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THE ELECTRIC ECOLOGY OF BUMBLEBEES

CLARA MONTGOMERY

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Doctor of Philosophy in the Faculty of Life Sciences.

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ABSTRACT

The positive electric charge carried by bumblebees (*Bombus terrestris*) has been shown to affect the movement of pollen between bee and flower during pollination and is likely to affect the sensitivity of bumblebees to electrical stimuli. In this thesis, I investigate how bumblebees gain charge and how this charge affects their interactions with their environment. I show that bumblebees gain an electric charge during flight and when foraging on flowers by triboelectrification. I establish that bumblebees flying outdoors have similar charges to bees flying in the laboratory and further find that charge is affected by relative humidity. Validating previous models of pollen transfer, my measurements also demonstrate that the electric charge carried by bees in nature is sufficient to facilitate pollen transfer during pollination.

Electric charges are measured on the pollinivorous solitary bee species *Osmia bicornis* and on the social wasp *Vespula vulgaris*. *O. bicornis* is found to have similar magnitude positive charges and triboelectrical properties to *B. terrestris*. Positive charge is not found on *V. vulgaris,* suggesting that charge generation is an adaptation for pollinivory. This finding supports the hypothesis that cuticular hairs constitute an adaptation enhancing electrostatic pollen transfer.

Mellitophilous flowers advertise their presence to bees using the emission of floral volatiles, with peak volatile emissions corresponding with the peak activity period of their pollinators. In *Petunia integrifolia*, electrical stimulation by touching the flower with a positively charged nylon ball causes an increase in volatile emissions when using charges representative of highly charged bees. In *Antirrhinum majus* MTP flowers charge presentation does not result in increased volatile emissions. Finally, I show that *P. integrifolia* flowers visited by charged bumblebees show an increase in volatile emissions, suggesting that bumblebee charge acts as an indicator of pollinator presence. Previously unappreciated, this electrostatic communication channel is proposed to be adaptive as it enables petunia flowers to coordinate their peak volatile emissions with the activity of their pollinators.

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Thanks to my family for their support and their confidence in me, and for hosting mason bees in the walls of their house.

Finally, thanks to my partner Joseph Greenwood, who has stood by me from my first undergraduate experiment to my 1000th attempt to shave a bumblebee.

DECLARATION

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author. SIGNED: .... DATE: ....

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1 INTRODUCTION

1.1 INSECTS, FLOWERS AND THE ROLE OF ELECTROSTATICS IN POLLINATION

The study of insect pollination, especially that of bees, has received a huge interest in recent years, due to the steady decline of pollinator populations and increasing fears about global food security. Zoophily (predominantly entomophily) is either essential or beneficial for 85% of the 107 most important crop species (Klein et al. 2006) yet insect populations are declining worldwide with insect numbers decreasing 75% in the last 27 years (Hallmann et al. 2017). As prolific pollinators the decline in bees specifically has been of great concern, with the reduction in numbers thought to be due to increased pressures such as habitat loss, disease, parasites, pesticide use and lack of available food resources (Goulson, Lye, and Darvill 2008; Potts et al. 2010).

Being sessile, plants are dependent on biological and environmental vectors to disperse their gametes and successfully reproduce. For successful sexual reproduction in most angiosperms, the pollen on a flower must move from the anthers of that flower to the stigma of another. Multiple strategies have evolved to optimise the transport of pollen between flowers. Environmental pollination agents comprise wind (anemophily) and water (hydrophily), wherein pollen is produced in great quantities and carried by the wind or water to receptive conspecific flowers. Biotic agents of pollination include bats and birds but by far the greatest pollinators are insects (Klein et al. 2006). Environmental vectors are typically highly inaccurate, requiring the production of huge volumes of pollen, few of which will reach receptive flowers. Animal pollination is much more effective and targeted, with nectivorous and pollinivorous insects readily transferring pollen between flowers as they forage. Flowers exhibit vibrant signals such as bright colours and attractive scent to attract receptive insects and to advertise their pollen and nectar availability. Floral morphology is often optimised to maximise the pollen transferred onto the insect as it squeezes past stamens to reach the nectar at the base of the flower. A pollen-bearing pollinator then visits other flowers, often of the same species. As the insect lands and attempts to access the nectar, some of the pollen it is carrying is transferred to the stigma of the receptive flower. Some insects, especially bees, exhibit floral constancy where they preferentially visit one flower type (Wilson and Stine 1996). These are especially valuable pollinators as pollen they carry is more likely to reach a receptive flower of the same species, increasing the probability of successful pollination.

