. Pollen from both of the pollinator's pollen loads can be deposited on the stigmas of visited flowers. The number of non-focal plant pollen grains deposited on the stigma, the outcross pollen receipt, of flower f is Do_f. As two processes can result in self-pollen deposition, autogamy and geitonogamy, the simulation keeps track of how the focal plant pollen was deposited. Let Dg_f and Da_f be the focal plant pollen load deposited on flower f by geitonogamy and autogamous pollen transfer facilitated by the pollinator respectively. Pollen can also be lost from the system, becoming unavailable for transfer by grooming or falling off the pollinator during flight (Harder & Wilson, 1998; Richards et al., 2009). Pollen is also lost after pollen removal due to failure to adhere to a pollinator (Harder & Thomson, 1989; Rademaker et al., 1997). Ws denotes the number of the focal plant's pollen grains lost during the simulation.

When a pollinator visits a flower, the fates of pollen on the pollinator and on the plant are determined by a sequence of transfer probabilities, which are described in table 2.1. The model depicts pollen transfer during each flower visit, as summarised in figure 2.2. Using this pollen transfer model and the pollinator movement model, pollen transfer resulting from several pollinator visits to the plants can be simulated. This simulation allows calculation of pollen fates and total pollen deposited on plants at the end of the simulation and the self- and outcross-pollen receipt by each of a plant's flowers. Table 2.2 describes how pollen fates are calculated. Table 2.3 describes the final pollen receipts of each flower and of the whole plant.

Table 2.1: The pollen transfer fractions of the pollen transfer simulation in the order in which they occur. The value used in the simulation is also listed. These values are obtained from Rademaker et al. (1997), except k_A, which is estimated from Routley & Husband (2006). Figure 2.6 demonstrates how these transfer fractions are applied in the simulation to allow pollen movement between flowers and pollinators.

Order of events	**Parameter**	**Description**	**Value used**
START: *Pollinator begins visit to flower*			
1	k_T	The fraction of the pollinator's pollen loads (both focal and non-focal) that is deposited on the stigma during a flower visit.	0.1
2	k_R	The fraction of the pollen on a flower's anthers that is removed by a pollinator during a flower visit.	0.16
3	k_L	The fraction of the pollen removed from a flower's anthers that fails to adhere to the pollinator and is lost from dispersal.	0.5, reduced to 0.3
4	k_A	The faction of the pollen that adheres to the pollinator that is deposited autogamously on the flower's stigma.	0.1
5	k_G	The fraction of the pollinator's pollen load that is lost by passive or grooming loss between flower visits.	0.1
FINISH: *Pollinator visits another flower or departs from inflorescence*			

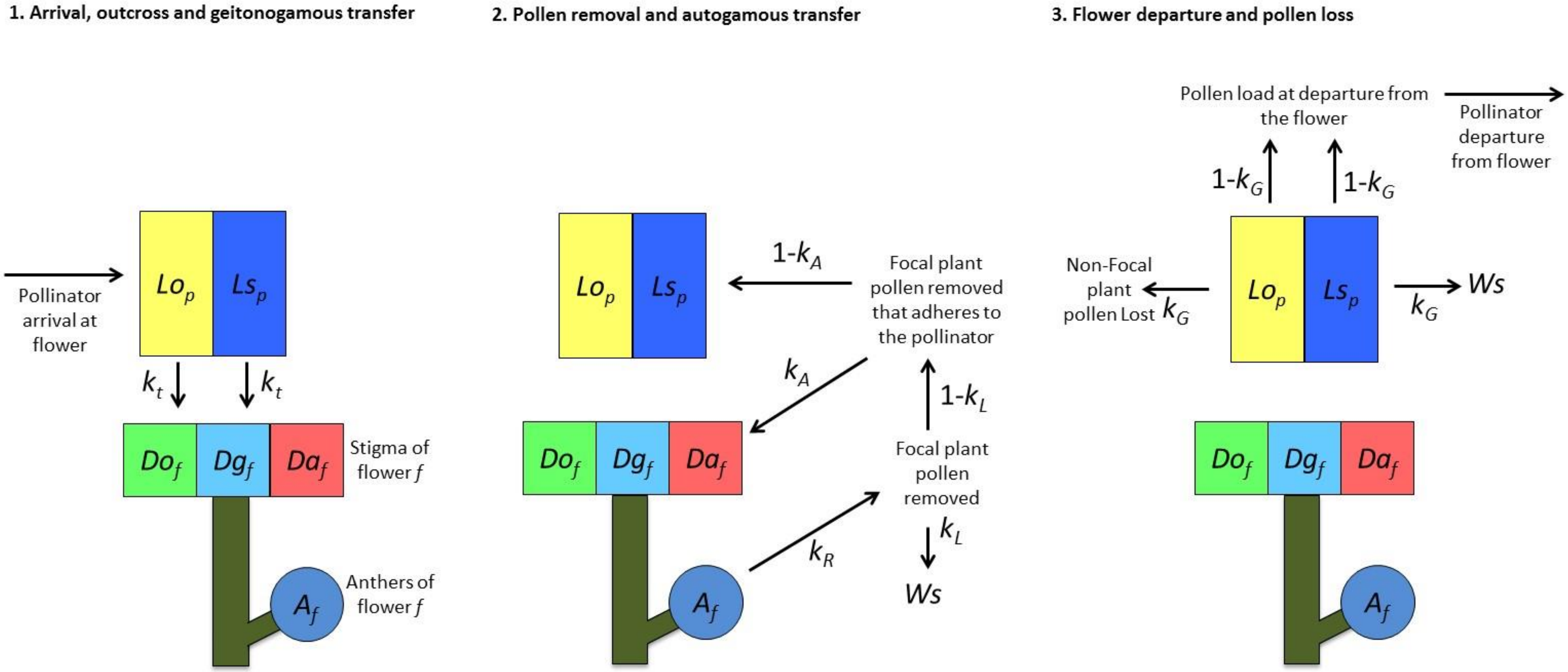


Figure 2.2: The sequence of modelled pollen transfer during each flower visit. Arrows depict pollen transfer between pollen states (squares, the stigma; circles, the anther; rectangles, the pollinator). The amount of pollen transport depends on the amount of pollen present in each state at the time of the flower visit. Transfer occurs in a set order (sequence depicted left to right): 1) following arrival at a flower outcross and geitonogamous pollen deposition occur; 2) Then pollen is removed from anthers, pollen removed may fail to adhere to the pollinator or be deposited autogamously; and 3) Passive loss of pollen occurs at departure from the flower.

Table 2.2: The final pollen fates of the whole plant and how they are calculated from states of the simulation. All pollen loss and deposition values are those at the end of the simulation. Similarly the pollen load values, for pollen export, are those carried at the departure of that pollinator from the plant.

Definition	Description
$T_A = \sum_{f=1}^{n} A_f,$	Total pollen grains remaining on all the anthers of a plant at the end of the simulation.
$T_{Dg} = \sum_{f=1}^{n} Dg_f$	Total number of focal pollen grains deposited on all the stigmas of a plant by geitonogamy at the end of the simulation, the geitonogamous pollen receipt of the plant.
$T_{Da} = \sum_{f=1}^{n} Da_f$	Total number of focal pollen grains deposited on all the stigmas of a plant by autogamy at the end of the simulation, the autogamous pollen receipt of the plant.
$T_E = \sum_{p=1}^{m} Ls_p$	Total number of focal pollen grains exported by *m* pollinators that visit the plant over the whole simulation. Where *m* is the total number of pollinators that visit the plant.
$T_L = Ws$	Total number of focal pollen grains lost from the plant over the period simulated.

Table 2.3: The final pollen receipt variables for each of the plant's flowers and the plant as a whole. All deposition values are those at the end of the simulation.

Definition	Description
Do_f	The number of non-focal plant pollen grains deposited on the stigma of flower *f* at the end of the simulation, the outcross pollen receipt of flower *f*.
Dg_f	The number of focal plant pollen grains deposited by geitonogamy on the stigma of flower *f* at the end of the simulation, the geitonogamous pollen receipt of flower *f*.
Da_f	The number of focal plant pollen grains deposited by autogamy on the stigma of flower *f* at the end of the simulation, the autogamous pollen receipt of flower *f*.
$T_{Do} = \sum_{f=1}^{n} Do_f$	Total non-focal plant pollen grains deposited on all stigmas on a plant at the end of the simulation, the outcross pollen receipt of the plant.
$T_{Dg} = \sum_{f=1}^{n} Dg_f$	Total number of focal plant pollen grains deposited on all the stigmas of a plant by geitonogamy at the end of the simulation, the geitonogamous pollen receipt of the plant.
$T_{Da} = \sum_{f=1}^{n} Da_f$	Total number of focal plant pollen grains deposited on all the stigmas of a plant by autogamy at the end of the simulation, the autogamous pollen receipt of the plant.

Impact of different pollinator foraging behaviour

The pollen transfer fractions and initial flower pollen loads estimated for *Echium vulgare* (Boraginaceae) were used for these simulations (Rademaker et al., 1997). This data set was chosen because it was the most complete data set of pollen transfer fractions available for a single species. Autogamy was not measured in this system so the autogamous transfer fraction, k_A, had to be estimated based on data from bisexual flowers of *Chamerion angustifolium* (Onagraceae) (Routley & Husband, 2006). The values for these pollen transfer fractions are shown in table 2.1. Initial simulations showed pollen loss to be the main pollen fate in simulations. This was due to the high k_L (0.5) found by Rademaker et al. (1997). To view changes in selfing and export more easily, k_L was reduced to 0.3 for these simulations. This change had no effect on the qualitative outcome of the simulations.

In each simulation plants were 6-flowered and each flower had an equal initial anther load, A_{f0}, of 40,000 pollen grains (Rademaker et al., 1997). During the simulation 10 pollinators that all followed the same foraging behaviour (as determined below), dictated by the following rules, visited the plant. Initially each pollinator carried no focal plant pollen and Lo_{p0} non-focal plant pollen grains. This initial non-focal plant pollen load was calculated using

$$Lo_{p0} = \frac{1-(1-k_T)(1-k_G)}{k_R A_{f0}(1-k_L)(1-k_A)(1-k_G)} \ , \qquad (2.5)$$

which describes a dynamic equilibrium between pollen pickup and deposition. The consistency of foraging behaviours of visiting pollinators was altered by either, arrival position or movement pattern. Pollinator arrival was either random (q_1 to $q_6 = 1/6$) or fixed at flower 1 (q_1 = 1, whereas q_2 to q_6 = 0). I simulated four separate movement behaviours of varying predictability, which are described in table 2.4, resulting in 8 combinations of arrival and movement behaviours. Simulations of ten pollinators following one of these eight foraging behaviours were replicated 30,000 times. This high number of replicates was performed in order to ensure that the mean values given by these simulations represent the true mean (repeated runs of these simulations did not yield notably different mean outcomes, thus statistical analysis was not necessary). The mean number of pollen grains in each state at the end of the simulation was calculated and used to quantify male success of simulated plants. Mean pollen receipt by plants was used as a measure of female success. Mean pollen receipt of each flower was also compared to assess whether patterns of deposition develop when pollinator movement is biased.

Table 2.4: The 4 simulated movement behaviours and associate parameter values. When pollinators follow the bias movement rule d_f always equals 0, so departure depends on movement of the pollinator in these foraging behaviour types (see page 27).

Movement behaviour	Movement rule followed	Parameter values	Departure probabilities
Random movement	Random movement	See equation 2.1	d_1 to d_6= 1/6
Weak upward movement	Biased movement	i=5, x=0.75, c=0	d_1 to d_6= 0
Strong upward movement	Biased movement	i=5, x=1, c=0	d_1 to d_6= 0
Uncertain upward movement	Biased movement	i=5, x=1, c=0.5	d_1 to d_6= 0

Results

Flower visitation

Changing pollinator foraging behaviour and arrival behaviour affected the number of pollinator visits received by each flower of the plant and the mean number of flowers each pollinator visited, its bout length, (table 2.5 and 2.6).

Table 2.5: Average flower visit frequencies for plants visited by 10 pollinators in relation to pollinator movement rule when initial flower position was chosen at random. Numbers in brackets indicate standard deviations of the mean visits per pollinator.

Flower	**Random movement**	**Weak upward movement**	**Strong upward movement**	**Uncertain upward movement**
Mean visits per pollinator	6.02 (±1.73)	5.85 (±1.45)	3.50 (±0.54)	5.22 (±1.36)
6	10.02	12.25	10.00	10.68
5	10.05	14.10	8.32	11.41
4	10.02	12.48	6.65	11.08
3	10.06	9.73	4.98	9.05
2	10.00	6.58	3.32	6.49
1	10.02	3.30	1.67	3.59
Total	60.17	58.44	34.94	52.31

Table 2.6: Average flower visit frequencies for plants visited by 10 pollinators in relation to pollinator movement rule when flower 1 is always visited first. Numbers in brackets indicate standard deviations of the mean visits per pollinator.

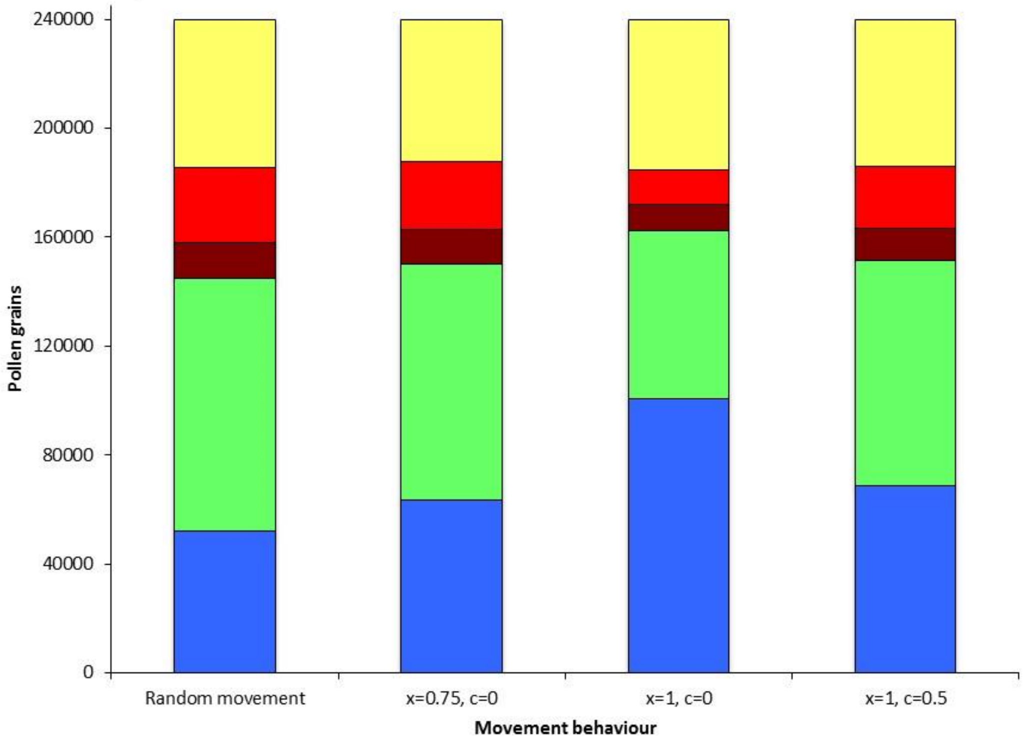
Flower	**Random movement**	**Weak upward movement**	**Strong upward movement**	**Uncertain upward movement**
Mean visits per pollinator	6.00 (±1.74)	7.32 (±1.67)	6.00 (±0.00)	6.43 (±1.52)
6	8.36	8.87	10.00	7.41
5	8.38	11.83	10.00	9.31
4	8.35	12.82	10.00	10.83
3	8.38	13.14	10.00	11.51
2	8.34	13.24	10.00	11.59
1	18.34	13.31	10.00	13.66
Total	60.15	73.21	60.00	64.31

Male reproductive success

Different arrival and movement behaviour altered the mean pollen fates of plants at the end of the simulation (figure 2.3). Changing arrival position from random to the lowest flower had little impact on the pollen fates of a plant when the pollinator moves randomly within the inflorescence. However, pollen fates were affected when pollinators followed the biased movement rule.

When pollinators initiated inflorescence visits at random flower positions pollen removal was lower when pollinators consistently moved upward, rather than randomly among flowers (sum of all but the blue bars in figure 2.3i). Removal was most limited when pollinators moved directionally because flowers received fewer visits, on average (table 2.5) (Klinkhamer et al., 1994). However, the plants visited by pollinators that show strong upward movement exported as much pollen as other plants despite a reduction in bout length (see yellow bars in figure 2.3i), largely because of reduced pollen discounting.

Figure 2.3ii shows how changing movement behaviour influenced pollen fates of plants when pollinators consistently arrived at the lowermost flower. In this case, pollen export increased consistently with pollen removal when pollinators moved predictably, with the strongest effect when pollinators show strong directional movement. Overall increased predictability in pollinator foraging, in terms of both arrival and movement within the inflorescence, increased export by the plant and therefore increased male reproductive success.


i) Random arrival

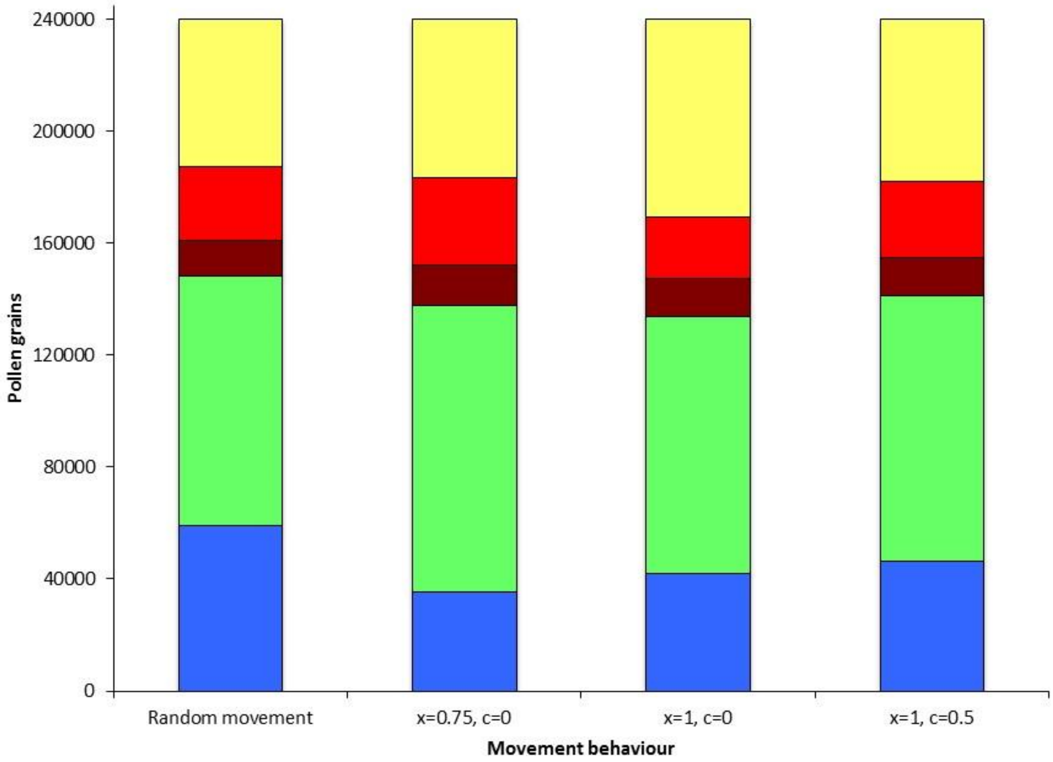

ii) Fixed arrival

Figure 2.3: The effect of foraging movement behaviour on mean plant pollen fates after 10 pollinator visits to inflorescences with (i) random arrival positions and lower (ii) fixed arrival at the bottom flower. Movement rules are depicted in table 4.4. Colours indicate the final pollen fates of the plant's pollen: pollen that remains un-removed in anthers, T_A (blue); lost, T_L (green); pollen deposited on focal plant stigmas by autogamy, T_{Da} (dark red); pollen deposited on focal plant stigmas by geitonogamy, T_{Dg} (bright red) and pollen exported from the plant on departing pollinators, T_E (yellow).

Female reproductive success

The total pollen receipt of simulated plants visited by pollinators showing each foraging behaviour is displayed in figure 2.4. When arrival was random (figure 2.4i) more predictable movement decreased self-pollen deposition from both sources, especially geitonogamy. This decrease in selfing was quite small unless movement within the inflorescence is heavily biased; however, this benefit comes at the cost reduced outcross pollen receipt. When pollinators always arrived on the lowest flower (figure 2.4ii) geitonogamy was reduced if vertical movement is more certain. Interestingly, when pollinators showed strong directional movement and arrival at bottom flower, plants received increased outcross pollen receipt and reduced selfing from both sources compared to when pollinators move randomly among flowers.

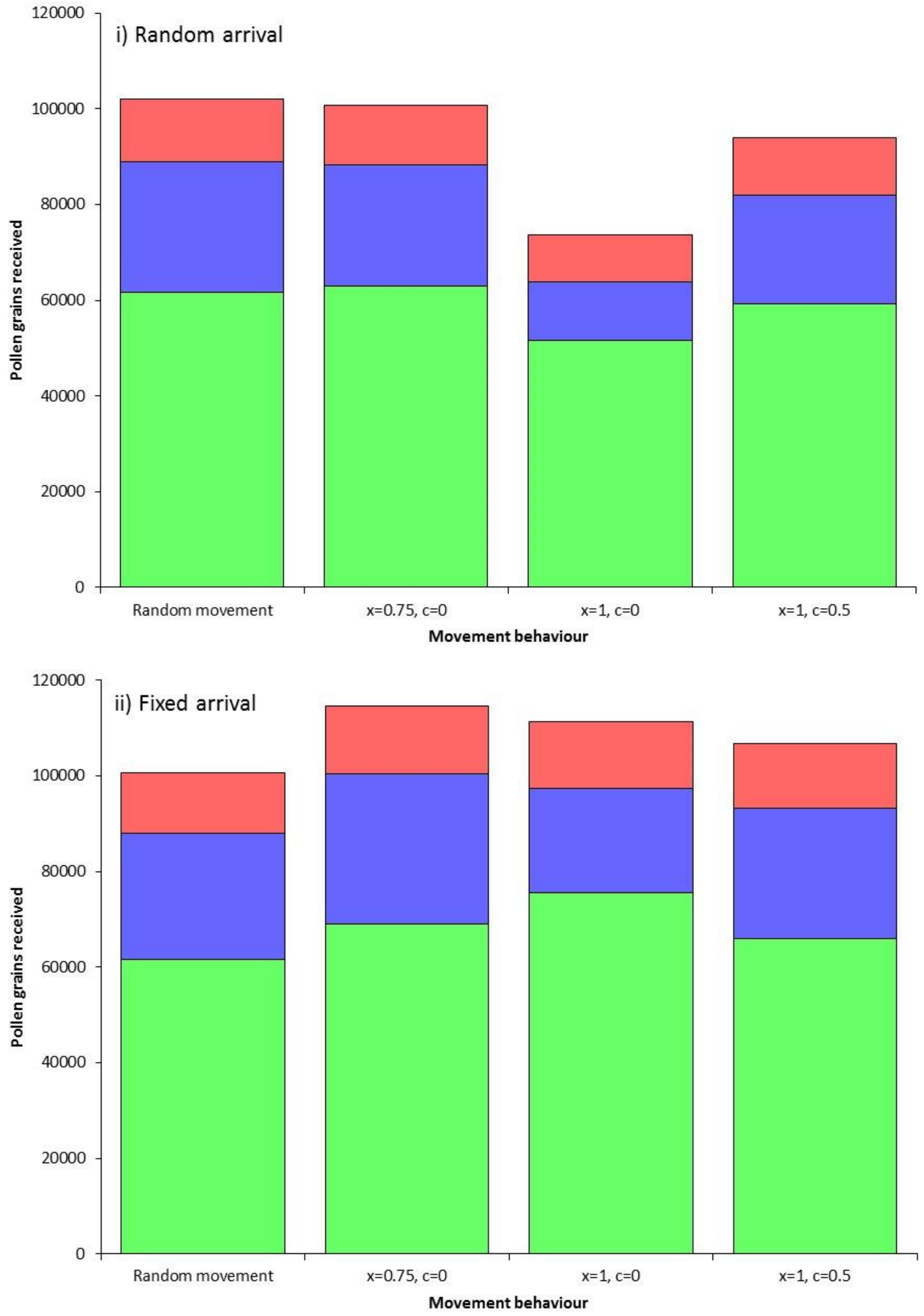


Figure 2.4: The effect of foraging movement behaviour on the mean total pollen receipt on simulated plants after 10 pollinator visits to the inflorescence. Upper panel (i) depicts random arrival and lower panel (ii) depicts fixed arrival to the bottom flower. Movement rules are depicted in table 4.4. Colours correspond to the source of pollen deposition: outcross pollen deposition, T_{Do} (green); geitonogamy, T_{Dg} (blue); autogamy, T_{Da} (red).

Pattern of pollen deposition within inflorescences

Differing pollinator movement behaviours altered the pattern of pollen deposition between flowers of the same plant, depending on the order and frequency of visits that flowers received (figure 2.5).When pollinators arrived at a random position on the inflorescence and then move randomly within it, the mean pollen deposition was evenly distributed over the plant. In contrast, pollinators that moved upwards in a more predictable pattern generated greater selfing, through geitonogamy, in upper flowers. Interestingly, following arrival at a random position, biased upward movement increased cross-pollination from lower to upper flowers.

When pollinators always arrived at flower 1, lower flowers tend to receive more outcross pollen than upper flowers. When pollinator movement was biased and arrival fixed to flower 1 the levels of geitonogamous pollen deposition increased in higher flowers in the same manner as we saw when movement was biased and arrival was random. As before, geitonogamy tended to increase when movement became more uncertain.

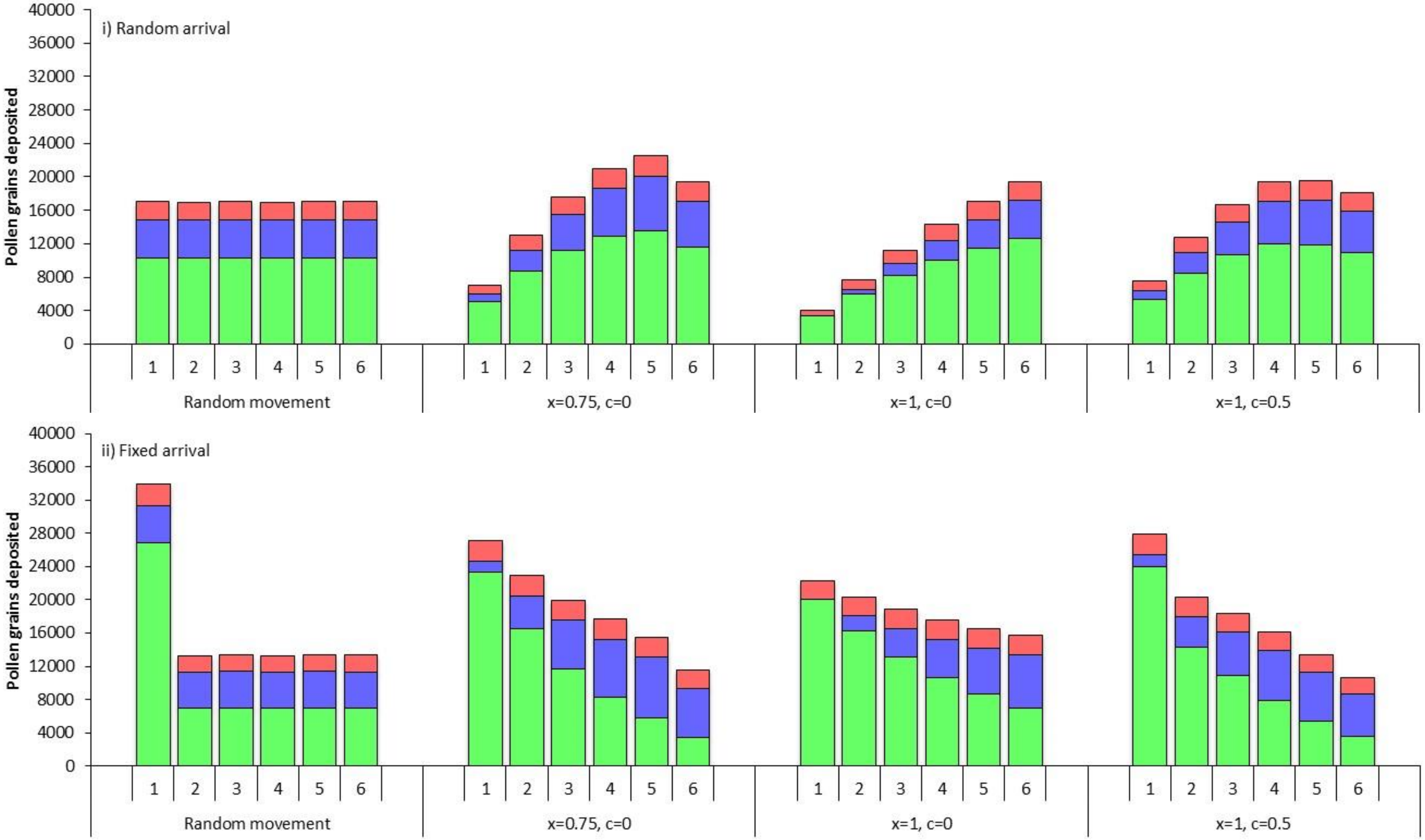


Figure 2.5: Patterns of pollen deposition in flowers at different positions within plant (1=lowest, 6=highest) after inflorescence visits by 10 pollinators that i) arrive at random positions, or ii) arrive consistently at flower 1. Each graph represents a differing foraging behaviour type labelled above. Numbers below columns correspond to the flower number. Colours correspond to the mode of pollen deposition: outcross pollen deposition (green); geitonogamy (blue); autogamy (red).

Discussion

The presented results indicate that increased predictability in pollinator foraging behaviours should result in increased pollen transfer success for plants. In addition I show, pollinator movement in a predictable direction should cause differential pollen receipt of flowers depending on flower position. I now discuss the evolutionary consequences of these findings.

Predictable movement and male and female success

The presented simulations indicate that predictable pollinator movement should improve the pollen transfer outcomes of adichogamous plants (figure 2.3 and 2.4), and should therefore improve both male and female success. The mean number of flowers visited per inflorescence visit did not differ when pollinators moved randomly, or started at bottom flowers and then moved consistently upward (table 2.6). Yet the advantages in terms of pollen receipt and export in plants visited by pollinators that move upward consistently are still seen. Therefore the benefits consistent pollinator movement provides in terms of pollen transfer are likely to be independent of the observed trade-offs in terms of selfing, export and outcross receipt associated with pollinator bout length (Klinkhamer et al., 1994; de Jong & Klinkhamer, 2005).

Simulated plants received more outcross pollen and exported more pollen when pollinators moved predictably (figure 2.3 and 2.4) primarily because of the increased variability in the bout length of pollinators that moved randomly. As the number of outcross pollen grains deposited on each flower decreases with increased bout length, leading to a decelerating relationship between bout length and outcross pollen receipt

(Engel & Irwin, 2003). Similarly pollen that can be exported declines with elevated bout length (Klinkhamer et al., 1994). Higher variability about the same mean bout length decreases in the mean outcross receipt and exported pollen, because of a mathematical property of decelerating relationships known as Jensen’s inequality (Jensen, 1906; Smallwood, 1996; Ruel & Ayres, 1999; Richards et al., 2009).

Predictable pollinator movement increased pollen export by pollinators in my simulations, this is likely due to the effect of reduced variability in bout length but also may be influenced by reduced the incidence of flower revisits. This reduction should increase pollen export when pollinators move more predictably (figure 2.3). Note that pollinators that initiate arrival at random positions should export as much pollen if they exhibit strong upwards movement as if they move randomly, despite reduced removal (figure 2.3i). Plants visited by randomly arriving, but predictably moving pollinators received fewer flower visits in my simulations, because the strong directional movement behaviour prevents visits to flowers below their arrival point and guarantees departure once flower 6 is reached. If instead directionally foraging pollinators always start at bottom flowers, they visit each flower once. That plants visited by randomly arriving pollinators that show a directional tendency are able to export as much as plants visited by randomly moving pollinators, despite the difference in flower visitation, verifies that predictably moving pollinators should export more pollen per flower visit then inconsistently moving pollinators. This increase in export per visit further supports the expectation that predictable pollinator movement increases male reproductive success due to reduced revisits (also see Jordan & Harder 2006).

Total self-pollen deposition via geitonogamy varied with the number of visits that plant receives from each pollinator (table 2.5 and figure 2.4), a well reported response (Barrett et al 1994). Therefore predictable movement may benefit plants by limiting bout length, thereby reducing self-pollen deposition, in addition to limiting variability in bout length and therefore increasing outcross pollen receipt. Similar adaptions to regulate bout length include non-rewarding flowers (Jersakova & Johnson, 2006; Jersakova et al., 2006) and deterrent nectar metabolites (Johnson et al., 2006; Irwin & Adler, 2008). However predictable movement reduces geitonogamy independently of bout length in the presented simulations. For example, compare the geitonogamy caused by randomly moving pollinators with that of pollinators that arrived at flower 1 and showed strong upward movement (figure 2.4). Plants in both situations received the same number of visits per pollinator, but plants visited by predictably moving pollinators experienced less geitonogamy. The reasons for this are unclear but predictable pollinator movement reduces the incidence of revisits and causes flower visits to involve predominately geitonogamous transfer (due to depletion of anther loads and non-focal pollen loads) potentially explaining the reduction in geitonogamy when pollinators move predictably. Further investigation into this unpredicted advantage is needed.

The results presented here suggest that adichogamous plants benefit from reduced flower revisits in addition to consistent bout length. However some studies have shown revisits occur rarely in nature (Jordan & Harder, 2006; Ishii et al. 2008; Dreisig, 2012). This can be due to scent marking left behind on flowers after bees have visited (Goulson et al., 2001; Stout & Goulson, 2001). Thus it is possible that in natural systems the advantage of reduced geitonogamy when pollinator move consistently, which occurs predominantly due to revisits, may be minor. Although, benefits of consistent pollinator movement to plant in terms of export and outcross pollen receipt, which occur due to reduced variability in bout length should remain even when revisits do not occur.

The potential advantages of predictable pollinator movement would influence the evolution of plants. Advantages in export and favourable pollen receipt, as a result of predictable movement, have been seen in plants with non-random arrangements of male and female flowers (Harder et al. 2000, Routley & Husband, 2003; Jersakova & Johnson, 2007; see chapter 1). The presented results suggest that similar advantages are also seen in adichogamous plants that show no special segregation of sexual function. Although these advantages are probably reduced compared to plants with non-random arrangements of male and female flowers (Jordan & Harder, 2006; Jersakova & Johnson, 2007), the pollen transfer advantage gained from predictable pollinator movement should promote selection for plant traits that encourage predictable foraging behaviour, including vertically arranged inflorescence architecture (Routley & Husband, 2003; Jordan & Harder, 2006), reward gradients along inflorescences (Pyke, 1978; Fisogni et al., 2011 but see Waddington & Heinrich, 1979 and de Jong et al. 2011), horizontal or pendant flower orientation and bilateral flower symmetry (Fenster et al., 2009). These results are therefore consistent with the occurrence of such directional foraging traits in true hermaphrodites (e.g. Ishii & Sakai, 2001; Cao et al., 2011).

Pollinator species differ in how much they show predictable directional tendencies (chapter 1; Jordan & Harder, 2006; Fisogni et al., 2011). The results suggest that selection should encourage traits that increase attraction of predictably moving pollinators. Many floral characteristics such as flower colour, shape and nectar composition have been associated with attraction of certain pollinator groups as part of pollination syndromes (Fulton & Hodges, 1999; Castellanos et al., 2004; Fenster et al., 2004; Thomson & Wilson, 2008). Conversely, some plant traits deter certain types of pollinators. For example, narrowing of flower corollas (Galen & Cuba, 2001; Castellanos et al., 2004) and absence of flower-lips or landing spots reduces bee attraction (Stout et al., 1998; Castellanos et al., 2004). Similarly the presence of secondary metabolites in floral nectar deters some flower visitors that find these compounds unpalatable (Adler & Irwin, 2005; Johnson et al., 2006). The simulation's results suggest that attractive or deterrent traits could be selected to either attract predictably foraging pollinators or repel unpredictably foraging pollinators. However, how different pollinator species vary in the consistency of directional movement is little studied (this is discussed further in chapter 4).

Predictable movement and the pattern of pollen receipt

Consistent upward movement leads to different patterns of pollen receipt among a plant's flowers in both theoretical studies (figure 2.5: Harder & Barrett, 1996; Rademaker et al., 1997; Kudo et al., 2001; Jordan & Harder, 2006) and field studies (Harder et al., 2000; Zeng et al., 2009). Geitonogamy was typically higher in flowers visited later in visit sequences, as expected. The expected decline in outcross pollen deposition in upper flowers when pollinators moved upwards occurred in my simulation only when arrival position was fixed at flower 1 (figure 2.5ii). When pollinators arrived at random positions and moved upward, upper flowers received more outcross pollen (figure 2.5i).

The opposite of the expected pattern of outcross pollen receipt arose when pollinators arrived at random positions. When pollinators always arrived at flower 1, flowers were always visited in vertical order starting with the lowest. However, with random arrival each flower has a 1 in 6 chance of being visited first. Thus, random arrival allows pollinators to visit upper flowers earlier in the visit sequence than if they arrived at the bottom flower. The earlier a flower is visited in sequence the more non-focal plant pollen is deposited on that flower, so greater outcross pollen receipt (Barrett et al., 1994; Engel & Irwin, 2003). Furthermore, a higher likelihood that a pollinator will travel upwards lowers the chance that it will deliver pollen to flowers below the arrival position. The combination of these two effects causes lower flowers to be visited less, whereas higher flowers tend to be visited more and earlier in the visit sequence compared to when pollinators arrive at the bottom flower, thus causing a reversal of the expected trend.

The predicted patterns of pollen deposition among flowers within plants (figure 2.5) suggest that lower flowers on plants visited by pollinators that arrive at bottom flowers and move upward would benefit if they expressed greater female function. This advantage arises because lower flowers receive less self-pollen and more outcross pollen. Similarly upper flowers, which receive less outcross pollen and more self-pollen, should allocate less to female function (Brunet & Charlesworth 1995).

Additionally, upper flowers likely export more of their pollen, as pollinators visit fewer additional flowers after visiting them, limiting pollen discounting. Thus, selection should also favour male expression in higher flowers and a decrease in male function in lower flowers. My simulations therefore support previous work (Lee, 1988; Thomson, 1989) that suggests these patterns of pollen deposition, which occur as a result of upward pollinator movement, might have driven the evolution of these non-random floral arrangements through dichogamy, such as Darwin's syndrome. Additionally, a similar pattern of allocation of reproductive resources occurs commonly in non-dichogamous racemes; male function being greater in higher flowers, female function being greater in lower flowers (Kudo et al., 2001; Ishii & Sakai, 2002; Zeng et al., 2009; Tang & Ren, 2011). However other non-exclusive explanations for these allocation patterns in adichogmaous plants include resource limitation due to lower flowers being able to access resources before higher flowers (Vallius, 2000; Kliber & Eckert, 2004) and architectural constraints on flowers of different positions (Wolfe, 1992; Diggle, 1995). The results of the simulations suggest such sex allocation patterns could be favoured in adichogamous plants, in the same manner as Darwin's syndrome, as a result of pollen deposition if pollinators show predictable upward tendencies.

When pollinators arrive at bottom flower, but then move randomly, the first flower visited always receives the large outcross pollen load (figure 2.5ii, random movement). This result may help explain patterns of expression of sexual function in Asteraceae capitula (included within heads in table 1.1) (Burtt, 1977; Webb, 1981). For these capitate plants, the outer flowers function as females, whereas the male-phase flowers are located centrally through dichogamy. As outer flowers of such nonvertically arranged flowers are always visited first and subsequent movement is less predictable (Jordan & Harder, 2006), the flowers most likely to receive the most pollen, those typically visited first, should emphasise female function.

When pollinators arrive less predictably, but move upward, outcross pollen deposition is greater in upper flowers (figure 2.5i). Thus, in this case selection might favour allocation of all reproductive resources to higher flowers as they should perform better as both males and females. However, because self-pollen deposition is still greater in upper flowers in these racemes, may still suffer costs to female success such as inbreeding depressions, stigma clogging and ovule discounting (Sage et al., 1994; Husband & Schemske, 1995; Zhang et al., 2008). Thus, selection may still favour patterns of sex allocation similar to those described above for plants on which pollinators arrive at lower flowers and move upward. Thus, the simulation results suggest that similar expression patterns of sexual function to those seen in nature would occur when pollinators show less predictable movement.

Conclusion

My simulations revealed that predictable upward pollinator movement should cause favourable pollen transfer in adichogamous plants, which is an advantage previously discussed for only plants that show dichogamy (Harder et al., 2000; Jordan & Harder, 2006). These findings also identify the nature of selection that may have favoured plant traits associated with predictable movement seen in adichogamous plants. Additionally, results confirm that predictable upward movement can generate differential pollen receipt across the inflorescence, which may underlie patterns of sex expression seen in nature. The presented simulations demonstrate the importance of considering the order and manner of pollinator foraging within the inflorescence in addition to how much movement is made (Harder et al., 2004). Furthermore, I highlight the need to consider predictable foraging in not only plants with spatial separation of sexual function within the inflorescence, via a non-random arrangement of sex function like Darwin’s syndrome, but also within adichogamous plants.

Chapter 3: The evolutionary advantage conveyed by Darwin's syndrome

Introduction

Many plants show a combination of dichogamy and sequentially ordered flower development within their inflorescences (chapter 1). These characteristics can create non-random arrangements of male- and female-phase flowers. One such arrangement, known as Darwin's syndrome (after Darwin, 1862), is a combination of racemous inflorescence topology, protandry and acropetalous flower development. This combination results in flowers arranged vertically with flowers in the male-phase (male flowers) positioned above older flowers in the female-phase (female flowers). Pollinators, particularly bumblebees, tend to move upwards through racemes from the bottom to the top of the inflorescences (Waddington & Heinrich, 1979; Corbet et al. 1981; Routley & Husband, 2003; Fisogni et al., 2011). This foraging has the result that in Darwin's syndrome male flowers tend to be visited after female flowers. This order of visitation was first suggested by Darwin (1862) as a mechanism to reduce the possibility that a plant's own pollen is carried to the plant's own stigmas, thereby reducing geitonogamy. Reductions in self-pollen deposition will improve the female fitness of plants when self-fertilization leads to high inbreeding depressions and poor progeny survival (Husband & Schemske, 1995 and 1997) and even when self-incompatibility prevents self-fertilisation if self-pollen interferes with outcross pollen fertilisation (Sage et al., 1994; Zhang et al., 2008). Directional movement from female to male flowers in other non-random floral arrangements should convey a similar advantage (Harder et al., 2004). In this study I investigate, by use of a simulation model, how non-random arrangements of male and female flowers in alter plant success relative to inflorescences of adichogamous flowers.

Understanding how pollinator movement alters reproductive success of plants showing non-random arrangements of male and female flowers will help answer why such arrangements evolved. Darwin’s (1862) prediction that non-random arrangements reduce selfing when pollinators move from female to male flowers has only been recently tested empirically. Harder et al. (2000) created artificial arrangements in the, normally adichogamous, racemes of *Eichhornia paniculata* (Pontederiaceae) by removal of stigmas and stamens to make functionally male and female flowers. The fraction of self-pollen receipt as a part of the total receipt were compared to unaltered adichogamous racemes. Self-pollen deposition increased with higher flower position from the bottom in unaltered inflorescences (Harder et al., 2000). Inflorescences with female flowers above males showed consistently higher selfing across all their stigmas, comparable to that of the top flowers of unaltered inflorescences. Inflorescences exhibiting Darwin’s syndrome had consistently lower levels of selfing throughout the inflorescence than adichogamous plants and inflorescences with female flowers above males. Darwin’s (1862) prediction that non-random floral arrangements can reduce geitonogamy has also been supported by modelling studies based on observed pollinator foraging on artificial racemes (Jordan & Harder, 2006).

In contrast, other observational studies have not found Darwin’s syndrome reduces the incidence of self-pollination. In experiments similar to Harder et al. (2000), Jersàkovà & Johnson (2007) confirmed that Darwin’s syndrome resulted in a reduction in the total amount of self-pollen received by orchid *Satyrium longicauda*. However, protandry also resulted in a reduction in outcross pollen receipt, due to reduced pollinator attraction and fewer receptive stigmas at any one time. This additional reduction in outcross pollen receipt in the protandrous plants resulted in the same proportions of geitonogamous pollen receipt as in non-sexually segregated plants. A lack of change in the proportion of self-pollen receipt minimises the benefit of reduced total self-pollination gained from Darwin’s syndrome (Jersàkovà & Johnson, 2007). Furthermore, the resultant decrease in total outcross pollen receipt will likely be detrimental to the plant, due to reduced ovule fertilisation (Engel & Irwin, 2003).

Darwin’s syndrome has been linked to a pollen export advantage. The lower total self-pollen receipt by Darwin’s syndrome plants allows more of a plant’s own pollen to be available for export (Klinkhamer et al., 1994; Harder et al., 2007; Jersàkovà & Johnson, 2007). This increase in export occurs regardless of any changes in outcross receipt (Jersàkovà & Johnson, 2007) and should still benefit plants even is self-pollen deposition does not affect female success (Harder & Barrett, 1996). Similarly, Routley & Husband (2003), in an observational experiment, found Darwin’s syndrome did not convey a significant female outcrossing advantage compared to adichogamous plants but did convey a twofold advantage in their estimate of seeds sired on other plants. The evidence presented by these studies indicates that a male reproductive advantage, increased export, as opposed to a female reproductive advantage, decreased self-fertilisation, drove evolution of Darwin’s syndrome and presumably other non-random arrangements (Jersàkovà & Johnson, 2007). This review thus demonstrates that studies have found similar but inconsistent results when investigating the adaptive value of Darwin’s syndrome and other non-random floral arrangements.

Geitonogamy causes complete pollen discounting (Lloyd 1992; Harder & Wilson 1995), so the advantages of reduced self-pollination and increased pollen export are not exclusive. Jordan & Harder’s (2006) model predicted, in addition to a reduction in selfing, that pollinators carried more of a plant’s pollen at departure from plants that exhibit Darwin’s syndrome. Similarly Harder et al. (2000) found some evidence of increased export in Darwin's syndrome plants, in addition to a selfing advantage, although this advantage was not consistently seen across years.

In addition to inconsistences in findings when studying the adaptive value of non-random floral arrangements, few studies address how predictable a pollinator's movement in a set direction needs to be to confer these advantages (but see Jordan & Harder, 2006). Although Darwin's syndrome is perhaps the most common non-random arrangement, other arrangements created by a combination of similar traits exist. Inflorescences of *Besseya bullii* (Scrophulariaceae) show racemeous topology and acropetalous flower development, but show protogyny as opposed to protandry (McKone et al., 1995), resulting in a reverse arrangement to Darwin's syndrome. In such racemes upward pollinator movement from male flowers to female flowers (but see McKone et al., 1995). Similarly non-random arrangements are seen in topologies other than racemes. Asteraceae have capitulate inflorescences (included within heads in table 1.1), which show female flowers on the periphery of the inflorescence and protandrous flowers in the centre, resulting in a set of male-phase flowers surrounded by female flowers for much of the inflorescence flowering period (Burtt, 1977; Webb, 1981). In these inflorescences movement from female to male flowers would be expected to be less predictable (chapter 1). Additionally, patterns of pollinator movements within a single topology, such as racemes, differ among pollinator species (Fisogni et al., 2011; chapter 1). Bumblebees typically move predictably upward (Darwin, 1862; de Jong et al., 2011), but other species like hummingbirds (Grant & Grant, 1968; Harder et al., 2004), honeybees and solitary bees (Fisogni et al., 2011) are reported to move less predictably. Given such variation in the predictability of pollinator foraging in inflorescences and in non-random arrangements, how is the advantage of non-random arrangements influenced by unpredictable pollinator movement? The answer is needed to quantify the adaptive advantages of such non-random flower arrangements, and how dependent they are on highly predictable pollinator behaviour. The effect of variable pollinator foraging on such plants may also reveal an explanation for the inconsistency between the studies investigating non-random arrangements' influence on pollen transfer. These studies may disagree because certain advantages depend on highly predictable pollinator behaviours. Here I address how predictability in pollinator movement alters pollen transfer within plants with non-random floral arrangements explaining the inconsistencies in results of past investigations. When pollinator movement from female flowers to male flowers is less predictable, geitonogamy should increase, as a pollinator is more likely to visit a female flower after a male flower. Pollinator movement in a way that promotes high geitonogamy should also reduce plant export, due to pollen discounting.

In this study I use a simulation model to investigate pollen transfer when plants show differing arrangements of male, female or bisexual flowers. I investigate how non-random arrangements of male- and female-phase flowers alter pollen transfer and therefore male and female success of plants compared to adichogamous racemes. Furthermore, I address how pollen transfer within these plants differs when pollinators show increasingly unpredictable foraging behaviour.

Methods

To investigate the impact of variable predictability in directional movement, I carried out a set of simulations identical to those described in chapter 2, except that the sex role of flowers in the simulated plants could be changed.

Flowers could function in one of 3 different sex types. Bisexual flowers functioned as both males and females simultaneously. These flowers had active anthers and stigmas, and thus functioned in the same way as the flowers simulated in chapter 2 (see figure 2.2). Non-bisexual flower phases were modelled by allowing only one stage of the pollen transfer sequence for an individual flower: female flowers had receptive stigmas, but did not possess pollen (figure 3.1); whereas male flowers donated pollen, but did not receive it (figure 3.2). The different flower sex types were then assembled to create three inflorescence arrangements (figure 3.3). Plants showing no sexual segregation had all flowers functioning as bisexuals, like those simulated in chapter 2. Darwin's syndrome plants had the lower half of plants function as females and the upper half as males. The "protogynous" syndrome had the upper half of flowers as females and lower half as males (like *B. bullii* racemes).

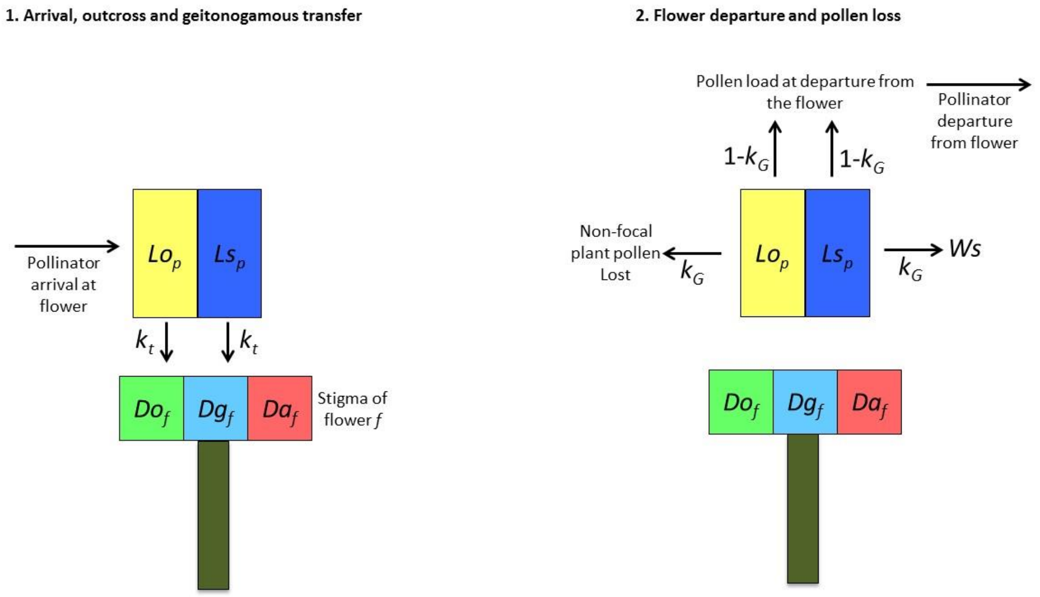


Figure 3.1: The sequence of pollen transfer for female flowers. Female flowers have no active anthers precluding pollen removal and autogamous self-pollination.

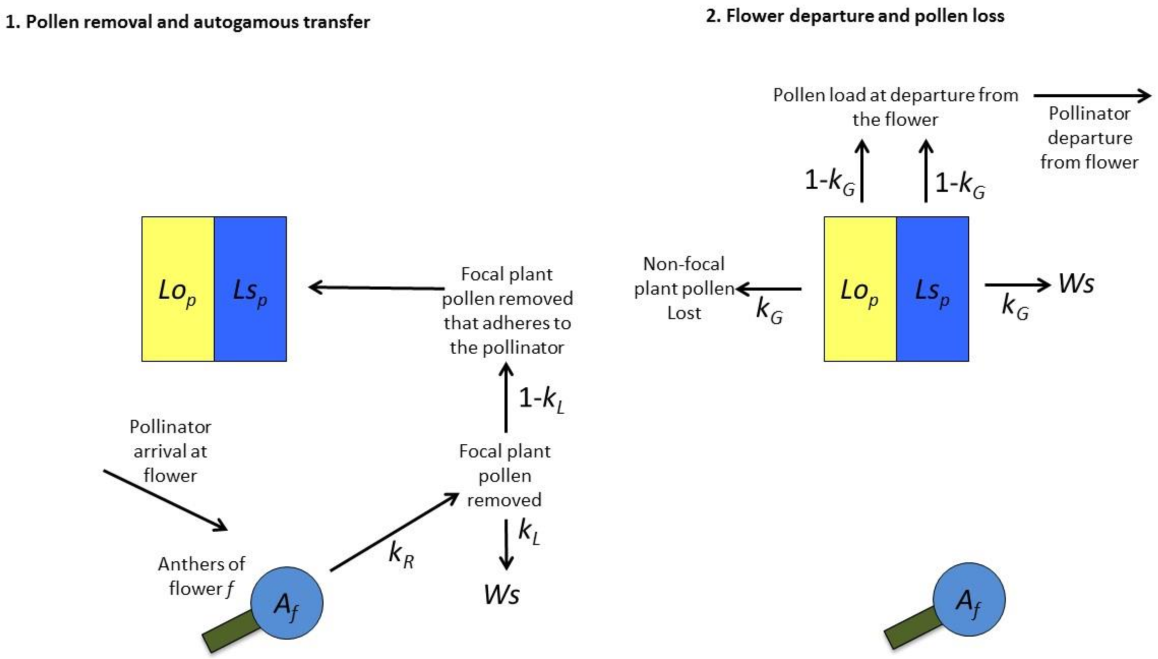


Figure 3.2: The sequence of pollen transfer for male flowers. Male flowers lack stigmas, so pollen is not deposited on these flowers and pollen is only removed.

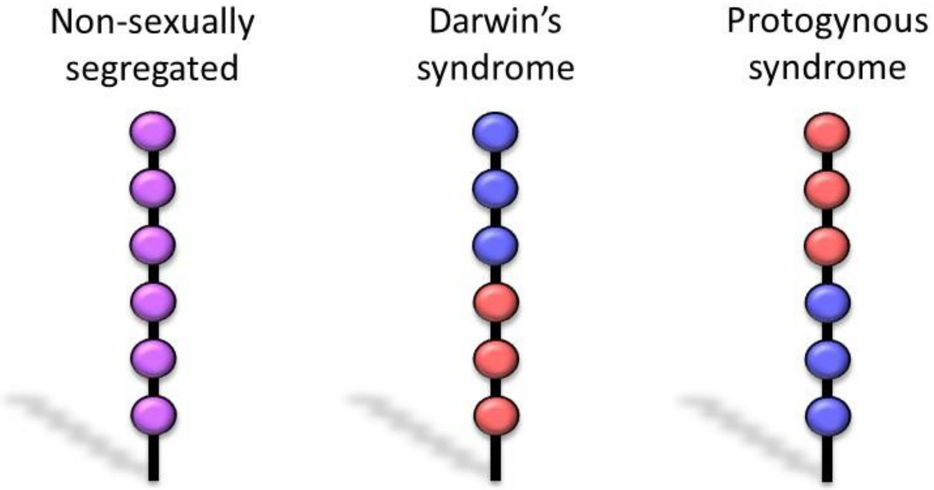


Figure 3.3: The different inflorescence types used in the simulations with *n*=6 flowers. The circles on each raceme represent flowers with the colours distinguishing flower types; Purple, bisexual; Red, female; and Blue, male.

Sets of simulations were carried for each inflorescence arrangement as described in chapter 2, including pollen transfer fractions and parameter values (based on Rademaker et al., 1997 and Routley & Husband, 2006). Ten pollinators showing the same foraging behaviour visited simulated racemes. Foraging behaviours differed in arrival position (either random or fixed to flower 1) and subsequent movement (see table 2.4), resulting in eight foraging scenarios per inflorescence type. As A_{f0} was the same for pollen-donating flowers of all arrangements (40,000 grains), comparing the total export and pollen receipts as a measure of male and female success, respectively, would bias the results in favour of non-sexually segregated plants, as they have more flowers active as pollen donors and receivers. Thus, all pollen fates and pollen receipt were compared on a per pollen donor flower basis. I also compared the fraction of the total pollen received by the stigmas of the whole plant that is self-pollen, the selfing fraction,

$$SF = \frac{T_{Dg}+T_{Da}}{T_{Dg}+T_{Da}+T_{Do}}\,. \tag{3.1}$$

Results

Female success

Mean pollen receipts differed depending on inflorescence arrangement and predictability in pollinator foraging behaviour (figure 3.4). Autogamy was impossible for plants with sexual segregation (Darwin's syndrome and the protogynous syndrome). Protogynous plants experienced geitonogamy comparable to non-sexually segregated plants, irrespective pollinator behaviour. Plants with Darwin's syndrome received much less self-pollen by geitonogamy compared to non-sexually segregated plants. This difference was greater when pollinators predictably move upward, to the extent that strict upward movement eliminated geitonogamy. When pollinators always arrived at flower 1, little impact was made on the amount of self-pollen deposition in plants that show Darwin's syndrome.

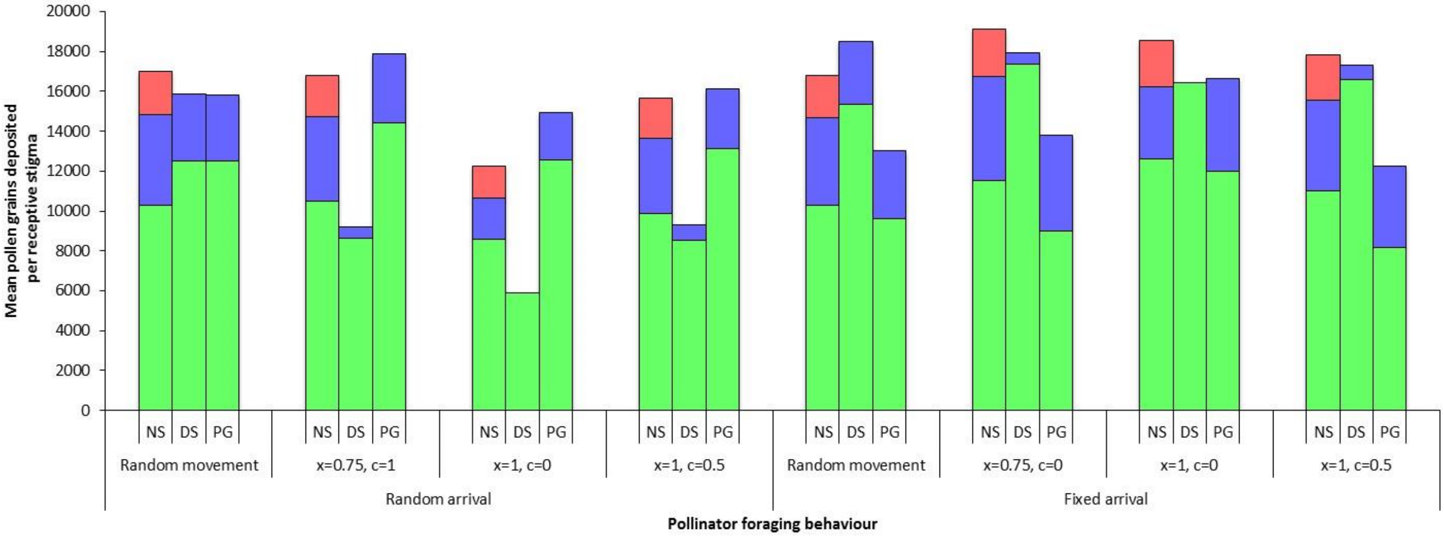


Figure 3.4: Effects of inflorescence type, pollinator arrival position and movement pattern on mean pollen receipt per pistillate flower. Colours correspond to the source of pollen deposition: Green, outcross pollen deposition; Blue, pollen received by geitonogamy and Red, pollen received by autogamy. Labels NS, DS and PG refer to non-sexually segregated, Darwin's syndrome and protogynous inflorescences, respectively.

Mean receipt of outcross pollen per stigma of a non-sexually segregated plant depended strongly on pollinator behaviour (figure 3.4). Whereas outcross receipt was the same between sexually segregated arrangements when arrival and movement were random, outcross pollen receipt differed between the two sexually segregated arrangements when pollinators arrived at flower 1 and moved randomly. When pollinators arrived at random positions and predictably moved upward, mean outcross pollen receipt decreased with increased movement predictability for plants with Darwin’s syndrome compared to non-sexually segregated plants. When pollinator arrival was always at the bottom flower, plants showing Darwin’s syndrome received more outcross pollen per flower than non-sexually segregated inflorescences and protogynous inflorescences.

The patterns of selfing fraction (figure 3.5) followed patterns expected from figure 3.4. Darwin’s syndrome typically reduced geitonogamy more than other arrangements. The selfing fraction was also lower for Darwin’s syndrome plants when pollinators moved more predictably and arrived at the bottom flower. Protogynous plants experienced about the same selfing fraction as non-sexually segregated plants

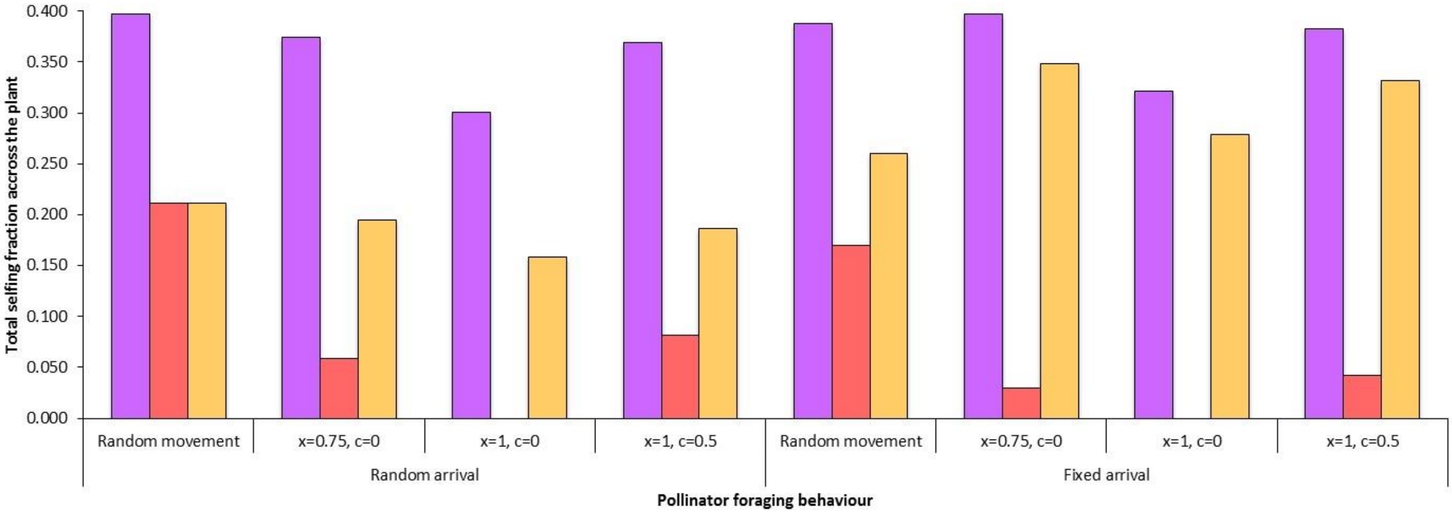


Figure 3.5: The mean selfing fractions of plants showing different floral arrangements after being visited by 10 pollinators showing a single foraging behaviour and arrival is random or fixed on flower 1. Column colours indicate the floral arrangement plants display: non-sexually segregated plants (purple column); Darwin’s syndrome (red column); protogynous arrangement (orange column).

when arrival was fixed at flower 1 and movement biased. When arrival was random protogynous plants showed about half the level of selfing as non-sexually segregated plants.

Male success

Sexually segregated plants tended to gain an export advantage over non-sexually segregated plants when pollinators moved randomly (figure 3.6), due to prevention of autogamy and therefore pollen discounting. Fates of pollen from plants with Darwin's syndrome were always preferable to those of non-sexually segregated plants. Darwin's syndrome promoted pollen removal and pollen export per donor flower compared to non-sexually segregated. This advantage increased slightly when movement was more predictable. Fixing arrival at bottom flowers had little effect on pollen export from plants with Darwin's syndrome compared to the same movement with random arrival. In contrast, for the protogynous arrangement, biased pollinator movement reduced pollen exported per flower compared to non-sexually segregated plants due to more pollen discounting through geitonogamy and pollen loss occurring as a result of more flower visits preceding pollen removal.

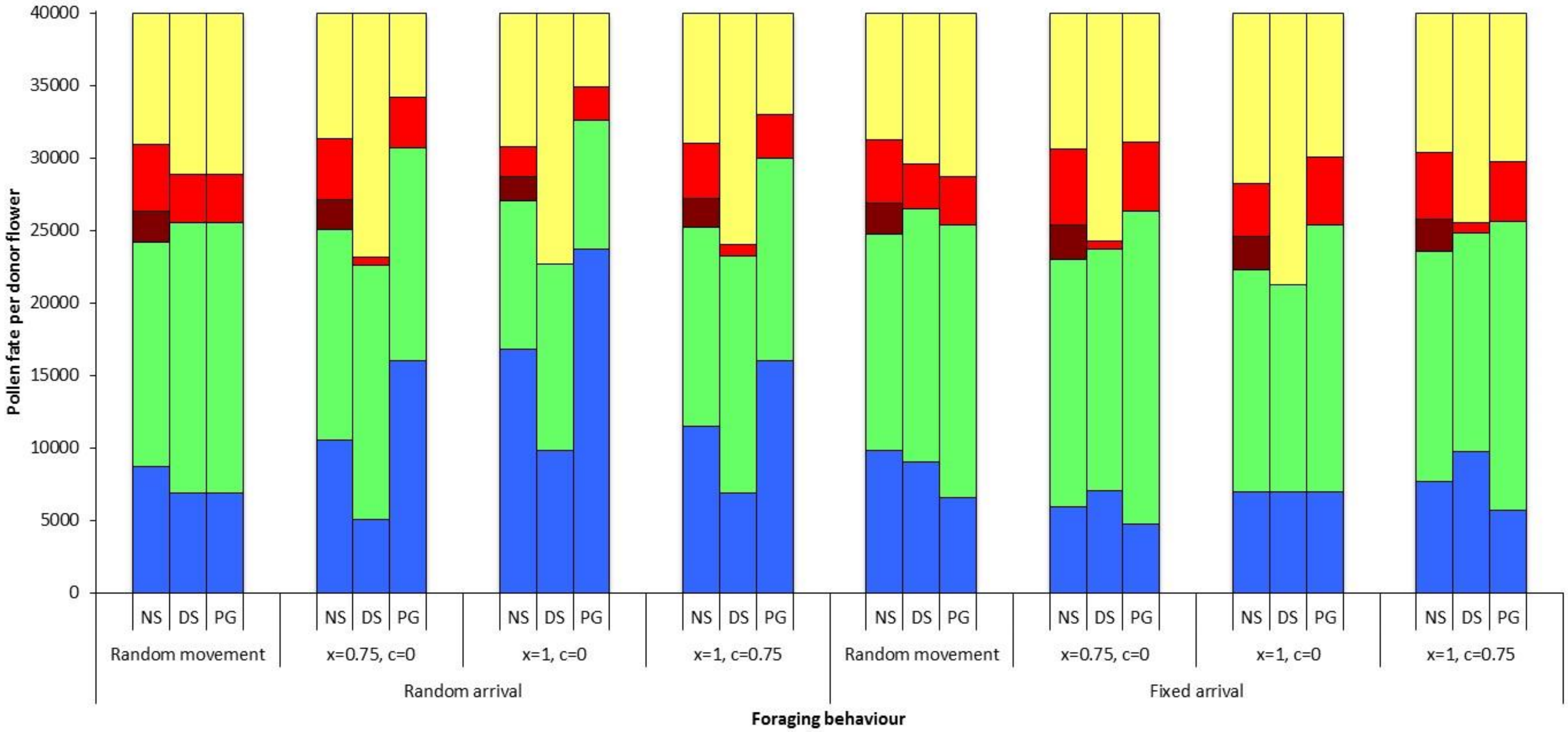


Figure 3.6: The mean pollen fates per donor flower of plants of different arrangements when visited by pollinators showing different foraging behaviours. Colours indicate final pollen fates of plants exposed to pollinators of a certain foraging behaviour type (x axis): Blue, is pollen that remains un-removed from anthers (T_A); Green, pollen lost to the system (T_L); Dark Red, focal plant pollen deposited on stigmas by autogamy (T_{Da}); Bright Red, focal plant pollen deposited on stigmas by geitonogamy (T_{Dg}) and Yellow, pollen exported from the plant on departing pollinators (T_E). Labels NS, DS and PG refer to the different inflorescence arrangements; Non-sexually segregated, Darwin's syndrome and Protogynous respectively.

Discussion

My simulations demonstrate that non-random floral arrangements should promote pollen transfer, but it are not sufficient to gain these advantages: directional movement of pollinators from female to male flowers is also required. When simulated pollinators moved predictably from female to male flowers within sexually segregated arrangements, plants gained the following advantages over non-sexually segregated, bisexual, plants: i) reduced self-pollination, through prevention of autogamy and reduction in geitonogamy; ii) increased export of pollen per donor flower as a result of reduced pollen discounting and pollen loss; and iii) increased receipt of outcross pollen when pollinator arrived first at female flowers. Plants with Darwin’s syndrome realize these benefits when pollinators arrive predictably and move upwards. Most of these advantages occur if movement is biased, but a potential cost of non-random floral arrangement arises when arrival position is not fixed. If arrival is unpredictable and pollinators move upward, then plants experience reduced outcross pollen receipt per flower. Additionally, the simulations of protogynous syndrome plants show that plants with non-random arrangements incur several costs in terms of selfing, export and outcross receipt when pollinators move consistently from male flowers to female flowers. These costs seen in our simulations of upward movement in a protogynous syndrome plant would also be incurred if a pollinator were to move downward in a plant showing Darwin’s syndrome. In this section I first explain how non-random arrangements of male and female flowers and predictable pollinator foraging interact to cause these effects. I then show how the effects of variable pollinator behaviour alter pollen transfer, clarifying apparently inconsistent interpretations of the adaptive advantage of Darwin’s syndrome. Lastly, I discuss the evolutionary consequences of non-random floral arrangements.

Went pollinators moved less predictably the pollen transfer advantages associated with non-random arrangements of male and female flowers decreased. An increase in geitonogamy and therefore pollen discounting was expected for plants with Darwin's syndrome when upward movement becomes less predictable, resulting in downwards movements from male to female flowers. This effect has been observed in past studies of Darwin's syndrome (Harder et al., 2004; Jordan & Harder, 2006). This effect is particularly evident for the protogynous arrangement (which is identical to downward movement on a Darwin's syndrome plant). When pollinator arrival position was unpredictable, Darwin's syndrome plants suffered reduced outcross pollen receipt as pollinator movement became more consistent. This reduction results from reduced visitation to lower flowers (see visitation numbers in tables 2.5 and 2.6) causing less outcross deposition. When arrival was fixed, plants with Darwin's syndrome received more outcross pollen per flower than plants with bisexual flowers because the few lower female flowers always received more outcross pollen during the first few flower visits (see Rademaker et al., 1997; Engel & Irwin, 2003; chapter 2).

Resolution of inconsistency in past investigations

Reduced self-pollination (Darwin, 1862; Harder et al., 2000) and increased pollen export (Harder et al., 2000; Routley & Husband, 2003; Jersàkovà & Johnson, 2007) associated with Darwin's syndrome have been observed previously. However, often they have not been observed together (but see Jordan & Harder, 2006) as my simulations suggest. The present simulations support the prediction that upwards pollinator movement in plants showing Darwin's syndrome (or female to male movement in other plants with non-random arrangements) should have the dual benefit of reduced selfing and increased export. Such a finding is unsurprising, based on the known interaction between self-pollen deposition and pollen discounting with export (Lloyd, 1992; Klinkhamer et al., 1994; Harder & Wilson, 1995; Harder et al., 2007). The potential negative effects on outcross pollen receipt have also been discussed (Jersàkovà & Johnson, 2007) as a consequence of reduced visitation and reduced stigma number. A reduction in outcross pollen receipt in plants showing Darwin's syndrome in my simulations was observed to occur if pollinator arrival was unpredictable. I will now attempt to explain the apparent inconsistencies between past studies of the adaptive advantage of Darwin's syndrome within the context of the presented simulations.

My simulations of pollinator movement suggest, in accordance with previous studies (Darwin, 1862; Harder et al., 2000; Jordan & Harder, 2006) that when pollinator movement is biased and arrival is predictable, plants with Darwin's syndrome benefit from increased export and reduced selfing. Jordan & Harder (2006) observed movement by three bumblebee species (*Bombus huntii*, *B. impatiens* and *B. occidentalis*) within artificial racemes that correspond to simulated pollinators that show predictable arrival and weak directional movement (see table 2.4); 75% of arrivals involved the lowest whorl of flowers and pollinators moved upwards during 71% of flower transitions. Similarly Harder et al. (2000) observed that *B. fervidus* and *B. vagans* visiting *Eichhornia paniculata* inflorescences arrived consistently at the lowest flowers of racemes and move upwards during 90% of flights. This behaviour is very similar to simulated pollinators with predictable arrival and strong upward movement behaviour. Both of these studies found that such consistent movement within plants with Darwin's syndrome reduced selfing compared to adichogamous racemes, in agreement with the presented simulation's findings (figure 3.4). Jordan & Harder (2006) found a similar export advantage, again in accordance with the above simulation (figure 3.6). Harder et al. (2000) found plants with Darwin's syndrome to realize enhanced pollen export during only one of two years. This inconsistency may be a consequence of reduced pollinator visitation in the first year, resulting in low pollen export (Harder et al., 2000).

In contrast to my simulation results and other studies of Darwin's syndrome, Jersáková & Johnson (2007) found equivalent selfing fraction between natural *Satyrium longicauda* plants and those that they had manipulated to resemble Darwin's syndrome, although absolute self-pollination was significantly lower in the latter plants. However, Jersáková & Johnson (2007) did find an export advantage in plants showing Darwin's syndrome. This apparent inconsistency can be explained when by the details of pollinator behaviour. The *Batsiothia scheki* hawkmoth pollinators observed by Jersáková & Johnson (2007) typically move upwards, but 'frequently' begin foraging on middle and upper whorls of the raceme and, perhaps, foraged like simulated pollinators showing random arrival and strong directional movement. In the simulations, such behaviour reduced outcross pollen receipt for Darwin's syndrome plants compared to non-spatially segregated plants can be seen (figure 3.5), a result also observed by Jersáková & Johnson (2007). Therefore, although the findings of Jersáková & Johnson (2007) appear inconsistent with other studies, when the likely pattern of movement is considered these apparently contradictory findings can be explained. This also demonstrated that considering the predictability of both arrival and movement is equally important when understanding the impacts on pollen transfer in plants showing non-random floral arrangements.

Routley & Husband (2003) also observed results inconsistent with my simulation findings. Although the bumblebee pollinator movements observed by Routley & Husband (2003) on *C. angustifolium* racemes are similar to simulations where arrival at the lowest flower is certain and pollinators show weak directional movement, no significant selfing reduction was associated with Darwin's syndrome. However, the arrangement of sexual function of flowers used by Routley & Husband (2003) differs from that used in other studies of Darwin's syndrome, and the above simulations (figure 3.3). Routley & Husband's (2003) Darwin's syndrome treatment had male flowers above bisexual flowers as opposed to females to balance pollen-ovule ratios, allowing comparisons of total pollen transfer. This different arrangement caused patterns of deposition in lower flowers identical to plants with only bisexual flowers with no sexual segregation. Therefore, Routley & Husband's (2003) different Darwin's syndrome arrangement, failed to alter self-pollen deposition in lower flowers, but would still promote export compared to non-sexually segregated plants, as upper flowers still avoided pollen discounting. Routley & Husband's (2003) results demonstrate that high overlap in sexual phases of sequential blooming inflorescences may reduce the benefits of non-random arrangements. Thus, Routley & Husband's (2003) result suggests that further investigation on the prevalence and impact of sex role overlap in non-random floral arrangements is needed to understand the pollen dispersal consequences of non-random floral arrangements.

Evolutionary consequences of non-random floral arrangements

Many of the advantages of Darwin's syndrome, (i.e. reduced self-pollination and increased export) are maintained when pollinator foraging is less predictable, as long as movement upwards remains biased (figure 3.4 and 3.6). Thus, selection should favour a non-random arrangement when pollinators exhibit directional movement, even when this directionality is weak. This might explain the common occurrence of Darwin's syndrome across many plant families (Bertin & Newman, 1993). Furthermore selection for additional plant traits, to encourage more predictable and biased pollinator movements, would be expected. Such traits would further improve directional pollinator movement may include bilateral symmetry of flowers, pendent or horizontal flowers (Fenster et al., 2009) or perhaps reward gradients (Fisogni et al., 2011). Alternatively, as pollinator species differ in consistency of directional movement (Wolf & Hainsworth, 1986; McKone et al., 1995; Harder et al., 2004), plants displaying non-random floral arrangements may also favour traits that attract pollinators with greater directional consistency or deter less predictable pollinators (e.g., Castellanos et al., 2004) (see chapter 2).

High outcross pollen receipt in Darwin's syndrome plants requires predictable arrival. My simulations suggest that strong selection for increasing predictably of pollinator arrival position in plants with non-random arrangements of the sex roles. This arrival predictability might be achieved by stronger attractive signals in female flowers (e.g. Lunau & Maier, 1995; Riffell et al., 2008; Clarke et al., 2013). More nectar in lower flowers in racemes have been observed in several species, including those showing Darwin's syndrome (Carlson & Harms, 2006; Fisogni et al., 2011), and promotes consistent arrival (Galen & Plowright, 1985). These nectar gradients have also been suggested to possess a role in encouraging directional movement behaviour (Fisogni et al., 2011). However, several studies demonstrate that nectar gradients do not alter the direction of pollinator foraging within inflorescences (Waddington & Heinrich, 1979; de Jong et al., 2011).

When simulated pollinators moved randomly, a non-random arrangement of male and female flowers, Darwin's syndrome or protogynous syndrome, is favourable. As geitonogamy is reduced compared to non-sexually segregated plants and autogamy prevented. Additionally, plants with non-random arrangements export more pollen than non-sexually segregated plants when pollinators move randomly. Thus, even when pollinator movement is random we might still expect selection to favour such an arrangement perhaps through dichogamy. This result might explain why non-random arrangements achieved by protandry and protogyny are still favoured by plants when pollinators seem to move unpredictably (McKone et al., 1995). For example, the occurrence of protandrous and protogynous capitula (Burtt, 1977; Webb, 1981), in which pollinator visit sequences are unpredictable. Thus, my simulations explain the occurrence of non-random arrangements even when pollinators do not show highly predictable movement behaviour.

The model implemented in this study does not allow for the pollination environment to change over the simulation. In natural systems the abundance and identity of species visiting plants (Kunin, 1993; Sargent & Roitberg, 2000), the total number of available flowers (Kunin, 1993) and the number of male and female flowers in dichogamous species (Charlesworth & Charlesworth, 1999; Zhao et al., 2008) vary over time. Although changes in pollinator complement may alter pollen transfer and plant success (Kunin, 1993), especially if pollinator behaviour differs between species (Fisogni et al., 2011), the robustness of the advantages of increased export and reduced selfing of non-random arrangement shown here suggest that non-random arrangements should still be preferable. If changes in pollen or stigma availability may alter the success of flowers early and late in the flower sequence, because one sex (phase) is more common in the population (Charlesworth & Charlesworth, 1999; Zhao et al., 2008), the advantages should be maintained to some degree, but further analysis is required to quantify this. However, sequentially blooming plants, like those showing Darwin’s syndrome, have been linked to ‘bet hedging advantages’ when optimal pollinator conditions are seasonally variable (Prusinkiewicz et al., 2007). Sequentially blooming plants tend to flower longer and therefore are more likely to be in flower during optimal pollination conditions. Thus, if variation in pollination conditions were included in the model, the relative benefits of non-random arrangements would perhaps be increased further.

Conclusion

The simulations presented in this study reveal differing benefits of non-random floral arrangements. Consideration of several aspects of pollinator foraging behaviours is required to understand the selective advantage that is likely to be driving the selection for these underlying non-random floral arrangements. However non-random floral arrangements should be favourable in several aspects compared to adichogamy under less predictable pollinator foraging. This advantage may explain the wide range of plants that show non-random arrangements of male and female flowers in their inflorescences. Although my simulations provide explanations for much of the variety in non-random arrangements and impacts of differing pollinator foraging within such plants, two aspects that require further investigation include the impacts of sex phase overlap on success of non-random arrangements and temporal variation in pollinator community. These topics may be linked, as low pollinator visitation may drive increased overlap of sex phases in protandrous species (Sargent & Roitberg, 2000; Routley & Husband, 2003; Iwata et al, 2011).

Chapter 4: Variation and causes of pollinator behaviour in racemes

Introduction

Pollinators foraging on vertical inflorescences commonly arrive at a bottom flower and move upwards (chapter 1). This relatively consistent upward movement decreases geitonogamous self-pollination and associated pollen discounting in plants with Darwin’s syndrome, to the extent that female(-phase) flowers are visited before male(-phase) flowers (chapter 3). This combination of behaviour and inflorescence organization can promote plant fitness as females by reduced inbreeding (Husband & Schemske, 1995). Additionally reduced pollen discounting increases male fitness of plants by pollen export (Harder et al., 2007), an advantage that will occur even if the plant suffers no inbreeding costs due to incompatibility or low inbreeding depression (Harder & Barrett, 1996). Such advantages apply to a lesser extent for racemose plants with adichogamous flowers (chapter 2).

The advantages of directional pollinator movement should select for floral and inflorescence traits that encourage upward foraging by their pollinators. Such traits, in addition to un-branching racemose inflorescence topology, may include horizontal or pendant flower orientation, bilateral flower symmetry (Fenster et al., 2009) and low flower helical angle (Iwata et al., 2011). When pollinators differ in predictability of upward movement, traits that encourage visitation of the more predictably foraging pollinators, and deter unpredictably foraging pollinators, should also be favoured (chapter 2 and 3). Traits that encourage visitation by efficient pollinators and discourage visitation of poor-quality pollinators have been seen in many systems (Galen & Cuba, 2001; Castellanos et al., 2004; Adler & Irwin, 2005; Thomson & Wilson, 2008, discussed in chapter 2).

Many efficient pollinators forage consistently upward in racemes, including bumblebees, *Bombus sp.* (Darwin, 1862; Routley & Husband, 2003; Fisogni et al., 2011; de Jong et al., 2011), hawkmoths (Jersakova & Johnson, 2007), leaf cutter bees (Iwata et al., 2011), and bombyliid flies (Knoll 1921). Other plant visitors have been reported to move upwards albeit less predictably; such as honeybees (Delvin & Stephenson, 1985; Fisogni et al., 2011). Some other pollinators such as solitary bees have been observed to show no directional tendency (Fisogni et al., 2011) and *Dilalictus* bees have been seen to forage laterally about tiers of racemes (McKone et al., 1995). Thus, pollinator species differ in their upward movement tendency. However, direct comparison of movement between pollinator species foraging on different plants is difficult, as plants differ in floral characteristics and methods quantifying movement behaviours vary.

How the consistency of pollinator movement in racemes varies among pollinator species is little studied. Some studies have observed foraging of multiple pollinator species on the same inflorescences, but often species are grouped together by genus (Fisogni et al., 2011; de Jong et al., 2011: but see Haynes & Mesler, 1984 and Jordan & Harder, 2006), which may conflate differences in foraging behaviour within a genus. Nevertheless, Jordan & Harder (2006) noted that three species of bumblebee foraged similarly on artificial plants of several topologies. As many plants are pollinated by multiple species (Waser et al., 1996), often belonging to the same genus or similar functional group (Fenster, Armbruster, Wilson, Dudash, & Thomson, Pollination syndromes and floral specialization, 2004), the extent to which pollinator species differ in foraging behaviour could affect the mode and intensity of selection on plant traits. Quantifying how pollinators differ in their movement could also affect pollen fates in plant communities. If pollinators differ in their movement enough to change the incidence of self-pollination, changes in the frequency of visitation of different species will alter the selfing and pollen discounting plants experience (Herrera, 2000; Keller & Waller, 2002). Pollinator communities are changing worldwide due to global changes in climate (Potts et al., 2010), human changes in land use (Goulson & Williams, 2001; Potts et al., 2010) and species introductions (Paton, 1993; MacFalane & Gurr, 1995; Inoue et al., 2008). Thus, an understanding of movement differences should have useful applications in conservation and invasion ecology.

Why pollinators move upward is not understood, precluding explanation of variation in behaviours between species. The most studied explanation for upward foraging is that pollinators travel through the inflorescence down gradients in floral nectar rewards. In this scenario, pollinators arrive where rewards are highest and then visit less rewarding flowers, continuing up the raceme to depart when rewards are low (Pyke, 1978; Best & Bierzychudek, 1982; Charnov, 1982). The level of floral rewards pollinators encounter has been observed to influence foraging behaviour. Typically, the probability of departure is linked to the level of rewards encountered, departure being more likely when rewards are low (Hodges, 1986; Biernaskie et al., 2009; Taneyhill, 2010) or highly variable (Lefebvre et al., 2007). Many racemose plants show reward gradients in nectar rewards that decrease from the bottom of the raceme to the top; however, racemes lacking reward gradients or showing reverse gradients have also been observed (Carlson & Harms, 2006, lists examples of each). If pollinators showed directional foraging because they follow nectar gradients, we should expect pollinators move downwards through the raceme when visiting plants showing nectar gradients that decreased from top to bottom. While pollinators show predictable upwards foraging behaviour when racemes possess bottom-to-top reward gradients (Best &

Bierzychudek, 1982; Fisogni et al., 2011), pollinators still show this behaviour when nectar gradients are in the opposite direction (Waddington & Heinrich, 1979; Delvin & Stephenson, 1985; de Jong et al., 2011). We also still see upwards directional pollinator foraging when nectar rewards are constant across the inflorescence (Jordan & Harder, 2006) and when rewards are absent (Waddington & Heinrich, 1979; Li et al., 2011). Such evidence disproves the theory that pollinator directional foraging occurs due to nectar gradients and has led to a support for the reward independent hypotheses.

Pollinators may forage directionally in racemes for several reasons. Upwards tendencies may reflect an innate response to vertically arranged floral signals (Jander & Jander, 1970; Lloyd & Webb, 1986). This is supported by the tendency of bumble bees to forage vertically on vertical floral arrangements other than racemes, like panicles (Jordan & Harder, 2006). Upwards foraging may also be more efficient perhaps allowing faster travel between flowers. Such an efficiency advantage may be due to the orientation of the pollinator with the flower (Waddington & Heinrich, 1979; Corbet et al., 1981) or because insects have better flight control when travelling upwards (Lloyd & Webb, The avoidance of interference between the presentation of pollen and stigmas in angiosperms, 1986). Although many pollinator behaviours are driven by increasing their foraging rate (Hodges, 1986; Pleasants, 1989) and foraging efficiency (Schimid-Hempel et al., 1985; Rasheed & Harder, 1997; Dedej & Delaplane, 2005), the link between upward foraging and efficiency has not been quantified.

In this study I undertake a two-part investigation. First, I quantify and compare directional foraging of insects visiting *Chamerion* (=*Epilobium*) *angustifolium* (L.) Holub flowers. These patterns of behaviour are then incorporated in a foraging model to estimate how each species' behaviour (e.g. upward tendency or arrival position) might influence its potential to cause geitonogamy. Second, I investigate potential causes of upward foraging. The extent to which *C. angustifolium* shows a nectar reward gradient which is evaluated and the relation between pollinator movement direction and travel time between flowers is investigated to identify the consequences of this behaviour for foraging efficiency.

Methods and Results

Study species

Chamerion angustifolium, commonly known as rosebay willowherb (UK) or fireweed (North America), is a perennial member of the Onagraceae. Individual plants stand about 2 m tall with large raceme inflorescences (see table 1.1), which at any one time present a variable number of flowers (of displays observed $\overline{x}$±SD = 19.3 ±4.4 flowers). Rosebay willowherb is self-compatible, but has strong inbreeding depression, approximately 95% (Husband & Schemske, 1995 & 1997), which should favour mechanisms of outcrossing (Lande & Schemske 1985; Harder et al., 2007).

Chamerion angustifolium racemes show Darwin's syndrome, a non-random arrangement of male-phase flowers above female-phase flowers, created by dichogamy and acropetalous (bottom to top) flower development. Each flower has eight anthers, which initially shed blue-green pollen (Myerscrough, 1980). When anthers begin to shed pollen, the style is strongly deflexed with the four stigma lobes closed. This male phase normally lasts 2 to 3 days by the end of which the anthers are depleted (Routley & Husband, 2005). Female phase begins when the style straightens bringing the stigma into the centre of the flower. After pollen receipt the flowers begin to wither, become discoloured and drop off when seedpod development begins.

Differences in pollinator behaviour: methods

Data collection

I video-recorded pollinator foraging on *C. angustifolium* during nine days between July 16 and August 20, 2013. Plants were observed in the fields between Little High wood and Greater High wood, near Durham University's School of Biosciences (54^{o}45'N; 1^{o}34'W). Filming took place while the weather was generally fine with, little wind and no rain (as rain damaged equipment). Weather data from the Durham University weather observatory (Burt T.P., personal communication, July 2013) for each day that filming took place is presented in the appendix. Tripod-mounted cameras (Models: Canon R306, Canon R36 or a Panasonic V700) were positioned to view multiple inflorescences side on. Hour-long recordings were made throughout the day with 60-min intervals between about 9 am and 6 pm, weather permitting. 45 h of video-recording was taken of 27 different inflorescences.

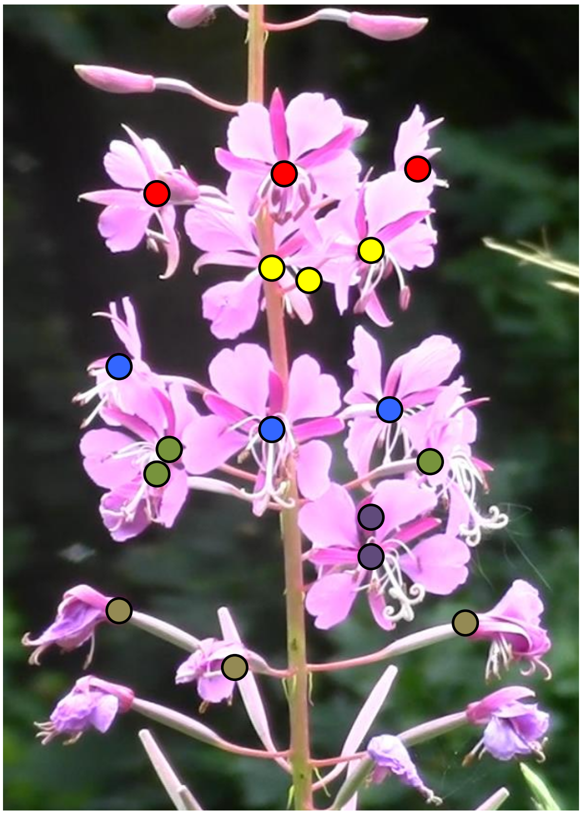
C. angustifolium racemes differ in length and number of flowers, which could influence pollinator behaviour, so the impacts of this variation in these factors on pollinator movement is considered in my analysis. Inflorescence architecture of each plant was characterised as a series of horizontal flower tiers arranged above one another (figure 4.1). Tiers were numbered from the bottom, tier 1 being the lowest and so forth. As plants differed in tier number, I calculated the relative vertical position of tier r in the raceme (r = 1 indicated the bottom tier) using

$$\rho_r = \frac{r-1}{R-1}, \qquad (4.1)$$

where R is the total number of tiers in the inflorescence. For each tier, the number of open, turgid flowers was counted, along with the sex phase of each flower. This classification allowed calculation of the percentage of each tier's flowers that were in male-phase. These plant characteristics determined from the video-recordings at the beginning of each hour-long observation period. Sequential opening and withering of flowers caused plant characteristics to change between video observations. Preliminary trials showed that assessing these plant characteristics from video-recordings yielded the same result as direct measurement in the field.

Pollinator movement within an inflorescence was observed by reviewing video recordings. The sequence of tiers between which a pollinator moved was recorded. I also noted the tiers at which each pollinator arrived and departed, and the number of flowers the pollinator had visited on the inflorescence before each movement, this is referred to as the current bout length, *b*.

Pollinator species were identified based on their colour patterns, using a key published by the Natural History Museum, London (www.nhm.ac.uk, accessed July 2013). Five main visitors to *C. angustifolium* were identified (table 4.1). However,

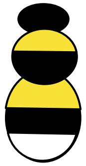
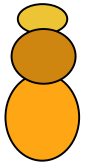
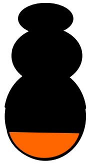
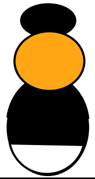
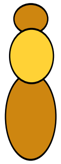


Tier	ρ	f_r	Number of ♂-phase flowers	 ♀-phase flowers	% male-phase flowers
🔴 6	1	3	3	0	100%
🟡 5	0.8	3	3	0	100%
🔵 4	0.6	3	2	1	66.6%
🟢 3	0.4	3	3	0	0%
🟣 2	0.2	2	2	0	0%
🟤 1	0	3	3	0	0%

Figure 4.1: An example of how the inflorescence structure of *C. angustifolium* was characterised. Flowers of the same tier are marked with dots of the same colour. This plant has $R = 6$ whorls. Note that below whorl 1 (the lowest whorl marked by brown dots) flowers were too withered to be visited and were not considered an active part of the inflorescence.

many bumblebee species are highly similar in appearance to other less common species and the video recordings were not sufficiently high quality to distinguish very similar species confidently, thus some individuals may have been misidentified. However, species group are usually dominated by a single species, with similar appearing species being rarer or absent (Natural History Museum, London, 2014), so that misidentification of the most common species is unlikely (Wolf et al., 2010). A possible exception involves the *B. lapidarius* group, which includes the equally common *B. lapidarius* and *B. pratorum*, which may still have be mistaken for each other. Henceforth these species groups are referred to by the name of their most common member.

Table 4.1: Species groups used in the analysis of pollinator behaviour based on similar groupings used by Fussel & Corbet (1992), Osborne et al. (2008) and Lye et al. (2012). Groups are characterised by the most common species and rarer species, which may have been mistaken for the main species of each group are also indicated (based on Natural History Museum, London, 2014). Also presented are the likely incidence of the rarer species in the study site (North-East UK) and the likelihood of mistaking the more common species for the rarer species.

Species	Subgenus	Description		Rarer species	Rarer species subgenus	Comparison
Bombus terrestris (Linnaeus)	*Bombus*	Two golden-yellow bands with a white tail		*B. lucourum*	*Bombus*	Differs slightly in colour. Less common but just as widespread as *B. terrestris*. Misidentifications of *B. lucourum* as *B. terrestris* are more likely than the reverse (Wolf et al., 2010), suggesting our sample contains few of these species.
				B. soroeensis	*Kallobombus*	Often differs slightly in banding pattern, possessing gaps in yellow bands. Rarer but present in the North East UK.
Bombus pascuorum (Scopoli)	*Thoracobombus*	Entire body is an orange-brown colour. Abdominal segment often has black hairs		*B. muscorum*	*Thoracobombus*	Similar appearance, rare but present in the North East UK.
				B. humilis	*Thoracobombus*	Similar appearance, but very rare in the North East UK.
Bombus lapidarius (Linnaeus)	*Melanobombus*	Black body with a red tail. Occurs in a pale form with a frontal yellow band.		*B. pratorum*	*Pyrobombus*	Appears identical to the *B. lapidarius* pale form. Appears even more similar as colours fade over the season. Equally common as *B. lapidarius*.
				B. ruderarius	*Thoracobombus*	Differ in head shape and leg structure. Rarer but present in the North East UK.
Bombus hypnorum (Linnaeus)	*Pyrobombus*	Bright orange thorax, black abdomen with white tail		An invasive species from Europe. No UK native species resemble its distinctive colours.		
Apis mellifera (Linnaeus)	*Apis*	Typical honeybee, yellow-brown in appearance		Several honeybee subspecies occur in the UK; however *Apis mellifera mellifera* (European dark honeybee) is by far the most common.		

Models of pollinator foraging

To characterise the dependence of movement patterns on flower position in the inflorescence, bout length and inflorescence size foraging model (figure 4.2) was fit to the observed sequences of pollinator movements. The model presented in this section, represents foraging as a series of binary choices after arrival (departure, lateral, upward and downward movement). The pollinator foraging data collected does involve repeated observations of the same pollinators on the same plant; it is non-independent. Implementing non-independence requires a more complex model (still representing a work in progress), however preliminary results of the non-independent model suggests that this change does not greatly impact the parameter estimates of this model. Therefore for simplicity this model treats the observations as independent.

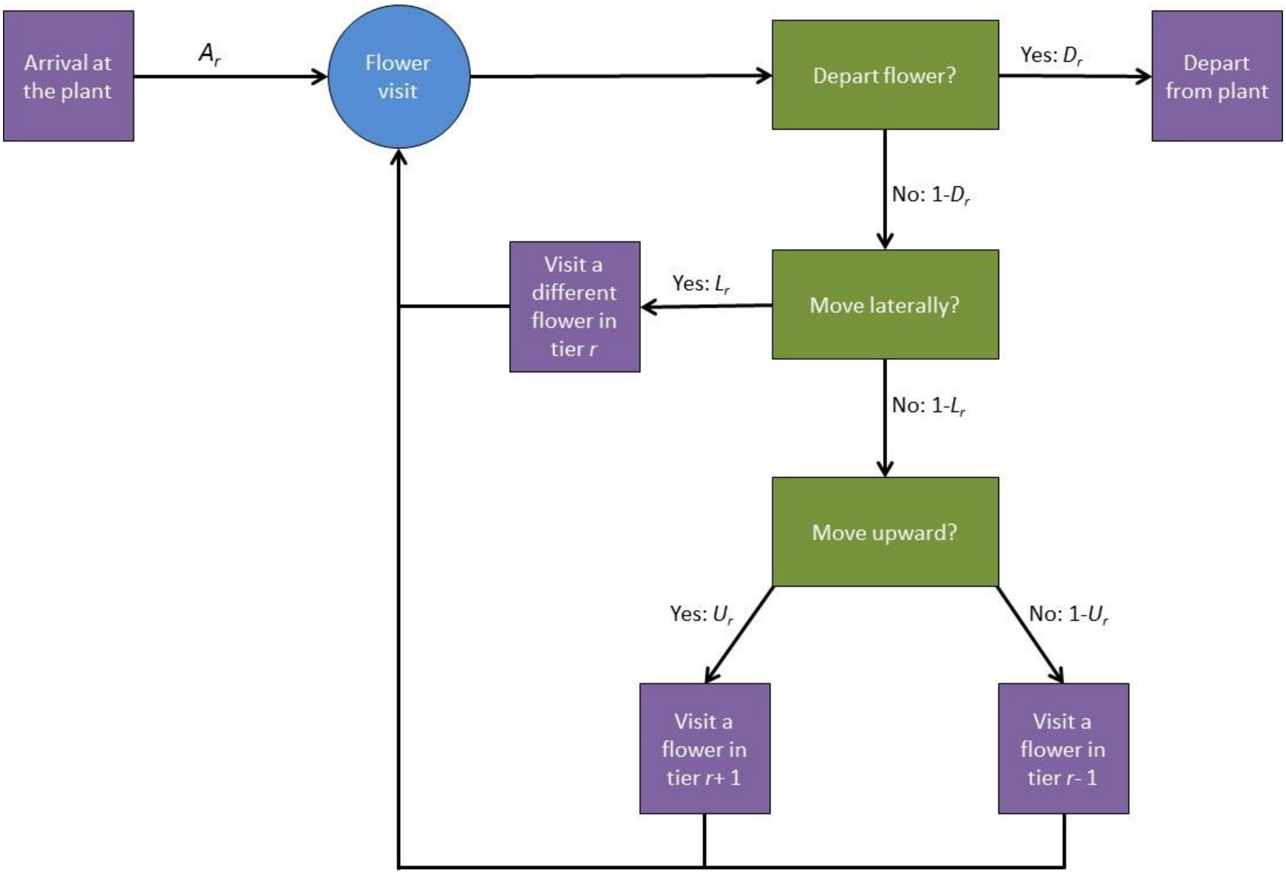


Figure 4.2: A representation of the series of foraging decisions each pollinator makes while visiting an inflorescence.

The pattern of arrival to an inflorescence was depicted as follows. If pollinators randomly choose their initial flower, then they arrive at tier r with probability $A_r(\boldsymbol{f}) = f_r/F$, where $\boldsymbol{f}$ is the vector of flower numbers at each tier, and F is the total number of flowers on an inflorescence ($F = \sum_{i=1}^{R} f_i$). Alternatively, the probability of arrival might differ among tiers, as represented by w_r, the relative weight associated with tier r. I assume that these weights can be described by the logit transformation:

$$\text{logit}\, w_r = \sum_{i=1}^{3} \Lambda_i \rho_r^i \,, \qquad (4.2)$$

where the Λ_i are parameters to be estimated and ρ_r^i is the relative position of tier r at the ith pollinator arrival. The arrival probabilities are then given by

$$A_r(f) = \frac{w_r f_r}{\sum_{i=1}^{R} w_i f_i} \,. \qquad (4.3)$$

Note that this model reduces to the random flower visit assumption when all the Λ_i are set to zero.

Inflorescence departure may be influenced by position of the pollinator within the inflorescence, inflorescence size and the pollinator's current bout length. I assume that these variables affect the departure logit linearly,

$$\text{logit}\, D_r = \beta_0 + \beta_\rho \rho_r + \beta_F F + \beta_b b + \beta_v v \,, \qquad (4.4)$$

where β_i is the effect size of predictor i on departure probability (i can be ρ, F, b or v). v represents the proportion of flowers on an inflorescence visited ($v=b/F$) and provides an alternative measure of bout length (see below).

If a pollinator does not depart, they move either laterally or vertically. The conditional lateral movement probability in tier r is calculated similarly,

$$\text{logit}\, L_r = \theta_0 + \theta_\rho \rho_r + \theta_F F + \theta_b b + \theta_v v. \qquad (4.5)$$

Alternatively, the pollinator moves vertically either up or down. However, a vertical movement within the raceme must be upward from the lowest tier (when $\rho =$

0), or downward from the top tier ($\rho = 1$). Thus, the probability of moving up at tier r, U_r, is always bounded by $U_1 = 1$ and $U_R = 0$. The probability of upward movement from tier r considering the effect of relative tier position alone is calculated by

$$u_r = (1 - \rho_r)^{-\xi_\rho}, \tag{4.6}$$

where ξ_ρ is the effect of ρ_r on the probability of moving upwards. Correspondingly, the logit of upward movement from a tier at position ρ_r is

$$H_r = \ln\left(\frac{u_r}{1-u_r}\right), \tag{4.7}$$

which allows inclusion of other effects in the calculation of the overall upward movement probability using

$$\operatorname{logit} U_r = H_r + \xi_F F + \xi_b b + \xi_v v, \tag{4.8}$$

where ξ_i is the effect of parameter i on U_r. Note that when all $\xi_i = 0$ in equation 4.8, U_r equals u_r.

Model selection

Parameters of the arrival model ($\Lambda_1, \Lambda_2, \Lambda_3$) were estimated as follows. Let R_i and $\boldsymbol{f}_i$ denote the number of tiers on plant i and the distribution of flowers on the plant respectively. Also let s_i denote the vector of arrival positions by pollinators to each tier of plant i. The total number of arrivals to the plant is $S_i = \sum_r s_{ir}$. The probability of observing this distribution of visits to each plant is given by a multinomial distribution. The log-likelihood of observing all arrival visits is

$$LL(\Lambda_1, \Lambda_2, \Lambda_3) = \sum_{i=1}^{I}\left(\ln\Gamma(S_i + 1) + \sum_{r=1}^{R_i}\left(s_{ir}\ln A_r(\boldsymbol{f}_i) - \ln\Gamma(s_{ir} + 1)\right)\right). \tag{4.9}$$

where Γ is a gamma function. For each pollinator species, four models of arrival were fitted: random arrival (all $\Lambda_i = 0$), a linear, a quadratic, and a cubic form with respect to

ρ_r. The best fitting model for each pollinator species was identified using AIC, based on the method described by Richards (2008).

The parameters of the departures and lateral and upward movements are also estimated using a similar approach. Let x_i donate the action of the pollinator for the i^{th} observed decision that could have been a departure:

$$x_i = \begin{cases} 0, \text{no departure;} \\ 1, \text{departure;} \end{cases}. \tag{4.10}$$

The log-likelihood of observing all possible departure decisions is:

$$LL(\beta_0, \beta_\rho, \beta_F, \beta_v, \beta_b) = \sum_i \ln\left(D_i x_i + (1 - D_i)(1 - x_i)\right). \tag{4.11}$$

Similarly let y_i denote the action for the i^{th} observation where a lateral movement decision could have been made:

$$y_i = \begin{cases} 0, \text{vertical movement;} \\ 1, \text{lateral movement;} \end{cases}. \tag{4.12}$$

The log-likelihood of seeing all behaviours in this context is

$$LL(\theta_0, \theta_\rho, \theta_F, \theta_v, \theta_b) = \sum_i \ln\left(L_i y_i + (1 - L_i)(1 - y_i)\right). \tag{4.13}$$

The observed upward or downward movement decisions are donated as

$$z_i = \begin{cases} 0, \text{downward movement;} \\ 1, \text{upward movement;} \end{cases}. \tag{4.14}$$

The log likelihood of seeing all vertical movements is

$$LL(\xi_\rho, \xi_F, \xi_v, \xi_b) = \sum_i \ln\left(U_i z_i + (1 - U_i)(1 - z_i)\right). \tag{4.15}$$

Likelihood-ratio tests were used to test the effects of parameter associated with departure, lateral and upward movement. These tests compared likelihoods of the full model and that of a reduced model from which the factor of interest had been excluded (i.e. setting that parameter to zero). v and b both are highly correlated measures of bout length, so I one was included in a particular model. To identify which bout length parameter should be included in the models, models containing each parameter were tested against models without bout length. If a bout length parameter significantly affected the respective transition probability, the parameter with the strongest effect (based on P values) was included in the full model for likelihood-ratio testing for the other parameters. Using the above tests, I identified the best model for each foraging action, for each species. This analysis was repeated with the data combined into one group to allow tests for differences in foraging behaviour between species using AIC.

The best models for the probabilities of pollinator arrival position, departure, lateral and upward movements were then used to simulate pollinator foraging behaviours in order to identify how differing movement affected each species' ability as a pollinator. Pollinators sometimes skipped tiers while moving between tiers, but much less often than between adjacent tiers, approximately 30% of all vertical movements were to non-adjacent tiers. For simplicity only vertical movements to adjacent teirs were included in the simulation. Plants visited in the simulation had six teirs each with three flowers, so inflorescences had F = 18 flowers in total. Flowers in tier four and above were male, and those below were female. This arrangement of flowers and sex function in the inflorescence was chosen so that inflorescences were typical of those in the field (tiers on average becoming male at $\rho = 0.6$). For each simulation I recorded the numbers of visits to male and female flowers by each pollinator, and the number of female flowers visited after a pollinator had visited a male flower (potentially geitonogamous visits). These data were used to quantify the potential effects of foraging behaviour on plant fitness. Simulations were replicated 20,000 times for each species. This high number of replicates ensured that in these simulations the mean visitations remained largely unchanged between repeats of each simulation, thus for the purposes of this thesis it was felt that there was no need to analyse them further with statistical tests.

Differences in pollinator behaviour among species: results

Bees did not arrive randomly within inflorescences and arrival position differed significantly between species (Table 4.2). All species tended to avoid arriving in the upper and lower rows of plants, favouring arrival at the lower-middle section of the inflorescence (figure 4.3; top row). Arrival position was most predictable in *B. hypnorum*, as seen by the high and narrow peak in figure 4.3 top row, and least predictable in *B. lapidarius*, as seen by the broad curve in figure 4.3 top row.

Table 4.2: Model selection results for bee arrival position, including analyses with all species combined and separate models for each species. Bold indicates the best AIC model. The combined model (bottom row) is based on the best AIC model for each species. Values under the headings Λ_i, LL, K represent the estimated parameter values of Λ_i, the log-likelihood values and the number of parameters in each model respectively.

	Parameter			**Species separated**			**Species combined**		
Model	Λ_1	Λ_2	Λ_3	***LL***	***K***	AIC	***LL***	***K***	AIC
Species combined									
random	0	0	0				-2740.4	0	5480.7
linear	-0.89	0	0				-2687.3	1	5376.5
quadratic	9.49	-11.54	0				-2187.5	2	4378.9
cubic	26.01	-52.47	24.75				-2164.5	3	4335.1
Species separated									
Apis sp.									
random	0	0	0	-1104.1	0	2208.2			
linear	-1.17	0	0	-1061.1	1	2124.3			
quadratic	8.71	-11.35	0	-826.0	2	1655.9			
cubic	**30.57**	**-65.75**	**33.16**	**-808.3**	**3**	**1622.6**			
B. terrestris									
random	0	0	0	-1056.8	0	2113.6			
linear	-0.38	0	0	-1053.5	1	2108.9			
quadratic	11.79	-13.26	0	-830.0	2	1664.1			
cubic	**27.24**	**-51.56**	**23.00**	**-825.5**	**3**	**1657.1**			
B. pascuorm									
random	0	0	0	-232.6	0	465.2			
linear	-2.15	0	0	-212.5	1	426.9			
quadratic	**3.22**	**-6.28**	**0**	**-203.6**	**2**	**411.1**			
cubic	1.69	-2.02	-2.92	-203.4	3	412.9			
B. lapidarius									
random	0	0	0	-186.3	0	372.7			
linear	-0.38	0	0	-186.1	1	374.1			
quadratic	**12.27**	**-19.10**	**0**	**-160.7**	**2**	**325.3**			
cubic	14.17	-10.42	-6.00	-160.7	3	327.3			
B. hypnorum									
random	0	0	0	-160.5	0	321.0			
linear	-1.95	0	0	-146.8	1	295.6			
quadratic	5.64	-8.93	0	-132.6	2	269.2			
cubic	**15.37**	**-34.12**	**16.06**	**-131.3**	**3**	**268.6**			
							-2129.4	**15**	**4284.7**

Once bees arrived at inflorescences each species responded differently to ρ, F, b and v (Table 4.3) and all measured aspects of movement, D_r, L_r and U_r, differed between significantly between species (Table 4.4). Relative position in the inflorescence had a fairly consistent effect on the estimated probabilities (Table 4.3, Figure 4.3). In general, departure and lateral movement became increasingly more frequent as bees moved up inflorescences. On average (Figure 4.3) *A. mellifera* and *B. terrestris* showed the strongest tendencies to move upwards and depart from the top of plants, whereas this pattern was weakest for *B. lapidarius*. Additionally, like many previous studies, the probability of departure, $D_{r,}$ increased with bout length (Hodges, 1986; Lefebvre et al., 2007; Biernaskie et al., 2009; Taneyhill, 2010), and decreased with inflorescence size (Robertson & MacNair, 1995; Goulson et al., 1998; Karron et al., 2004). Responses to inflorescence size and bout length were not seen across all species (Table 4.3, Figure 4.3).

The observed differences in pollinator foraging were predicted by our model to influence the mean number of flower visits per inflorescences visit, for each species, and the fraction of female visits made that had the potential for geitonogamy (figure 4.4). For all pollinators, over half the simulated visits to female flowers were preceded by visits to at least one male flower on the same inflorescence. Pollinators with the stronger upward movement tendencies, *A. mellifera* and *B. terrestris*, engaged in fewer potentially geitonogamous flower visits. The other three species visited more flowers, including more visits that could cause geitonogamy.

Table 4.3: Estimated parameters for models of the probabilities of inflorescence departure, and lateral and vertical movements. Estimates for the effects of absolute (*b*) and relative (*v*) bout length are from models that included all parameters except the other bout length parameter. Estimates for other parameters are from the full model using the best bout-length parameter. P represents the results of likelihood-ratio tests. Bold indicates significant effects of the associated independent variable, which was therefore included in the best fitting model. Numbers in brackets indicate the number of observations. All likelihood ratio tests summarised in this table had 1 degrees of freedom.

		Species Combined		*A. mellifera*		*B. terrestris*		*B. pascuorum*		*B. lapidarius*		*B. hypnorum*	
Parameter		Full model value	P value	Full model value	P value	Full model value	P value	Full model value	P value	Full model value	P value	Full model value	P value
DEPARTURE: D_r		(17496)		(7582)		(7579)		(877)		(745)		(713)	
Bout length													
b	$\boldsymbol{\beta_b}$	0.031	<0.001	0.033	<0.001	0.033	0.001	0.002	0.947	0.036	0.244	0.114	<0.001
v	$\boldsymbol{\beta_v}$	0.629	**<0.001**	0.648	**<0.001**	0.610	**<0.001**	0.111	0.810	0.697	0.204	2.134	**<0.001**
Other parameters													
Baseline	$\boldsymbol{\beta_0}$	-2.238	NA	-2.161	NA	-2.102	NA	-3.015	NA	-3.460	NA	-1.811	NA
ρ	$\boldsymbol{\beta_\rho}$	1.925	**<0.001**	1.469	**<0.001**	2.269	**<0.001**	2.516	**<0.001**	3.227	**<0.001**	1.386	**<0.001**
F	$\boldsymbol{\beta_F}$	-0.012	**0.005**	-0.001	0.839	-0.030	**<0.001**	0.009	0.725	0.002	0.948	-0.054	**0.020**
LATERAL: L_r		(13368)		(5832)		(5686)		(703)		(578)		(569)	
Bout length													
b	$\boldsymbol{\theta_b}$	0.002	0.756	-0.027	**0.008**	0.030	**0.019**	-0.023	0.310	0.053	0.121	0.045	0.256
v	$\boldsymbol{\theta_v}$	0.103	0.448	-0.444	0.023	0.536	0.021	-0.522	0.316	0.933	0.114	1.061	0.172
Other parameters													
Baseline	$\boldsymbol{\theta_0}$	-1.870	NA	-1.456	NA	-1.927	NA	-2.223	NA	1.129	NA	-3.260	NA
ρ	$\boldsymbol{\theta_\rho}$	1.032	**<0.001**	1.026	**<0.001**	1.244	**<0.001**	0.590	0.089	1.013	**0.011**	1.341	**0.001**
F	$\boldsymbol{\theta_F}$	0.003	0.536	-0.003	0.631	-0.018	**0.031**	0.040	0.131	-0.158	**<0.001**	0.058	**0.009**
UPWARDS: U_r		(10355)		(4339)		(4583)		(536)		(438)		(459)	
Bout length													
b	$\boldsymbol{\xi_b}$	-0.055	**<0.001**	-0.094	**<0.001**	-0.017	0.230	0.031	0.314	-0.016	0.734	0.005	0.896
v	$\boldsymbol{\xi_v}$	-0.881	<0.001	-1.749	<0.001	-0.133	0.609	0.714	0.223	-0.231	0.777	0.580	0.505
Other parameters													
ρ	$\boldsymbol{\xi_\rho}$	-0.586	**<0.001**	-0.455	**<0.001**	-0.858	**<0.001**	-0.668	**<0.001**	-1.561	**<0.001**	-0.649	**<0.001**
F	$\boldsymbol{\xi_F}$	0.020	**<0.001**	0.008	0.335	0.417	**<0.001**	0.013	0.631	0.120	**0.001**	0.014	0.634

Table 4.4: Parameter estimates and AIC for models of foraging after arrival, including separate analyses for each species and a common analysis. Parameters included in the model are based on the analysis presented in table 4.3. The bold AIC value indicates the best fitting models (i.e. species specific models or species independent). Note that parameter estimates differ from those table 4.3 as these models exclude all non-significant parameters.

Parameter Values		Species Separated *A. mellifera*	*B. terrestris*	*B. pascuorum*	*B. lapidarius*	*B. hypnorum*	Species Combined
DEPARTURE: D_r							
Baseline	$\boldsymbol{\beta_0}$	-2.190	-2.102	-2.827	-3.422	-1.811	-2.237
ρ	$\boldsymbol{\beta_\rho}$	1.470	2.269	2.497	3.230	1.386	1.925
F	$\boldsymbol{\beta_F}$	0	-0.030	0	0	-0.054	-0.012
b	$\boldsymbol{\beta_b}$	0	0	0	0	0	0
v	$\boldsymbol{\beta_v}$	0.655	0.610	0	0	2.134	0.629
LL		-3995.640	-4010.042	-409.868	-362.133	-334.965	-9151.784
						-9112.647	-9151.784
k		3	4	2	2	4	4
total k						15	4
AIC						**18255.295**	18311.567
LATERAL: L_r							
Baseline	$\boldsymbol{\theta_0}$	-1.525	-1.927	-1.164	1.129	-3.260	-1.812
ρ	$\boldsymbol{\theta_\rho}$	1.027	1.244	0	1.013	1.341	1.033
F	$\boldsymbol{\theta_F}$	0	-0.018	0	-0.158	0.058	0
b	$\boldsymbol{\theta_b}$	-0.027	0.030	0	0	0	0
v	$\boldsymbol{\theta_v}$	0	0	0	0	0	0
LL		-3283.577	-2744.846	-385.144	-308.419	-271.170	-7055.713
						-6993.154	-7055.713
k		3	4	1	3	3	2
total k						14	2
AIC						**14014.309**	14115.426
UPWARDS: U_r							
ρ	$\boldsymbol{\xi_\rho}$	-0.400	-0.858	-0.552	-1.561	-0.536	-0.432
F	$\boldsymbol{\xi_F}$	0	0.042	0	0.120	0	0
b	$\boldsymbol{\xi_b}$	-0.093	0	0	0	0	-0.052
v	$\boldsymbol{\xi_v}$	0	0	0	0	0	0
LL		-2168.404	-2146.820	-259.056	-179.581	-202.589	-4998.190
						-4956.450	-4998.190
k		2	2	1	2	1	2
total k						8	2
AIC						**9928.901**	10000.381

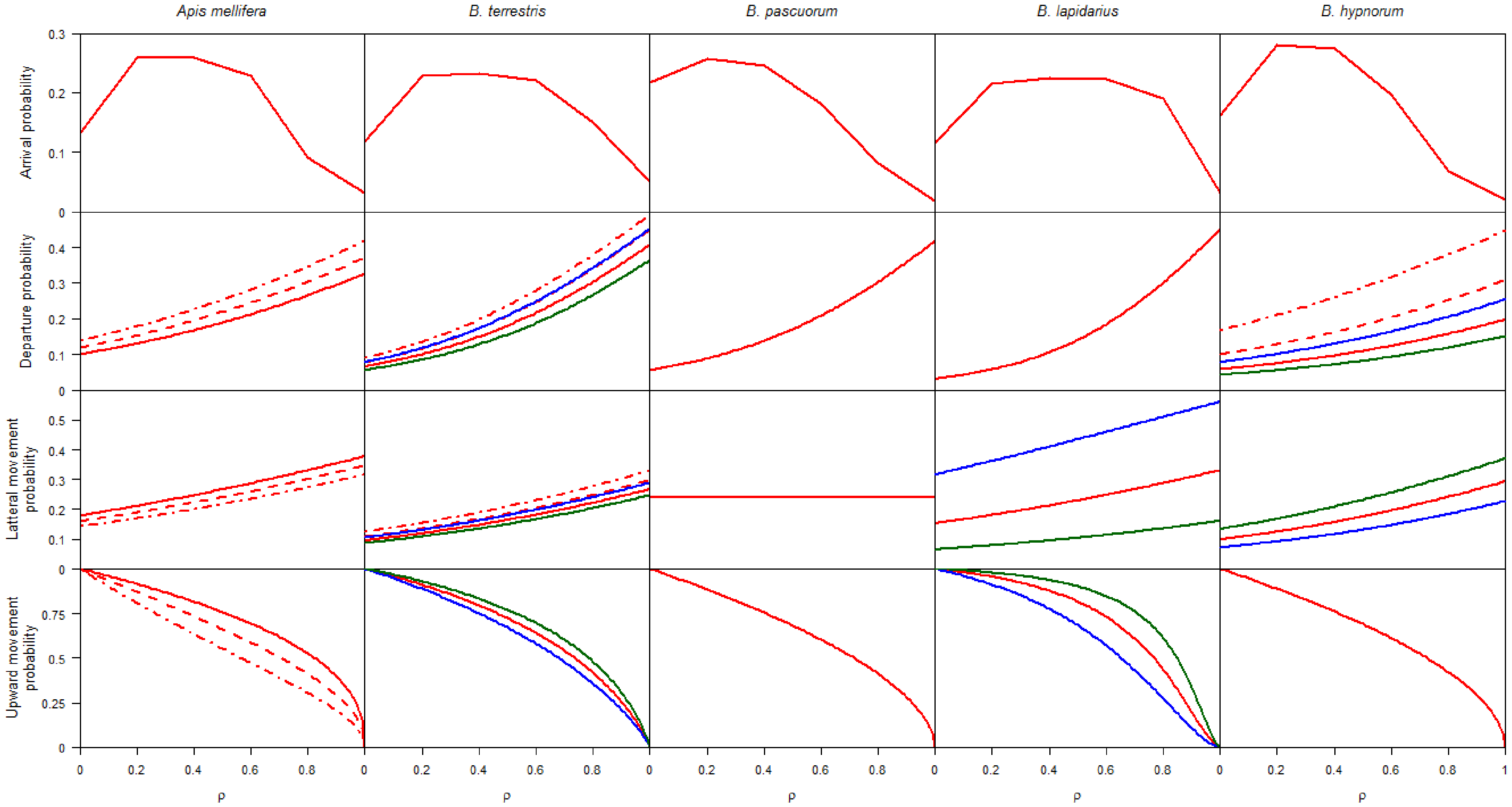


Figure 4.3: Relations of mean transition probabilities to a bee's relative position in the inflorescence, ρ, inflorescence size, F, and the position of a visit in the visit sequence, b. Colours and line dashing represent the effect of flower number and bout length on transition probabilities where these parameters have an influence on transition probabilities: the near average plant, F=18 flowers (red line); a larger plant, F=24 flowers (green line) and a smaller plant, F=12 flowers (blue line). Movement behaviours are given after the first flower visit (solid line), after 5 flower visits (dashed line) and after 10 flower visits (dotted and dashed line). When only solid or red lines are shown bout length and flower number have no influence on that pollinator behaviour.

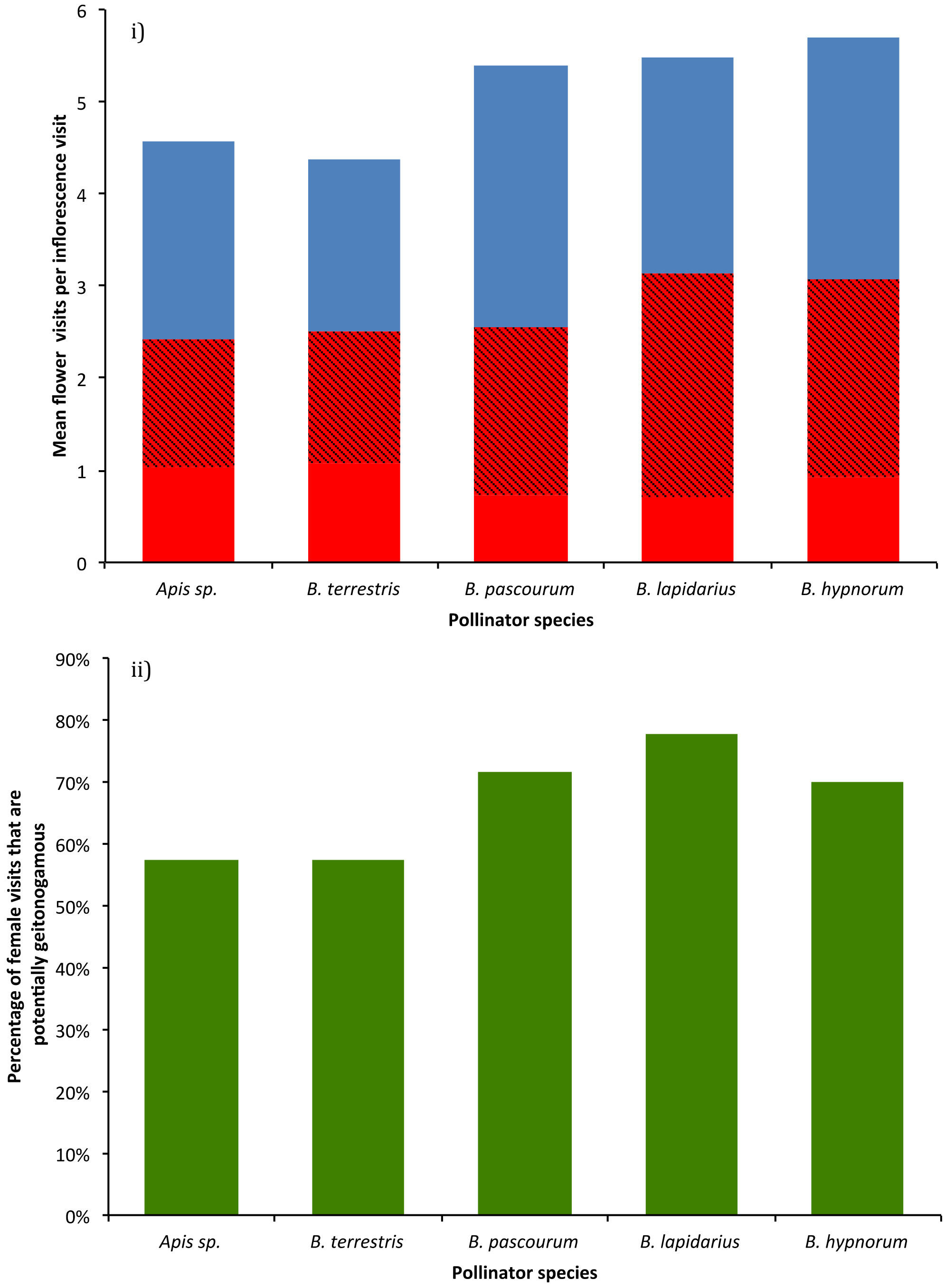


Figure 4.4: Simulated pollinator movement on 18-flowered inflorescences for each pollinator species, including: i) the mean visitation to female- (red) and male-phase (blue) flowers. Colour corresponds to the sex phase of the flower visited: male flowers (blue) and female (red); and ii) the percentage of visits to female-phase that were preceded by visits to male-phase flowers (potentially geitonogamous). Cross-hatched female visits in panel i) represent those that are potentially geitonogamous.

The causes of directional pollinator foraging: methods

Nectar gradients

I assessed the existence of a gradient in nectar availability within *C. angustifolium* based on standing crop (volume) (nectar concentration is relatively constant for this species: (Komlos, 1999; Routley & Husband, 2005)). From all 579 flowers on 55 randomly selected, exposed plants (July 18 – September 12, 2013), I extracted nectar was extracted from exposed flowers with 0.5μl microcapillary tubes. The height of the nectar column in the tube was measured to quantify volume (Potts et al., 2004). I also recorded the tier location (ρ) of each flower. It is possible that the accuracy of this measure of nectar volume may be limited, and the exact volume of nectar extracted may be different from my estimate using the microcapillaries. However, this inaccuracy should be consistent across all measurements, thus estimate of nectar volume should still be suitable as a relative measure of nectar volume. No nectar could be extracted from many sampled flowers, which were considered to be empty.

Statistical analyses considered separate gradient models for the incidence of empty flowers and the standing crop of non-empty flowers. Let $n_{\rho t}$ be the probability that a flower in relative tier position ρ does not contain nectar if sampled on sampling day, t (taken as the number of days since July 18th). Accordingly,

$$\text{logit}\, n_{\rho t} = \eta_0 + \eta_1 \rho_r^1 + \eta_2 \rho_r^2 + \eta_t t, \qquad (4.16)$$

where η_i are the parameters to be estimated. The standing nectar crop in non-empty flowers in relative position ρ on sampling day t is described by

$$c_{\rho t} = \exp(\omega_0 + \omega_1 \rho_r^1 + \omega_2 \rho_r^2 + \omega_t t), \qquad (4.17)$$

where ω_i are the parameters to be estimated.

These models were fit to the nectar data as follows. Let x_i denote the nectar volume in flower i, given that it was not empty, and let q_i denote whether it was empty or not, denoted by

$$q_i = \begin{cases} 0, \text{rewarding;} \\ 1, \text{unrewarding;} \end{cases}. \tag{4.18}$$

The log-likelihood is then

$$LL(\eta_0, \eta_1\eta_2, \eta_t) = \sum_{i=1}^{I} \left(n_i q_i + (1 - n_i)(1 - q_i)\right). \tag{4.19}$$

The analysis of standing crop considered a gamma distribution, so the log-likelihood function for $c_{\rho t}$ was

$$LL(\omega_0, \omega_1, \omega_2) = \sum_{i=1}^{I} \ln \mathrm{fg}(x_i, c_{pt}, \phi), \tag{4.20}$$

where x_i is the observed nectar crop given nectar was present, fg is the probability density function of the gamma distribution and ϕ represents the variation about the mean of this gamma distribution (see Richards, 2008). The model best describing the pattern of nectar standing crop was selected using AIC, as recommended by Richards (2008).

Foraging speed

To investigate whether upwards movement provides any advantage in foraging time, I analysed two hour-long videos of bees foraging on *C. angustifolium* inflorescences. These videos were recorded on July 26 and 31, 2013, beginning at 9.30 am. These observation periods were chosen to minimise the effects of time of the day or season. Recordings were viewed at 25% speed and movements between flowers of each pollinator were timed with a stopwatch. This time was then rescaled to give the true movement time. In addition to movement time, movement direction (up, down, lateral) bee species and bee ID (assuming each bee that entered the field of view was a different bee) were recorded. I additionally recorded the number of tiers travelled as a measure of distance (roughly 2 to 3 cm per tier). Movements where the bee inspected a flower but did not land were excluded. Additionally movements where the bee walked as opposed to flew, were excluded. Walking requires 90% less energy than flight (Rothe & Nachtigall, 1989), so an effect of direction should have little energetic consequence.

Only *B. terrestris* and *A. mellifera* were represented by sufficient observations for statistical analysis. Lateral movements, where the pollinator stayed in the same tier, were excluded from the analysis, leaving 321 vertical movements by 93 *B. terrestris* pollinators, and 254 vertical movements by 79 *A. mellifera* pollinators. Movement duration was log-transformed to normalise variation about the means prior to analysis. Mixed effects linear models of the effects of direction and distance moved (in tiers) on log movement duration between flowers were fitted to the data for each species. These models treated bee identity as a random effect. Likelihood ratio tests were used to test for the effects of direction and distance of movement and an interaction between these two effects.

The causes of directional pollinator foraging: results

Reward gradients

Analysis of nectar crops showed that flowers on the ends of the inflorescence were more likely to not contain nectar (table 4.5, figure 4.5i). However, for the flowers that contained nectar, volume did not vary with relative position in the raceme (table 4.5, figure 4.5ii). These two factors had the result that the mean nectar rewards pollinators encountered when visiting *C. angustifolium* were slightly greater in the middle of the inflorescence but for the most part remained largely constant (figure 4.5iii).

Table 4.5: The AIC values and estimated parameters of the nectar crop models. n_{pt} parameters are selected first then c_{pt} parameters. Bold model indicates the best model based on Richards's (2008) selection method

Model	η_1	η_2	η_3	η_t	ϕ	ω_1	ω_2	ω_3	ω_t	LL	k	AIC	ΔAIC
Vertical distribution of rewarding flowers, n_{pt}													
Constant n_r	-1.35				0.09	-2.38				342.63	3	-679.26	17.21
with η_t	-1.85			0.02	0.09	-2.38				344.82	4	-681.63	14.84
Linear n_r	-1.27	-0.17			0.09	-2.38				342.80	4	-677.60	18.87
with η_t	-1.76	-0.18		0.02	0.09	-2.38				345.01	5	-680.01	16.46
Quadratic n_r	**-0.84**	**-4.13**	**3.98**		**0.09**	**-2.38**				**351.45**	**5**	**-692.91**	**3.57**
with η_t	-1.38	-4.30	4.15	0.02	0.09	-2.38				354.24	6	-696.47	0
Vertical distribution of nectar in rewarding flowers, c_{pt}													
Constant c_r	**-0.84**	**-4.13**	**3.98**		**0.09**	**-2.38**				**351.45**	**5**	**-692.91**	**0**
with ω_t	-0.84	-4.13	3.98		0.09	-2.29			<-0.01	352.18	6	-692.35	0.04
Linear c_r	-0.84	-4.13	3.98		0.09	-2.31	-0.15			352.43	6	-690.86	1.87
with ω_t	-0.84	-4.13	3.98		0.09	-2.22	-0.15		<-0.01	353.17	7	-692.35	0.55
Quadratic c_r	-0.84	-4.13	3.98		0.09	-2.33	-0.01	-0.14		352.52	7	-691.04	0.56
with ω_t	-0.84	-4.13	3.98		0.09	-2.24	0.04	-0.19	<-0.01	353.33	8	-690.66	2.25

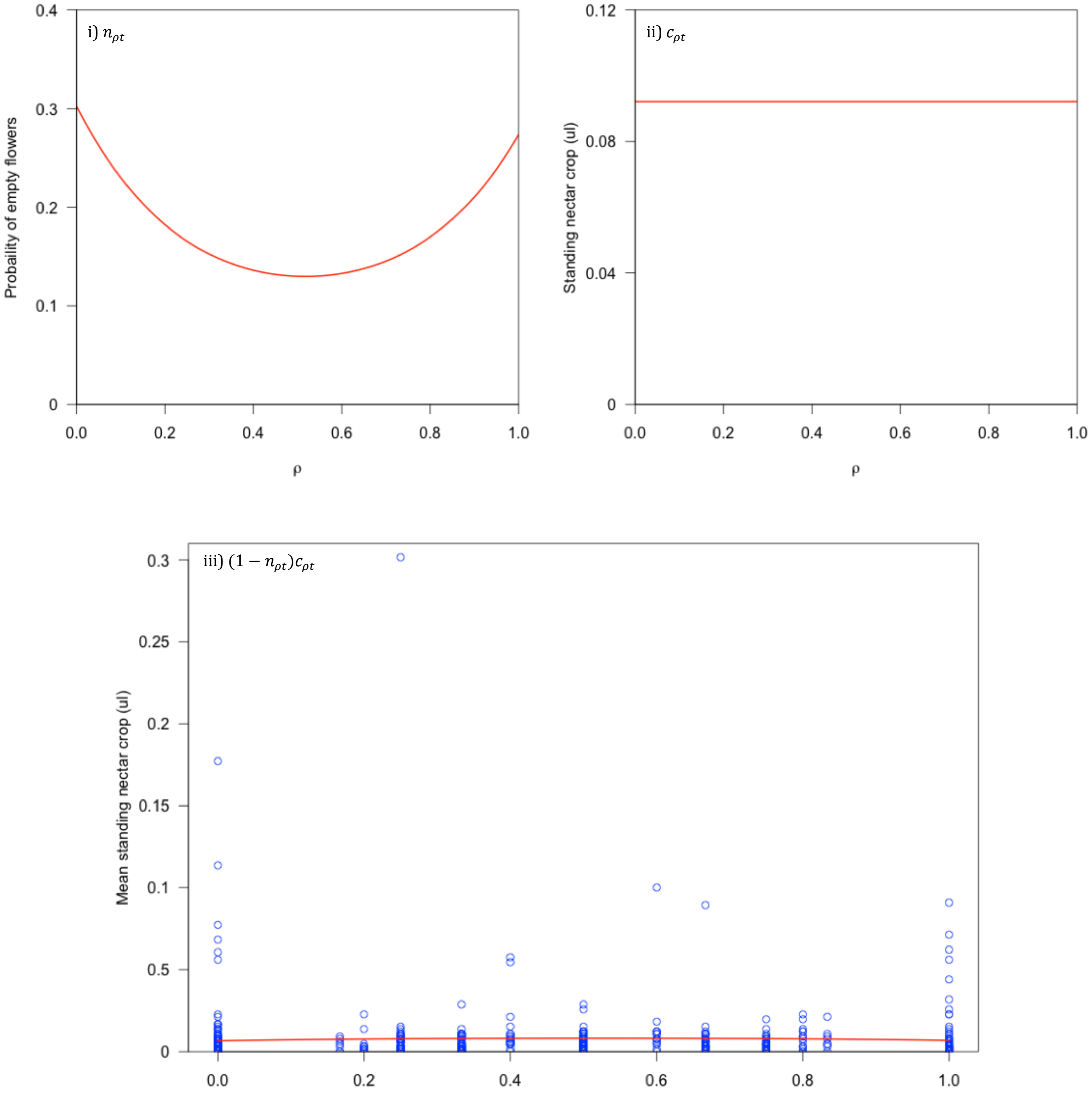


Figure 4.5: Variation in standing nectar crop throughout racemes of *C. angustifolium*, including i) the probability of a flower being empty, $n_{\rho t}$; ii) the standing crop in non-empty flowers, $c_{\rho t}$; and iii) the observed nectar crops (blue circles) and the expected mean rewards according to the best fitting model (red line).

Foraging speed

Analysis of pollinator movement duration revealed no interacting effects between distance and direction of movement on movement duration for either *B. terrestris* (likelihood ratio test, $G_{1,319}$=0.57, P=0.451) or *A. mellifera* (likelihood ratio test, $G_{1,252}$=0.26, P=0.608). Movement in an upward direction significantly reduced time spent travelling between flowers for both *B. terrestris* (likelihood ratio test, $G_{1,319}$=23.28, P<0.001) and *A. mellifera* (likelihood ratio test, $G_{1,252}$=16.61, P<0.001). *B. terrestris* and *A. mellifera* respectively flew upward 15.6% and 13.8% faster than downward (figure 4.6). Analysis also supported a weak positive effect of distance travelled on movement duration for both *B. terrestris* (likelihood ratio test, $G_{1,319}$=3.96, P=0.046) and *A. mellifera* (likelihood ratio test, $G_{1,252}$=5.94, P=0.015). These findings found the best models for *B. terrestris* movement duration to be

$$\ln(M) = -0.84 - (0.18x_1) + (0.06x_2), \tag{4.21}$$

and *A. mellifera* movement duration to be

$$\ln(M) = -0.53 - (0.15x_1) + (0.09x_2) \tag{4.22}$$

where M is movement duration in seconds, x_1 describes movement direction as

$$x_1 \begin{cases} 0, \text{downward movment;} \\ 0, \text{upward movment;} \end{cases}, \tag{4.23}$$

and x_2 is number of inflorescence tiers moved.

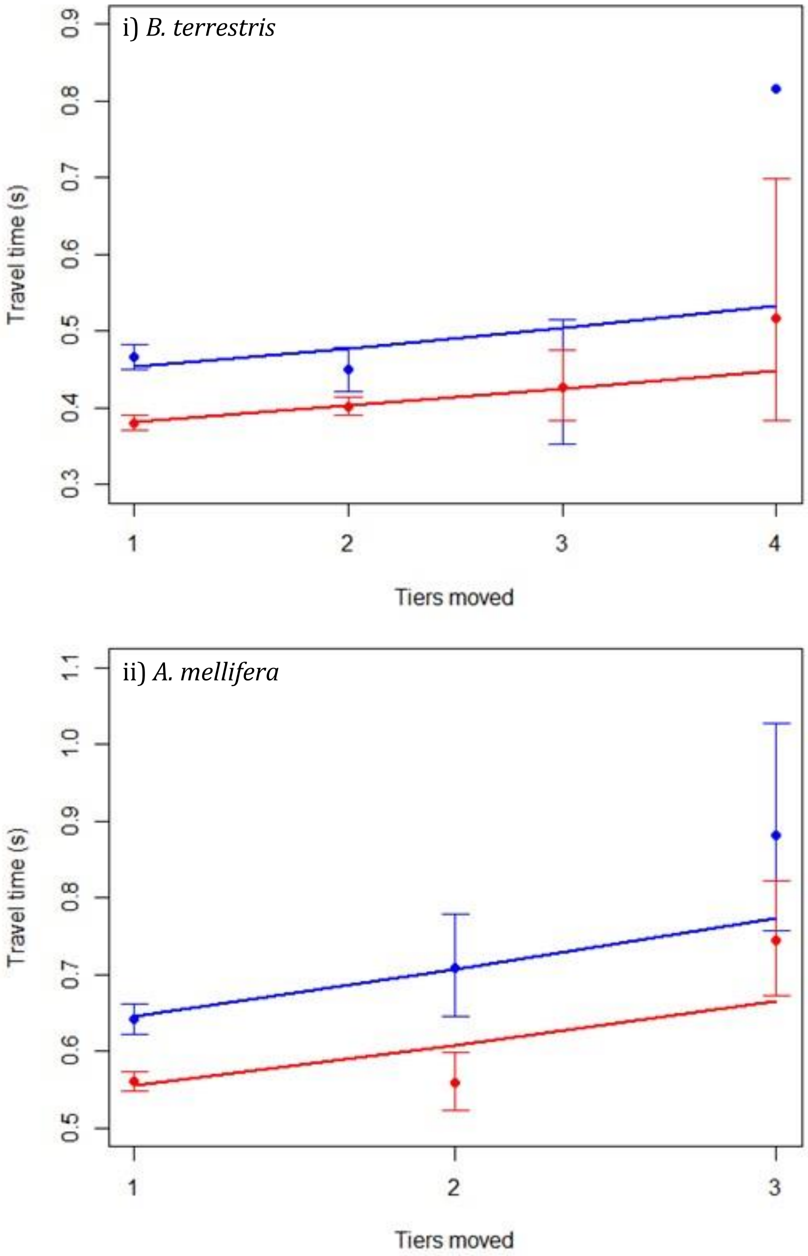


Figure 4.6: The mean vertical travel times of i) *Bombus terrestris* and ii) *Apis mellifera* dependent on the number of tiers moved (x-axis) and the direction of movement: red, upwards movements; blue, downwards movements. Error bars represent ±1 standard error. Lines represent travel times projected by our best fitting models for each species for upward and downward movement. Sample sizes of each mean are as follows: *B. terrestris* travelling 1 tier, upwards 135 and downwards 77; 2 tiers, upwards 75 and downwards 22; 3 tiers, upwards 5 and downwards 2; 4 tiers, upwards 4 and downwards 1. *A. mellifera* travelling: 1 tier, upwards 130 and downwards 77; 2 tiers, upwards 23 and downwards 16; 3 tiers, upwards 6 and downwards 2.

Discussion

The above analyses of pollinator foraging have revealed several new insights into how pollinators forage within inflorescences. The five main pollinator species in the studied *C. angustifolium* population showed similar directional tendency in racemose inflorescences, but differed somewhat in the influences on their foraging decisions (table 4.2 and 4.4). Our simulation of pollinator movements suggested that these differences should cause species to differ in their potentials to cause geitonogamy (figure 4.4). A separate study found no gradient in nectar standing crop in the *C. angustifolium* population, so the general upward foraging of bees visiting these plants must reflect other causes. In particular, *Apis mellifera* and *Bombus terrestris* travelled faster through inflorescences when foraging upwards, so upward movement likely enhances foraging efficiency reducing travel time. Additionally this difference in travel time could not be attributed to pollinators simply travelling further when moving down. I now discuss these observations and their consequences for pollen transfer.

Explanations of foraging behaviour

Nectar production rate in rosebay willowherb has been reported to be greater in the lower female-phase flowers than in male-phase flowers (Carlson & Harms, 2006), but no gradient was evident in nectar standing crop in my study population. If female-phase flowers produce nectar faster, this absence of vertical structure suggests that visitation is too frequent for such differences to maintain a vertical gradient. Instead, average nectar standing crop is slightly greater in central flowers of racemes owing to reduced chances of these flowers being empty (figure 4.5). The high frequency of empty flowers among bottom flowers likely reflects a cessation of nectar production prior to withering.

In contrast, the high incidence of empty flowers at the top of racemes likely reflects newer flowers, which have opened but have not begun nectar production.

As bees arrived at flowers in the lower-middle region of racemes and proceeded upward, they should have encountered the highest expected nectar availability as Galen & Plowright (1985) also observed for *C. angustifolium*. Many pollinating insects modify their foraging based on learned association of floral cues to favour greater rewards (Hammer & Menzel, 1995; Smithson & MacNair, 1997; Laloi et al., 1999; Gumbert, 2000; Chittka et al., 2001; Weiss & Papaj, 2003). The observed bees had probably learned to associate this region with greater incidence of non-empty flowers. Arrival position may differ between species due to a differing capacity to identify and respond to changes in nectar distribution (as observed in Irwin & Brody, 2000). Further analysis of the capacities of naïve pollinators to detect and respond to higher incidence of empty flowers vary between species might explain difference in arrival pattern.

Average nectar standing crop did not vary systematically among non-empty flowers within inflorescences (figure 4.5), and this did not create a gradient that could motivate the directional movement we have observed, thus favouring reward independent explanations of this behaviour. Therefore, the results of this study as consistent those of other studies (Waddington & Heinrich, 1979; Heinrich, 1979; Delvin & Stephenson, 1985; de Jong et al., 2011), that nectar reward gradients are not the primary cause of directional pollinator movement in raceme inflorescences, although nectar distribution may explain the patterns of bee arrival location.

In contrast, an advantage to upward movement from reduced travel time between flower visits was strongly demonstrated by both *B. terrestris* and *A. mellifera*. This faster travel between flowers suggests that upward foraging will be advantageous to bees in terms of how quickly they harvest nectar from each inflorescence. Rate of reward intake (Pleasants, 1989) or foraging efficiency (Rasheed & Harder, 1997), appears to drive the foraging strategies of many insect pollinators. Faster nectar accumulation will increase both the rate and efficiency of the bee foragers, as less time and energy is spent foraging. Thus, the results presented here support the hypothesis of Corbet et al. (1981) that pollinators show upward directional tendencies because this behaviour conveys an advantage of increased foraging efficiency.

The underlying causes of differences in pollinator behaviours are an interesting area for future research, as this will help further our understanding how inflorescence architecture influences pollinator behaviours. Pollinator departure, especially in terms of response to bout length, perhaps differs between species due to differing initial motivations to remain at inflorescences (Waage, 1979; Lefebvre et al., 2007; Taneyhill, 2010). Factors that might affect this motivation and departure (Charnov, 1976; Lefebvre et al., 2007) include: travel time between the hive and inflorescences (Knight et al., 2005); flower handling times (Harder, 1983; Kunte, 2007) and energetic costs of foraging (Nachtigall et al., 1995; Wolf et al., 1999). Additionally a pollinator’s ability to access food in flowers might differ with species (Inouye, 1980; Soltz, 1987), which may encourage earlier departure of species which have limited access. Variation between species in travel times between flower visits when pollinators travel up and down may reveal that certain pollinators show weaker directional tendencies because they gain less of a travel time advantage when foraging upward.

A species’ ability to evaluate the structure of the inflorescence may explain the differences in pollinator responses to relative position in the inflorescence and flower number. If a pollinator is unable to accurately identify its position in the inflorescence, as well as others, it may move differently. This potentially explains why some species tend to move down earlier or depart lower down the inflorescence. Pollinators that cannot accurately identify inflorescence size may misjudge their position in the raceme. This might cause a pollinator to misjudge when it has reached the top of the plant and choose to depart earlier or move downward sooner. Pollinator's ability to judge inflorescence structure remains largely unknown. However, *B. terrestris* detects groups of flowers better than honeybees (Wertlen et al., 2008) due to larger eyes, which improve its sensory ability (Spaethe & Chittka, 2003). These differences in ability to distinguish the inflorescence might explain the differing responses between *B. terrestris* and *A. mellifera* to inflorescence size (F).

Consequences for plants

A tendency to move upward was observed in all five bee species. This is consistent with previous studies (Darwin, 1862; Harder et al., 2000; Routley & Husband, 2003; Fisogni et al., 2011; de Jong et al., 2011). Lateral movement tended to increase with ρ, as did departure. Departure from the top of the inflorescence can be thought of as moving upward from the very top of the inflorescence (Harder et al., 2004; de Jong et al., 2011). Similarly, if a pollinator is prone to move up, or opposed to down, the observation of increased lateral movement is consistent with observations of an upward directional tendency.

A. mellifera and *B. terrestris* typically exhibited a stronger upward bias compared to the other species. These species typically arrived at the lower middle of the inflorescence and then moved upward with more consistency than other species. As movements down from male phase flowers to female phase flowers are less frequent for these species, they should cause less geitonogamy (figure 4.4). Despite these similarities, *A. mellifera* had slightly more predictable arrival, and a higher probability of lateral movement. Departure probability increased with bout length for both species, but *A. mellifera* increasingly moved vertically (non-lateral) and downward late during bouts, whereas *B. terrestris* moved laterally more often and upward movement did not vary with bout length. However, these differences did not influence predicted mean visitation or the predicted percentage of geitonogamous female visits. This lack of difference likely reflects *A. mellifera*'s typically short bouts, so that very few *A. mellifera* remain on a plant long enough for higher downward movement probabilities to take effect. Fisogni et al. (2011) found that *A. mellifera* foraging on *Dictamnus albus* had a weaker directional tendency than bumblebees. These contrasting results may indicate differences between the plants involved, in methods of quantifying movement, or *A. mellifera*'s tendency to move down as bout length progresses.

The pollinator species with weaker directional movement (i.e. *B. pascuorum* and *B. lapidarius*) tended to visit more flowers per inflorescence. These species were also expected to make more potentially geitonogamous visits (figure 4.4). *B. lapidarius* moved downward most frequently of all the species investigated, which increased potential geitonogamy. *B. pascuorum* moved laterally more frequently on lower tiers than the other species observed, so that once a geitonogamous visit occurred there was a higher likelihood of further geitonogamous visits. These results support previous findings that pollinators with less directional movement are more likely to move from female phase to male phase flowers within dichogamous inflorescences, causing more geitonogamy (chapter 3; Jordan & Harder, 2006; Jersakova & Johnson, 2007).

Importantly, my findings indicate that plants with non-random arrangements of male- and female-phase flowers do not completely decouple the costs of increased bout length on self-pollination (Klinkhamer et al., 1994; Karron et al., 2004), which has been reported previously for *C. angustifolium* (Schmid-Hempel & Speiser, 1988; Routley & Husband, 2003). The lack of influence of bout length on departure by *B. pascuorum* and *B. lapidaries* exacerbates the effect of weaker directional tendencies on geitonogamy, by increasing the number of flowers visited per inflorescence visit. Even when pollinators typically move upwards, as seen by *B. hypnorum*, potentially geitonogamous visits occurred commonly due to long bouts (figure 4.4).

Based on my simulations all bee species are expected to visit male- and female-phase flowers with equal frequency (figure 4.4), pollinators should not impose costs of disproportionate visitation to one sex phase (Le Croff et al., 1998; Charlesworth & Charlesworth, 1999). Instead, a pollinator's potential to cause geitonogamy is the most important factor differentiating the effects of pollinator species on plant fitness. Based on the results of the movement simulations, selection should favour traits of *C. angustifolium* that encourage visitation of *A. mellifera* and *B. terrestris* in a manner similar to the traits described by Castellanos et al. (2004) and Thomson & Wilson (2008) such as floral shape and colour (discussed in Chapter 2). As bumblebees carry pollen between plants better than honeybees (Adler & Irwin, 2006), these results suggest that *B. terrestris* should be favoured more. Furthermore, selection should favour traits that discourage visitation of *B. lapidarius*, *B. pascuorum* and *B. hypnorum* when *A. mellifera* and *B. terrestris* are present (discussed previously in chapter 2). As these three species visit *C. angustifolium* less commonly than *A. mellifera* and *B. terrestris* (table 4.3) and the differences in the potential to cause geitonogamy between species are not large and all five species show a common upward movment tendency selection favouring exclusion of these other species is probably weak. Analysis of pollinator movement also found that the invasive *B. hypnorum* has similar potential to cause geitonogamy as the native pollinators. If *B. hypnorum* transfers pollen similarly to other UK bumblebees, it should affect inbreeding of *C. angustifolium* like the native species.

Conclusion

This study has demonstrated that despite typically moving upward, the main 5 pollinators of *C. angustifolium* at my study site differ in their foraging behaviour which should lead to consequences for the incidence of geitonogamy. These differences in pollinator quality as a result of movement, suggests the existence of selection favouring pollinators with strong directional tendencies over pollinators with weaker directional tendencies. However, such selection may be weak, due to the common directional tendency and similar potentials to cause geitonogamy among species. Furthermore, the upwards tendency of pollinators on racemes is unlikely result from nectar reward gradients, but instead reflects an efficiency advantage owing to reduced travel time between flowers.

Chapter 5: Thesis conclusion

This thesis focuses on furthering understanding of how inflorescence architecture influences a plant's fitness. Although Darwin (1862) first reported that vertical architecture encourages upwards pollinator movement, the impacts of inflorescences and directional pollinator foraging have been largely ignored until recently. Instead the focus has been on pollinator behaviour and pollen transfer at the flower level (Harder et al., 2004). The lack of attention paid to inflorescence function means that the influences of inflorescence architecture on pollinator behaviour and pollen transfer within and between plants remain poorly understood. Furthermore, studies that consider inflorescence architecture disagree with regards to how vertical inflorescences influence pollinator movement (e.g. compare Fisogni et al., 2011 with Waddington & Heinrich, 1979 and de Jong et al., 2011) and its impact on pollen transport. Such patterns are especially important for plants with non-random arrangements of male(-phase) and female(-phase) flowers (e.g. Harder et al., 2000 and Jersakova & Johnson, 2007). As pollen transfer is essential for plant reproduction, evolution and crop production (chapter 1), this thesis addresses problems of key importance. This study sought to answer two main questions:

1) How does inflorescence architecture influence pollinator behaviour?
2) How do these influences on behaviour alter pollen transfer within and among plants?

This chapter summarises my findings and suggests directions for future study of inflorescence architecture that my research has raised.

Simulations of pollinator foraging within plants that show no separation of sexual function within the inflorescence (the adichogmaous plants in chapter 2) revealed that highly consistent pollinator arrival position, departure and between-flower movement should improve the quality of pollen transfer within plants compared to random movement. These improvements to pollen transfer included decreased self-pollen deposition, increased pollen export (figure 2.3) and outcross pollen receipt (figure 2.4). Such benefits have not previously been demonstrated for plants with adichogamous flowers and they suggest explanations for plant traits that encourage directional movement, such as racemose inflorescences, among such species.

Simulations of plants that express sex function non-randomly among flowers revealed that when pollinators move consistently they generate pollen-transfer advantages over plants without sexual segregation (chapter 3: also see Harder et al., 2000; Routley & Husband, 2003; Jordan & Harder, 2006). Some pollen transfer advantages, such reduced self-pollination and pollen export, are fairly robust to less consistent pollinator foraging, as long as female-phase flowers were visited before male-phase flowers (figure 3.6). However improved outcross pollen receipt for plants with non-random arrangements, compared to adichogamous plants, depended on the consistency of arrival position. With random arrival, outcross pollen receipt decreased compared to adichogamy (figure 3.4). The dependence of the outcross pollen receipt advantage on pollinator arrival at lower flowers in plants with non-random arrangements may explain why some studies have not observed female outcrossing advantage in plants with non-random arrangements (e.g. Jersakova & Johnson, 2007).

These theoretical studies of the impacts of directional foraging within inflorescences (like that observed when many pollinators forage on vertical inflorescences) show that directional foraging should endow similar advantages in terms of pollen transfer to adichogamous plants and plants with non-random arrangements. Consequently, selection should favour traits that encourage directional foraging, such as vertical raceme architecture, all else being equal (chapter 2 and 3).

However, these pollen transfer advantages should be greater and more robust in plants with non-random arrangements of floral sex roles, so selection should also favour non-random arrangements when pollinators behave consistently on inflorescences. Furthermore, selection should favour traits that increase attraction of consistently moving pollinators and deter less predictably foraging pollinators (see Castellanos et al. 2004; Johnson et al. 2006; chapter 2).

Observation of pollinator foraging in *C. angustifolium* showed that the five main pollinators generally moved upward, as has been reported previously for large-bodied bees (Jordan & Harder, 2006; Fisogni et al., 2011; de Jong et al., 2011). However, bee species differed in aspects of their behaviour on inflorescence (table 4.2 and 4.4, figure 4.3), which should be sufficient to cause differences in each species' potential to cause geitonogamy (figure 4.4) More consistently foraging species, such as *B. terrestris* and *A. mellifera*, were predicted to engage in fewer potentially geitonogamous visits, as expected based on the simulations in chapter 3. Fewer geitonogamous visits should increase pollen export (chapter 2 and 3; Harder & Barrett 1995and 1996; Rademaker et al., 1997). Thus, through differing foraging behaviour, pollinators influence plant fitness differently. Therefore, selection within racemose plants should favour attraction of more consistently foraging species and deter less consistently foraging species, if possible. However, in *C. angustifolium* selection favouring traits that attract and deter the different pollinator species is likely weak due to similarities in each species' potential for causing geitonogamy.

Additionally, nectar standing crop within *C. angustifolium* did not vary systematically. Despite this when confronted with no nectar gradient bees visiting *C. angustifolium* still showed an upward movement tendency within inflorescences (chapter 4). This finding adds to the growing evidence that nectar gradients do not cause directional foraging by bees (Waddington & Heinrich, 1979; Heinrich, 1979; Delvin & Stephenson, 1985; de Jong et al., 2011).

In contrast, arrival behaviour may have been influenced by nectar rewards. Arrival position of bees tended to correspond with the portion of the inflorescence with the fewest unrewarding flowers. Therefore, a foraging efficiency advantage (as suggested by Corbet et al., 1981; Lloyd & Webb, 1986), due to reduced travel time between flowers, may instead motivate upward pollinator movement on racemose inflorescences.

The results of the previous chapters demonstrate the need to consider the inflorescence to understand plant-pollinator interactions fully. How pollinators interact with the inflorescence, in terms of how they move within the inflorescence and where they arrive and depart, influences pollen transfer and therefore plant fitness and plant evolution (chapter 2 and 3). Foraging differences between species are sufficient to alter plant success (chapter 4). Furthermore, the arrangement of sexual function within inflorescences can further alter pollen transfer (chapter 3), indicating that the phenological aspects of inflorescence architecture should be considered in addition to topology and pollinator foraging behaviours (reviewed by Harder & Prusinkiewicz, 2013). Thus, my research adds to the growing evidence (Harder et al., 2000; Routley & Husband, 2003; Jordan & Harder, 2006; Jersakova & Johnson, 2007; Ishii et al., 2008; Iwata et al., 2011) supporting this conclusion. I also demonstrated that upward movement promotes pollinator foraging efficiency. Thus, pollen transfer, plant evolution and potentially how pollinators maximise foraging efficiency, all relate to how pollinators solve spatial problems that scale from the flower, through the inflorescence and the plant to the population as a whole.

A need to view pollen transfer at the inflorescence level presents a number of interesting directions for future study. Further investigation of how plant traits, especially inflorescence traits, influence pollinator foraging behaviours is still required. The simulations of pollen transfer presented in chapters 2 and 3 suggest that selection should favour traits that encourage more predictable foraging by pollinators. Thus, how different plant traits affect pollinator directionality warrants attention. How pollinators move within the broad inflorescence topologies, as summarized in table 1.1, has been described (Jordan & Harder, 2006; Ishii et al., 2008; Iwata et al., 2011; de Jong et al., 2011). However, the effects of aspects such as flower density (but see Ishii et al., 2008), flower helical angle (but see Iwata et al., 2011), and flower orientation remain relatively under studied. Similarly, how inflorescence branch length, such as the distinction between spikes and racemes (table 1.1), is unknown. Additionally, how many floral traits, normally considered at the individual flower level, interact with inflorescence architecture, potentially increasing or moderating the effects of the inflorescence on pollinator attraction and directional foraging, has received little attention, other than the effects of visual signals, including flower size, on visitation (Wertlen et al., 2008; Ishii & Harder, 2012). Other traits that could to be considered include olfactory signals (Shuttleworth & Johnson, 2009) and flower shape (Castellanos et al., 2004; Coombs & Peter, 2009).

The results presented in chapter 4 show bee species behave differently on inflorescences, with potential consequences for pollen transfer outcomes, plant fitness and evolution. This identifies a need for better understanding of the extent to which foraging behaviour differs between species, including their responses to different inflorescence traits. For this reason it may be useful to consider these foraging behaviours in terms of arrival, departure and between flower movement, in a manner similar to the model shown in figure 4.1. Knowledge of each of these aspects of movement is required for accurate predictions of pollen transfer outcomes (as shown in chapter 2 and 3). Although the invasive species, *B. hypnorum* behaved similarly to native species on *C. angustifolium* inflorescences, understanding how species vary in foraging behaviour, both within inflorescence and at a wider scale, may provide insights into the pollen transfer consequences of different species invasions. Additionally, identification of which pollinators behave most consistently on inflorescences and therefore would perform as the best pollinators of crop plants could be useful in implementing pollinator supplementation programmes.

Until recently, pollination studies have largely overlooked how pollinators behave while foraging within inflorescences; instead focusing more on the pollinator’s interactions with individual flowers. However, in this thesis I show that directional pollinator movement within an inflorescence differs between species and has complex impacts on pollen transfer both within and among plants. In addition, I show how downward movement within inflorescences imposes energetic costs on bees. In this way, I have demonstrated the relevance of inflorescence architecture and associated pollinator behaviour to pollen transfer, pollinator foraging efficiency and plant evolution.

Appendix

Table A.1: Weather data for Durham for each of the days where filming took place (dates given all are for 2013). All weather data was obtained by the Durham University Observatory (accessed 2013). Additionally the mean, maximum and minimum of each observation for all 9 days is given.

Date	Max Temp (◦c)	Min Temp (◦c)	Total rainfall (mm)	Dry Bulb Temp at 9:00	Average Humidity (%)	Total Sunshine (hours)	Average Dry Bulb Temp (◦c)
Jul-16	26.20	12.46	0.0	22.05	69	6.93	19.43
Jul-22	23.05	13.91	0.0	15.52	85	4.03	16.81
Jul-24	24.46	16.59	1.2	20.87	75	4.30	20.09
Jul-26	24.66	12.80	0.0	20.51	65	6.85	18.45
Jul-31	21.63	10.76	5.4	17.55	78	5.46	16.03
Aug-06	18.40	8.78	0.0	15.07	77	4.43	13.75
Aug-08	21.78	7.76	0.6	18.38	69	5.35	15.86
Aug-11	18.49	10.49	0.4	16.44	75	5.08	14.04
Aug-18	18.72	10.84	0.0	13.99	71	5.83	14.22
Mean	21.93	11.60	0.84	17.82	73.78	5.36	16.52
Maximum	26.20	16.59	5.40	22.05	85.00	6.93	20.09
Minimum	18.40	7.76	0.00	13.99	65.00	4.03	13.75

Bibliography

Adler, L.S. & Irwin, R.E., 2005. Ecological costs and benefits of defences in nectar. *Ecology*, 86(11), pp.2968-78.

Adler, L.S. & Irwin, R.E., 2006. Comparison of pollen transfer dynamics by multiple floral visitors: experiments with pollen and fluorescent dye. *Annals of Botany*, 97, pp.140-50.

Aizen, M.A. & Harder, L.D., 2007. Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology*, 88(2), pp.271-81.

Aizen, M.A.; Garibaldi, L.A.; Cunningham, S.A. & Klein, A.M., 2008. Long-term trends in crop yeild and production reveal no current pollination shortage but increasing pollinator dependency. *Current Biology*, 18, pp.1572-75.

Albert, M.J.; Iriondo, J.M.; Escudero, A. & Torres, E., 2008. Dissecting components of flowering pattern: size effets on female fitness. *Botanical Journal of the Linnean Society*, 156, pp.227-36.

Ashman, T.-L.; Knight, T. M.; Steets, J. A.; Amarasekare, P.; Burd, M.; Campbell, D. R.; Dudash, M. R.; Johnson, M. O.; Mazer, S. J.; Mitchell, R. J.; Morgan, M. T. & Wilson, W. G., 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology*, 85, pp.2408-21.

Barrett, S.C.H., Harder, L.D. & Cole, W.W., 1994. Effects of flower number ans position on self-fertilization in experimental populations of *Eichhornia paniculata* (Pontederiaceae). *Functional Ecology*, 8, pp.526-35.

Bateman, A.J., 1947. Contamination in seed crops. *Heredity*, 1, pp.303-36

Bertin, R.I., 1993. Incidence of monoecy and dichogamy in relation to self fertilization in angiosperms. *American Journal of Botany*, 80(5), pp.557-60.

Bertin, R.I. & Newman, C.M., 1993. Dichogamy in angiosperms. *Botanical Review*, 59, 112-152.

Best, L.S. & Bierzychudek, P., 1982. Pollinator foraging on foxglove (*Digitalis purpurea*): a test of a new model. *Evolution*, 36, pp.70-79.

Bhardwaj, M. & Eckert, C.G., 2001. Functional analysis of synchronous dichogamy in flowering rush, *Butomus umbellatus* (Butomaceae). *American Journal of Botany*, 88(12), pp.2204-13.

Biernaskie, J.M.; Walker, S.C. & Gegear, R.J., 2009. Bumblebees learn to forage like Bayesians. *The American Naturalist*, 174(3), pp.413-23.

Bluthgen, N. & Klein, A.-M., 2011. Functional complementarity and specialisation: the role of biodiversity in plant-pollinator interactions. *Basic and Applied Ecology*, 12, pp.282-91.

Brunet, J. & Charlesworth, D., 1995. Floral sex allocation in sequentially blooming plants. *Evolution*, 49(1), pp.70-79.

Burtt, B.L., 1977. Aspects of diversification in the capitulum. In Heywood, V.H., Harborne, J.B. & Turner, B.L. *The Biology and Chemistry of Compositae*. New York: New York Acedemic Press. pp.41-59.

Cao, G.; Xue, L.; Li, Y. & Pan, K., 2011. The relative importance of achitecture and resource competition in allocation to pollen and ovule number within inflorescences of *Hosta venticosa* varies with the resource pools. *Annals of Botany*, 107, pp.1413-19.

Carlson, J.E. & Harms, K.E., 2006. The evolution of gender-biased nectar production in hermaphoditic plants. *The Botanical Review*, 72(2), pp.179-205.

Castellanos, M.C.; Wilson, P. & Thomson, J.D., 2004. ‘Anti-bee’ and ‘pro-bird’ changes during the evolution of hummingbird pollination in *Penstemon* flowers. *Journal of Evolutionary Biology*, 17, pp.876-85.

Charlesworth, B. & Charlesworth, D., 1999. The genetic basis of inbreeding depression. *Genetics Reserch*, 74, pp.329-40.

Charnov, E.L., 1976. Optimal foraging, the marginal value theorem. *Theoretical Population Biology*, 9(2), pp.129-36.

Charnov, E.L., 1982. *The Theory of Sex Allocation*. Princeton, NJ: Princeton University Press.

Chittka L., Spaethe, J., Schmidt A., Hickelsberger A., 2001. Adaption, constraint, and chance in the evolution of flower color and pollination color vision. In: *Cognative Ecology of Pollination*, Cambridge University Press, Cambrige, UK, pp. 106-126.

Clarke, D.; Whitney, H.; Sutton, G. & Robert, D., 2013. Detection and learning of floral electric feilds by bumblebees. *Science*, 340, pp.66-69.

Coombs, G. & Peter, C.I., 2009. Do floral traits of *Strelitzia reginae* limit nectar theft by sunbirds. *South African Journal of Botany*, 75, pp.751-56.

Corbet, S.A.; Cuthill, I.; Fallows, M.; Harrison, M. & Hartley, G., 1981. Why do nectar-foraging bees and wasp work upwards on inflorescences? *Oecologia*, 89, pp.83-129.

Darwin, C.R., 1862. *On the Various Contrivances by which British and Foreign Orchids are Fertilised by Insects*. London: John Murray.

de Almeida, N.M.; de Castro, C. C.; de Lima Leite, A. V.; Novo, R. R. & Machado, I. C., 2013. Enantiostyly in *Chamaecrita ramosa* (Fabaceae- Caesalpinioideae): floral morphology, pollen transfer dynamics and breeding system. *Plant Biology*, 15, pp.369-75.

de Jong, T.J., 2000. From pollen dynamics to adaptive dynamics. *Plant Species Biology*, 15, pp.31-41.

de Jong, T. & Klinkhamer, P., 2005. Flower number and male reproductive success in animal-pollinated plants. In *Evolutionary Ecology of Plant Reproductive Strategies*. Cambrige: Cambridge University press. pp.42-52.

de Jong, T.J.; Klinkhamer, P.G.L. & Van Staalduinen, M.J., 1992. The consequences of pollination biology for selection of mass or extended blooming. *Functional Ecology*, 6, pp.606-15.

de Jong, T.J.; Klinkhamer, P.G.L.; Shmida, A. & Thuijsman, F., 2011. On the evolution of protandry and the direction between preference and rank order in pollinator visitation. *Evolutionary Ecology Research*, 13, pp.307-14.

Dedej, S. & Delaplane, K.S., 2005. Net energetic advantage drives honey bees (*Apis mellifera* L.) to nectar larceny in *vaccinium ashei* Reade. *Behavioural Ecology and Sociobiology*, 57, pp.398-403.

Delvin, B. & Stephenson, A.G., 1985. Sex differential floral longevity, nectar secretion, and pollinator foraging in a protandrous species. *American Journal of Botany*, 72(2), pp.303-10.

Diggle, P.K., 1995. Architectual effects and the interpretation of patterns of fruit and seed development. *Annual Review of Ecology, Evolution and Sytematics*, 26, pp.531-552

Diggle, P.K., 2003. Architectural effects on floral form and function: a review. In: *Deep Morphology: Toward Renaissance of Morphology in Plant Systematics* (eds. Stuessy, T.; Hörandl, E. & Mayer V.), Koeltz, Königtein, Germany, pp. 63-80.

Dreisig H., 2012. How long to stay on a plant: the response of bumblebees to encountered nectar levels. *Arthropod-Plant Interactions*, 6, 315-25.

Drumont, L.; Delle-Vedove, R.; Bessiere, J.-M.; Hossaert-McKey, M. & Schatz, B., 2010. Rare white-flowered morphs increase the reporductive success of common purple morphs in a food-deceptive orchid. *New Phytologist*, 185, pp.300-10.

Endress, P.K., 2010. Disentangling confusions in inflorescence morphology: patterns and diversity of reproductive shoot ramification in angiosperms. *Journal of Systematics and Evolution*, 48(4), pp.225-39.

Engel, E.C. & Irwin, R.E., 2003. Linking pollinator visitation rate and pollen receipt. *American Journal of Botany*, 90(11), pp.1612-18.

Fenster, C.B.; Armbruster, W. S.; Wilson, P.; Dudash, M. R. & Thomson, J. D., 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology*, 35, pp.375-403.

Fenster, C.B.; Armbruster, W.S. & Dudash, M.R., 2009. Specialization of flowers: is floral orientation an overlooked first step? *New Phytologist*, 183, pp.502-06.

Fisher, R.A., 1941. Average excess and average effect of gene substitution. *Annuals of Eugenics*, 11, pp.53-63.

Fisogni, A.; Cristofolini, G.; Rossi, M. & Galloni, M., 2011. Pollinator directionality as a responce to nectar gradient: promoting outcrossing while avoiding dichogamy. *Plant Biology*, 13, pp.848-56.

Fulton, M. & Hodges, S.A., 1999. Floral isolation between Aquilegia formosa and Aquilegia pubescens. *Proceedings for the Royal Society of London B.*, 266, pp.2247-52.

Fussell, M. & Corbet, S., 1992. The nesting places of some British bumblebees. *Journal of Apicultural Research*, 31, pp.32-41.

Galen, C. & Plowright, R.C., 1985. The effects of nectar level and flower development on pollen carry-over in inflorescences of fireweed (*Epilobium angustifolium*) (Onagraceae). *Canadian Journal of Botany,* 63(3), pp.488-91.

Galen, C. & Cuba, J., 2001. Down the tube: pollinators, predators, and the evolution of flower shape in alpine skypilot, *Polemonium viscosum*. *Evolution*, 55(10), pp.1963-71.

Gallai, N.; Salles, J.-M.; Settele, J. & Vaissiere, B.E., 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics*, 68, pp.810-21.

Galloway, L.F.; Cirigliano, T. & Gremski, K., 2002. The contribution of display size and dichogamy to potential geitonogamy in *Campanula america*. *International Journal of Plant Sciences*, 163(1), pp.133-39.

Goodwillie, C.; Kalisz, S.; Eckert, C.G., 2005. The evolutionary enigma of mixed mating systems in plants: occurance, theorectical explainations, and empirical evidence. *Annual Review of Ecology, Evolution and Systematics,* 36, pp.47-79.

Goulson, D. & Williams, P., 2001. *Bombus Hypnorum* (Hymenoptera: Apidae), a new British bumblebee? *British Journal of Entamology and Natural History*, 14, pp.129-31.

Goulson, D.; Stout, J.C.; Hawson, S.A. & Allen, J.A., 1998. Floral display size in comfrey, *Symphytum officinale* L. (Boraginaceae): relationships with visitation by three bumblebee species and subsequent seed set. *Oecologia*, 113, pp.502-08.

Grant, K.A. & Grant, V., 1968. *Hummingbirds and Their Flowers*. New York: Columbia University Press.

Gumbert, A., 2000. Color choices by bumble bees (*Bombus terrestris*): innate preferences and generalisation after learning. *Behavioural Ecology and Sociobiology*, 48, pp.36-43.

Hammer, M. & Menzel, R., 1995. Learning and memory in the honeybee. *The Journal of Neuroscience,* 15(3), pp.1617-30.

Harder, L.D., 1983. Flower handling efficiency of bumble bees: morphological aspects of probing time. *Oecologia*, 57, pp.274-80.

Harder, L.D., 1990. Pollen removal by bumblebees and its implications for pollen dispersal. *Ecology*, 71(3), pp.1110-25.

Harder, L.R. & Thomson, J.D., 1989. Evolutionary options for maximising pollen dispersal of animal-pollinated plants. *American Naturalist*, 133(3), pp.323-44.

Harder L.D. & Wilson, W.G., 1994. Floral evolution and male reproductive success: optimal dispensing schedules for pollen dispersal by animal-pollinated plant. *Evolutionary Ecology*, 8(5), pp.545-559

Harder, L.D. & Barrett, S.C.H., 1995. Mating costs of large floral displays in hermaphrodite plants. *Nature*, 373, pp.512-15.

Harder, L.D. & Wilson, W.G., 1995. A clarification of pollen discounting and its joint effect with inbreeding depression on mating system evolution. *The American Naturalist*, 152(5), pp.684-95.

Harder, L.D. & Barrett S.C.H., 1996. Pollen dispersal and mating patterns in animal pollinated plants. In: *Floral Biology: Studies on Floral Evolution in Animal Pollinated Plants* (eds. Lloyd, D.G. & Barrett, S.C.H.), Chapman and Hall, New York, pp.140-190.

Harder, L.D. & Wilson, W.G., 1998. Theoretical consequences of heterogeneous transport conditions for pollen dispersal by animals. *Ecology*, 79(8), pp.2789-807.

Harder L.D. & Aizen, M.A., 2004. The functional significance of synchronous protandry in *Alstroemeria aurea*. *Functional Ecology*, 18, pp.467-74.

Harder, L.D. & Johnson S.D., 2009. Darwin’s beautiful contrivances: evolutionary and functional evidence for floral adaptation. *New Phytologist*, 183, pp.530-545

Harder, L.D. & Prusinkiewicz, P., 2013. The interplay between inflorescence development and function as the crucible of architectural diversity. *Annals of Botany, 112: 1477-1493*

Harder L.D.; Barrett, S.C.H. & Cole, W.W., 2000. The mating consequences of sexual segregation within inflorescences of flowering plants. *Proceedings for the Royal Society of London Series B*, 267, pp.315-20.

Harder, L.D.; Jordan, C.Y.; Gross, W.E. & Routley, M.B., 2004. Beyond floricentrism: the pollination fuction of inflorescences. *Plant Species Biology*, 19, pp.137-48.

Harder, L.D.; Richards, S.A. & Routley, M.B., 2007. Effect of reproductive compensation gamete discounting and reproductive assurance on mating-system diversity in hermaphrodites. *Evolution*, 62(1), pp.157-72.

Haynes, J. & Mesler, M., 1984. Pollen foraging by bumblebees: foraging patterns and efficiency on *Lupinus polyphyllus*. *Oecologia*, 61, pp.249-53.

Heinrich, B., 1979. Resource heterogeneity and patterns of movement in foraging bumblebees. *Oecologia*, 40, pp.235-45.

Herrera, C.M., 2000. Flower-to-seedling consequences of different pollination regimes in an insect-pollinated shrub. *Ecology*, 81, pp.15-29.

Herrera, C.M., 2009. Multiplicity in unity: plant subindividual variation and interactions with animals. Chicago, IL: University of Chicago Press.

Hodges, C.M., 1986. Bumble bee foraging: Energetic consequneces of using a threshold departure rule. *Ecology,* 66, pp.188-97.

Husband, B.C. & Schemske, D.W., 1995. Magnitude and timing of inbreeding depression in a diploid population of *Epilobium angustifolium* (Onagraceae). *Heredity*, 75, pp.206-15.

Husband, B.C. & Schemske, D.W., 1997. The effect of inbreeding in diploid and teraploid populations of *Epilobium angustifolium* (Onagraceae): implications for the genetic basis of inbreeding depression. *Evolution*, 51(3), pp.737-46.

Inoue, M.N.; Yokoyama, J. & Washitani, I., 2008. Displacement of Japanese native bumblebees by recently introduced *Bombus terrestris* (L.) (Hymenoptera: Apidae). *Journal of Insect Conservation*, 12, pp.135-46.

Inouye, D.W., 1980. The effect of proboscis and corolla tube lengths on patterns and rates of flower visitation by bumblbees. *Oecologia,* 45(2), pp.197-201.

Irwin, R.E. & Brody, A.K., 2000. Consequences of nectar robbing for realised male function in a hummingbird-pollinated plant. *Ecology*, 81(9), pp.2637-43.

Irwin, R.E. & Adler, L.S., 2008. Nectar secondary compounds affect self-pollen transfer: implications for female and male reproduction. *Ecology*, 89, pp.2207-17.

Ishii, H.S. & Sakai, S., 2001. Effects of display size and position on individual floral longevity in racemes of *Narthecium asiaticum* (liliaceae). *Functional Ecology*, 15, pp.396-405.

Ishii, H.S. & Sakai, S., 2002. Temporal variation in floral display size and individual floral sex allocation in racemes of *Narthecium asiaticum* (Liliaceae). *American Journal of Botany,* 3, pp.441-46.

Ishii, H.S. & Harder, L.D., 2012. Phenological associations of within- and among-plant variation in gender with floral morphology and integration in protandrous *Delphinium glacucum*. *Journal of Ecology*, 100, pp.1029-38.

Ishii, H.S.; Hirabayashi, Y. & Kudo, G., 2008. Combined effects of infloresence architecture, display size, plant density and empty flowers on bumble bee behaviour: experimental study with artificial inflorescences. *Oecologia*, 156(2), pp.341-50.

Iwata, T.; Nagasaki, O.; Ishii, H.S. & Ushimara, A., 2012. Infloresence architecture affects pollinator behaviour and mating success in *Spiranthes sinensis* (Orchidaceae). *New Phytologist*, 193, pp.196-203.

Jander, R. & Jander, U., 1970. Uber die phylogenie der geotaxis innerhalb der bienen (Apoidea). *Zeitschrift für vergleichende Physiologie*, 66, pp.355-68.

Jensen, J.L., 1906. Sur les fonctions convexes et les inegualites entre les valeurs moyennes. *Acta Mathematica*, 30, pp.175-93.

Jersáková, J. & Johnson, S.D., 2006. Lack of floral nectar reduces self-pollination in fly pollinated orchid. *Oecologia*, 147, pp.60-68.

Jersáková, J. & Johnson, S.D., 2007. Protandry promotes male pollination success in a moth-pollinated orchid. *Functional Ecology*, 21, pp.496-504.

Jersáková, J.; Johnson, S.D. & Kindlmann, P., 2006. Mechanisms and evolution of deceptive pollination in orchids. *Biological Reviews*, 81(2), pp.219-35.

Johnson, S.D. & Anderson, B., 2010. Coevolution between food-rewarding flowers and their pollinators. *Evolution Education Outreach*, 3, pp.32-39.

Johnson, S.D.; Peter, C.I. & Agren, J., 2004. The effects of nectar addition on pollen removal and geitonogamy in the non-rewarding orchid *Anacamptis morio*. *Proceedings for the Royal Society of London Series B.*, 271, pp.803-09.

Johnson, S.D.; Hargreaves, A.L. & Brown, M., 2006. Dark, bitter-tasting nectar functions as a filter of flower visitors in a bird-pollinated plant. *Ecology*, 87(11), pp.2709-16.

Jordan, C.Y. & Harder, L.D., 2006. Manipulation of bee behaviour by inflorescence architcture and its consequences for plant mating. *The American Naturalist*, 167(4), pp.496-509.

Karron, J.D. & Mitchell, R.J., 2012. Effects of floral display size on male and female reproductive success in *Mimulus ringens*. *Annals of Botany*, 109, pp.563-570

Karron, J.D.; Mitchell, R. J.; Holmquist, K. G.; Bell, J. M. & Funk, B., 2004. The influence of floral display size on selfing rates in *Mimulus rigens*. *Heredity*, 92, pp.242-48.

Keller, L.F. & Waller, D.M., 2002. Inbreeding effects in wild populations. *Trends in Ecology and Evolution*, 17(5), pp.230-41.

Klein, A.-M.; Vaissiere, B. E.; Cane, J. H.; Steffan-Dewenter, I.; Cunningham, S. A.; Kremen, C. & Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society*, 274(1608), pp.303-13.

Kliber, A. & Eckert, C.G., 2004. Sequential decline in allocation amoung flowers within inflorescences: proximate mechanisms and adaptive significance. *Ecology*, 85, pp.1675-87.

Klinkhamer, P.G.L.; de Jong, T.J. & Metz, J.A.J., 1994. Why plants can be too attractive -- a discussion of measures to estimate male fitness. *Journal of Ecology*, 82(1), pp.191-94.

Knight, M.E.; Martin, A. P.; Bishop, S.; Osborne, J. L.; Hale, R. J.; Sanderson, R. A. & Goulson, D., 2005. An interspecific comparison of foragign range an nest density of four bumblebee (*Bombus*) species. *Molecular Ecology*, 14, pp.1811-20.

Knoll F., 1921. Insekten und Blumen: experimentelle Arbeiten zur Vertiefungunserer Kenntnisse uber die Wechselbeziehungen zwischen Pflanzen und Tieren. Heft 1: II. Bombylius fuliginosus und die Farbe der Blumen. *Abhandlungen der Zoologische-Botanische Gesellschaft in Wien* 12: 17–116.

Komlos, D.A., 1999. Vegetative and sexual life-history attributes of *Chamerion angustifolium* (fireweed) as influenced by arbuscular mycorrhizal fungi. *Master's Thesis, Department of Botany, University of Guelph.* [Online] Available at: http://www.collectionscanada.gc.ca/obj/s4/f2/dsk2/ftp01/MQ43178.pdf [Accessed 26 March 2014].

Kudo, G.; Maeda, T. & Narita, K., 2001. Variation in floral sex allocation and reproductive variation within inflorescences of *Corydalis ambigua* (Fumariaceae): pollination effciency or resource limitation? *Journal of Ecology*, 89, pp.48-56.

Kunin, W.E., 1993. Sex and the single mustard: population density and pollinator behaviour effects on seed-set. *Ecology*, 74(7), pp.2145-60.

Kunte, K., 2007. Allometry and functional constraints on proboscis lengths in butterflies. 21, pp.982-87.

Laloi, D.; Sandoz, J. C.; Picard-Nizou, A. L.; Marchesi, A.; Tasei, J. N.; Poppy, G. & Pham-Delegue, M. H., 1999. Olfactory conditioning of the proboscis extension in bumble bees. *Entomologia Experimentalis et Applicata*, 90, pp.123-29.

Lande, R. & Schemske, D.W., 1985. The evolution of self-fertilisation and inbreeding depression in plants I. Genetics models. *Evolution*, 39, pp.24-40.

Le Croff, J.; Agren, J. & Schemske, D.W., 1998. Floral display, pollinator discrimination, and female reproductive success in two monoecious *Begonia* species. *Ecology*, 79(5), pp.1610-19.

Lee, T.D., 1988. Patterns of fruit and seed prioduction. In: *Plant Reproductive Ecology: Patterns and Strategies* (eds. Lovett Doust, J. & Lovett Doust, L.), Oxford University Press, New York, pp.179-202.

Lefebvre, D.; Pierre, J.; Outreman, Y. & Pierre, J.-S., 2007. Patch departure rules in bumblebees: evidence of a decremental motivational mechanism. *Behavioural Ecology and Sociobiology*, 61, pp.1707-15.

Leshem, Y.; Keasar, T. & Shimida, A., 2011. Female-biased nectar production in the protandrous, hermaphroditic shrub *Salvia hierosolymitana* (Lamiaceae). *Austrailian Journal of Botany*, 59, pp.18-25.

Li, P.; Huang, B. Q.; Pemberton, R. W.; Luo, Y. B. & Cheng, J., 2011. Floral display influences male and female reporductive success of the deceptive orchid *Phaius delavayi*. *Plant Systematics and Evolution*, 296, pp.21-27.

Liu, K.-W.; Liu, Z.-J.; Huang, L.; Li, L.-Q.; Chen, L.-J. & Tang, G.-D., 2006. Self pollination strategy in an orchid. *Nature*, 441, pp.945-46.

Lloyd, D.G., 1992. Self- and cross-fertilisation in plants II. The selection of self fertiliation. *International Journal of Plant Sciences*, 153, pp.370-80.

Lloyd, D.G. & Bawa, K.S., 1984. Modification of the gender of seed plants in varying conditions. *Evolutionary Biology*, 17, pp.255-338.

Lloyd, D.G. & Webb, C.J., 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms. *New Zealand Jornal of Botany*, 24, pp.135-62.

Lunau, K. & Maier, E.J., 1995. Innate colour preferences of flower visitors. *Journal of Comparative Physiology A*, 177, pp.1-19.

Lye, G.C.; Osborne, J.L.; Park, K.J. & Goulson, D., 2012. Using citizen science to monitor *Bombus* populations in the UK nesting ecology and relative abundance in urban environment. *Journal of Insect Conservation*, 16(5), pp.697-707.

MacFalane, R.P. & Gurr, L., 1995. Distribution of bumble bees in New Zeland. *New Zealand Entomologist*, 18, pp.29-36.

Maloof, J.E., 2001. The effects of a bumble bee nectar robber on plant reproductive success and polliantor behaviour. *American Journal of Botany*, 88(11), pp.1960-65.

Marten-Rodriguez, S.; Quesada, M. & Momen, B., 2012. Effects of Local Plant Density and Incomplete Dichogamy on the Reproductive Success of the Rare Neotropical Palm *Geonoma epetiolata*. *Biotropica*, 44(5), pp.680-88.

McKone, M.J.; Ostertag, R.; Rauscher, J. T.; Heiser, D. A. & Russell, F. L., 1995. An exception to Darwin's syndrome: floral position, protogyny, and insect visitation in *Besseya bullii* (Scrophulariaceae). *Oecologia*, 101, pp.68-74.

Mitchell, R.J.; Karron, J.D.; Holmquist, K.G. & Bell, J.M., 2004. The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. *Functional Ecology*, 18, pp.116-24.

Myerscrough, P.J., 1980. Biological flora of the British Ilses. No. 148. *Epilobium angustifolium* L. (*Chamaenerion angustifolium* (L.) Scop.). *Journal of Ecology*, 68, pp.1047-74.

Nachtigall, W.; Hanauer-Thieser, U. & Morz, M., 1995. Flight of the honey bee VII: metabolic power versus flight speed relation. *Journal of Comparative Physiology B*, 165, pp.484-89.

Narbona, E.; Ortiz, P.L. & Arista, M., 2011. Linking self-incomplatibility, dichogamy, and flowering synchrony in two *Euphorbia* species: Alternative mechanisms for avoiding self fertilisation e. *PLos ONE*, 6(6). article ID e20668.

Natural History Museum, London, 2014. *British bumblebee identification guide*. [Online] Available at: http://www.nhm.ac.uk/nature-online/life/insects-spiders/identification-guides-and-keys/bumblebees/ [Accessed July 2013].

Ohashi, K. & Yahara, T., 2001. Behavioural responces of pollinators to variation in floral display size and their influences on the evolution of floral traits. In: *Cognative Ecology of Pollination* (eds. Chittka, L. & Thomson JD), Cambridge University Press, Cambridge, UK, pp.274-296.

Ollerton, J.; Winfree, R. & Tarrant, S., 2011. How many flowering plants are pollinated by animals? *Oikos*, 120, pp.321-26.

Osborne, J.L.; Martin, A. P.; Shortall, C. R.; Todd, A. D.; Goulson, D.; Knight, M. E.; Hale, R. J. & Sanderson, R. A., 2008. Quantifying and comparing bumblebee nest densities in gardens and contryside habitats. *Journal of Applied Ecology*, 45(3), pp.784-92.

Paton, D.C., 1993. Honeybees in the Austrailian Environment. *BioScience*, 43, pp.95-103.

Pauw, A.; Stofberg, J. & Waterman, R.J., 2008. Flies and flowers in Darwin's race. *Evolution*, 63(1), pp.268-79.

Pleasants, J.M., 1981. Bumblebee responces to variation in nectar avaiability. *Ecology*, 62(6), pp.1648-61.

Pleasants, J.M., 1989. Optimal foragigng by nectarivores: A test of the marginal-value theorem. *The American Naturalist*, 134(1), pp.51-71.

Potts, S.G.; Vulliamy, B.; Roberts, S.; O'Toole, C.; Dafni, A.; Ne'eman, G. & Willmer, P. G., 2004. Nectar resource diversity organises flower-visitor community structure. *The Neatherlands Enotomological society*, 113, pp.103-07.

Potts, S.G.; Biesmeijer, J. C.; Kremen, C.; Neumann, P.; Schweiger, O. & Kunin, W. E., 2010. Global pollinator declines: trends, impacts and drivers. *Trends in Ecology and Evolution*, 25(6), pp.345-53.

Prusinkiewicz, P., Erasmus, Y.; Lane, B.; Harder, L. D. & Coen, E., 2007. Evolution and development of inflorescence architectures. *Science*, 316, pp.1452-56.

Pyke, G.H., 1978. Optimal foraging in bumblebees and coevolution with their plants. *Oecologia*, 36, pp.281-93.

Rademaker, M.C.J., de Jong, T.J. & Klinkhamer, P.G.L., 1997. Pollen dynamics of bumblebee visitation on *Echium vulgare*. *Functional Ecology*, 11, pp.554-63.

Rademaker, M.C.J., de Jong, T.J. & Van Der Meijden, E., 1999. Selfing rates in natural populations of *Echium vulgare*: a combined empirical and model approach. *Functional Ecology*, 13, pp.828-37.

Ramirez, N. & Seres, A., 1994. Plant reproductive biology of herbaceous monoctos in a Venezuelan tropical cloud forest. *Plant Systematics and Evolution*, 190, pp.129-42.

Rasheed, S.A. & Harder, L.D., 1997. Economic motivation for plant specie preferences of pollen-collecting bumble bees. *Ecological Entomology*, 22, pp.209-19.

Renner, S.S., 2001. How common is heterodichogamy. *Trends in Ecology and Evolution*, 16(11), pp.595-97.

Richards, S.A., 2008. Dealing with overdispersed count data in applied ecology. *Journal of Applied Ecology*, 45, pp.218-27.

Richards, S.A.; Williams, N.M. & Harder, L.D., 2009. Variation in pollination: causes and consequences for plant reproduction. *American Naturalist*, 174(3), pp.382-98.

Riffell, J.A.; Alarcon, R.; Abrell, L.; Davidowitz, G.; Bronstein, J. L. & Hildebrand, J. G., 2008. Behavioural consequences of innate preferences and olfactory learning in hawkmoth-flower interations. *PNAS*, 105(9), pp.3404-09.

Robertson, A.W. & MacNair, M.R., 1995. The effect of floral display size on polliantor service to individual flower of *Myosotis* and *Mimulus*. *Oikos*, 72, pp.106-14.

Rothe, U. & Nachtigall, W., 1989. Flight of the honeybee: IV. Respiratory quotients and metabolic rates during sitting, walking and flying. *Journal of Comparative Physiology*, 158, pp.739-49.

Routley, M.B. & Husband, B.C., 2003. The effect of protandry on siring success in *Chemerion angustifoloium* (Onagraceae) with different inflorescence sizes. *Evolution*, 57(2), pp.240-48.

Routley, M.B. & Husband, B.C., 2005. Responses to selection on male-phase duration in *Chamerion angustifolium*. *Journal of Evolutionary Biology*, 18, pp.1050-59.

Routley, M.B. & Husband, B.C., 2006. Sexual interference within flowers of *Chamerion angustifolium*. *Evolutionary Ecology*, 20, pp.331-43.

Ruel, J.J. & Ayres, M.P., 1999. Jensen's inequality predicts effects of enviromental variation. *TREE*, 14(9), pp.361-66.

Sage, T.L.; Bertin, R.I. & Williams, E.G., 1994. Ovarian and other late-acting self incompatibility systems. In Williams, E.G., Clarke, A.E. & Knox, R.B. *Genetic Control of Self-Incompatability and Reproductive Development in Flowering Plants.* Dordrecht, The Netherlands: Kluwer Acedemic Publishers.

Sargent, R.D. & Roitberg, B.D., 2000. Seasonal decline in male-phase duration in a protandrous plant: a responce to increased mating oppitunities. *Functional Ecology*, 14(4), pp.484-89.

Schmid-Hempel, P. & Speiser, B., 1988. Effect of inflorescence size on polliantion in *Epilobium angustifolium*. *Oikos*, 53(1), pp.98-104.

Schimid-Hempel, P.; Kacelnik, A. & Houston, A.I., 1985. Honeybees maximize efficiency by not filling their crop. *Behavioural Ecology and Sociobiology*, 17, pp.61-66.

Schlessman, M.A., 1987. Gender modification in North American ginseng: Dichotomous choice versus adjustment. *BioScience*, 37(7), pp.465-75.

Seavey, S.R. & Bawa, K.S., 1986. Late-acting self-incompatility in angiosperms. *The Botanical Review*, 52, pp.196-219.

Shetler, S.G., 1979. Pollen-collectiong hairs of *Campanula* (Cmpanulaceae) 1: historical review. *Taxon*, 1 2/3, pp.205-15.

Shuttleworth, A. & Johnson, S.D., 2009. The importance of scent and nectar filters in a specialized wasp-pollination system. *Functional Ecology*, 23, pp.931-40.

Sinu, P.A. & Shivanna, K.R., 2007. Pollination biology of large cardamom (*Amomum sublatum*). *Current Science*, 93(4), pp.548-52.

Smallwood, P.D., 1996. An introduction to risk sensitivity: the use of Jensen's inequality to clarisy evolutionary arguements of adaptive constraint. *American Zoologist*, 36, pp.392-401.

Smithson, A. & MacNair, M.R., 1997. Negative frequency-dependent selection by pollinators on artificial flowers without rewards. *Evolution*, 51(3), pp.715-23.

Soltz, R.L., 1987. Interspecific competition and resource utilization between bumblebees. *The Southwestern Naturalist*, 32(1), pp.39-52.

Spaethe, J. & Chittka, L., 2003. Interindividual variation of eye optics and single object resolution in bumblbees. *Journal of Experimental Biology*, 206, pp.3447-55.

Stanton, M.L., 1987. Reproductive biology of petal colour variants in wild populations of *Raphanus sativus*: I. pollinator responce to color morphs. *American Journal of Botany*, 74(2), pp.178-87.

Stout, J.C.; Allen, J.A. & Goulson, D., 1998. The influence of relative plant density and floral morphological complexity on the behaviour of bumblebees. *Oecologia*, 117, pp.543-50.

Takayama, S. & Isogai, A., 2005. Self-incompatabilty in plants. *Annual Reveiw of Plant Biology*, 56, pp.467-89.

Taneyhill, D.E., 2010. Patch departure behaviour of bumble bees: rules and mechanisms. *Psyche*. Article ID 872736.

Tang, J.-Y. & Ren, M.-X., 2011. Sex allocation and functional bias of quaternay and quinary flowers on the same infloresence in hermaphrodite *Ruta graveolens*. *Acta Oecologica*, 37, pp.449-54.

Thomson, J.D., 1986. Pollen transport and deposition by bumble bees in *Erythronium*: influences of floral nectar and bee grooming. *Journal of Ecology*, 74, pp.329-341.

Thomson, J.D., 1988. Effects of variation in inflorescence size and floral rewards on the visitation rates of traplining pollinators of *Aralia hispida*. *Evolutionary Ecology*, 2, pp.65-76.

Thomson, J.D., 1989. Deployment of ovules and pollen amoung flowers within inflorecences. *Evolutionary Trends in Plants*, 3, pp.65-68.

Thomson, J.D. & Wilson, P., 2008. Explaining evolutionary shifts between bee and hummingbird pollination: convergence, divergence, and directionality. *International Journal of Plant Science*, 169(1), pp.23-38.

Troll, W., 1969. *Die Infloreszenzen: Topologie und stellung im Aufbau des vegetationskorpes Vol. 2. part 1*. Stuttgard: Fischer. Classification method translated by Endress 2010.

Vallius, E., 2000. Position-dependent reproductive success of flowers in *Dactylorhiza maculata* (Orchidaceae). *Functional Ecology*, 14, pp.65-68.

Waage, J.K., 1979. Foraging for patchily-distributed host by the parasitoid, *Nemeritis canescens*. *Journal of Animal Ecology*, 48(2), pp.353-71.

Waddington K.D. & Heinrich B., 1979. The foraging movements of bumblebees on vertical “inflorescences”: an experimental analysis. *Journal of Comparative Physiology*, 134, pp.113-17.

Waser, N.M.; Chittka, L.; Price, M. V.; Williams, N. M. & Ollerton, J., 1996. Generalization in pollination systems, and why it matters. *Ecology*, 77(4), pp.1043-60.

Webb, C.J., 1981. Andromonecism, protandry and sexual selection in Umbelliferae. *New Zealand Journal of Botany*, 19, pp.335-38.

Weiss, M.R. & Papaj, D.R., 2003. Colour learning in two behavioural contexts: how much can a butterfly keep in mind. *Animal Behaviour*, 65, pp.425-34.

Wertlen, A.M.; Niggebrugge, C.; Vorobyev, M. & Hempel de Ibarra, N., 2008. Detection of patches of coloured discs by bees. *Journal of Experimental Biology*, 211, pp.2101-04.

Wolf, L.L. & Hainsworth, F.R., 1986. Information and hummingbird foraging at individual inflorescences of *Ipomopsis aggregata*. *Oikos*, 46, pp.15-22.

Wolf, T.J., Ellington C.P. & Begley I.S., 1999. Foraging costs in bumblebees: field conditions cause large individual differences. *Insectes Sociaux*, 46, pp.291-295

Wolf, S.; Rohde, M. & Moritz, R.F.A., 2010. The reliability of morphological traits in the differentiation of *Bombus terrestris* and *B. lucorum* (Hymenoptera: Apidae). *Apidologie*, 41, pp.45-53.

Wolfe L.M., 1992. Why does the size of reproductive structures decline through time in *Hydrophyllum appendiculatum* (Hydrophyllaceae)? Developmental constraints vs. resource limitation. *American Journal of Botany*, 79, 1286-90.

Zeng, Y.-F.; Bai, W.-N.; Zhou, Y. & Zhang, D.-Y., 2009. Variation in floral sex allocation and reproductive success in sequentially flowering inflorescence of *Corydalis remota* var, *lineariloba* (Fumariaceae). *Journal of Integrative Plant Biology*, 51(3), pp.299-307.

Zhang, Z.-Q. & Li, Q.-J., 2008. Autonomous selfing provides reproductive assurance in an alpine ginger *Roscoea schneideriana* (Zingiberaceae). *Annals of Botany*, 102(4), pp.531-38.

Zhang, A.-Q.; Tan, D.-Y. & Zhu, J.-Z., 2008. The cross-fertilisation mechanism and reprodutive interference of self-pollen of Medicago varia 'Xinmu No. 1'. *Journal of Systematics and Evolution*, 46(6), pp.856-60.

Zhao, Z.-G.; Meng, J.-L.; Fan, B.-L. & Du, G.-Z., 2008. Reproductive patterns within racemes in protandrous *Aconitum gymanandrum* (Ranunculaceae): potential mechanism and amoung family variation. *Plant Systematics and Evolution*, 273, pp.247-56.