The movement of small particles such as pollen and powders is affected by electrostatics. Electrically charged particles in an electric field are subject to a Coulomb force. Coulomb's law dictates that like charges will be repelled away from each other whilst opposite charges attract. As pollen grains are small and light they can be easily and precisely directed by the application of an electric field. This directional application of Coulomb force to small particles is used in technologies such as photocopying, where charged ink particles are very accurately attracted to specific areas on paper. Given that the distribution of electric charge on a surface has such a profound effect on the movement of charged particles, it is not surprising that the electric environment provided by plants and pollinators has been found to vastly affect the movement of pollen during insect pollination (Corbet et al. 1982; Vaknin et al. 2000; Armbruster 2001; Vaknin 2001c; Clarke, Morley, and Robert 2017).

Entomophilic flowers often exhibit highly specialised morphologies for pollinator attraction and pollen transfer (Willmer 2011). This optimised morphology extends to their electric profile, with the shape of many flowers providing the strongest electric field around the stigma and the edges of petals (Clarke et al. 2013). Airborne or insect-borne pollen is attracted to these sites, increasing the efficiency of pollination by reducing the amount of pollen lost to the environment. Knowledge of the shape and strength of the floral electric field has been used to design charged pollen sprays, which allow the deposition of electrostatically charged pollen on flowers, increasing the efficiency of mechanical pollination on an agricultural scale (Vaknin et al., 2000; reviewed by Law 2001). Increasing the electrostatic charge on pollen grains has been shown to increase deposition on the stigma of receptive flowers and to enhance seed-set in experimental trials (Law et al. 2000; Vaknin et al. 2000; 2001a; 2001b). This electrostatic interplay has also been used for the targeted application of pesticides, allowing reduced waste and reduced agricultural runoff (Law 1983). Alongside the interest into the electrostatics of pollen movement, researchers began investigating the electric charge of pollinators. It was suggested (Erickson 1975) and then established (Corbet et al. 1982; Gan-Mor et al. 1995) that a charged pollinator could attract pollen from the stamens of a flower and transfer it more efficiently to the stigma of subsequent plants. Additionally, many pollinators are pollinivorous, and an electric charge would facilitate the transportation of significant quantities of pollen back to the colony for consumption.

Research of electrically charged insects has received periodic attention over the last 100 years, with the earliest measurements of insect charging published in 1929 by Heuschmann (Heuschmann 1929). Since then, the role of electric charging in insect pollination has been studied infrequently, with insects consistently exhibiting a weakly positive electric charge but with little knowledge of how this charge was generated or to what extent this charge affected pollen transfer. Recently, interest has been renewed with the discovery that bumblebees and honeybees could detect weak electrostatic fields (Clarke et al. 2013; Greggers et al. 2013). This sparked debate as to the roles of electrostatics in plant-pollinator communication, with bumblebees proposed to sense the strength and shape of the electric fields surrounding flowers and to use this information as a floral cue. In both bumblebees and honeybees the proposed sensory mechanism for electroreception was the deflection of mechanosensory structures; hairs in bumblebees (Sutton et al. 2016), antennae in honeybees (Es'kov and Sapozhnikov 1976; Greggers et al. 2013) in an electric field following Coulomb’s law. The electric force acting on the sensory structures is proportional to the strength of the electric field and the electric charge of the structure. Thus the electric charge on insects was proposed to have a secondary function, with the sensitivity to electrical stimuli increasing with the electric charge on the insect (Sutton et al. 2016). Insect electric charge therefore both affects the pollination efficiency of plants and is proposed to affect the sensitivity of insects to electric stimuli. With the natural electric environment in which bees forage now being heavily influenced by anthropogenic activity such as the construction of power lines, research into the role of electrostatics in insect ecology is of great value for agriculture and conservation ecology.

1.2 MEASUREMENTS OF INSECT ELECTRIC CHARGE

Electric charges on honeybees and other insects have been measured periodically over the last century using a wide range of techniques, and insects have typically been found to be positively charged (summarised in Table 1.1) although the exact mechanisms of insect charging have remained elusive. In the only field-based measurements of insect charge by Colin and colleagues in 1991, honeybees were found to be positively charged when flying back to the colony, but to also have an elevated positive charge when overwintering within the colony, suggesting charging within the hive (Colin, Richard, and Chauzy 1991). Honeybees were also positively charged during flight, with tethered flying honeybees being more positively charged than bees swung by a motor (Gan-Mor et al. 1995), suggesting flight is integral to charge generation. However tethered honeybees flying in a wind tunnel did not gain charge as they flew, with bee charge remaining steady over several minutes of flight (Lighthart, Prier, and Bromenshenk 2005). One suggested method by which insects could gain charge was by friction with materials they encountered, known as triboelectric charging. Flies, beetles and moths became positively charged when rubbed with various materials but became negatively charged when rubbed with asbestos (Edwards 1962; Table 1.1). Flies were also found to become charged when walking over most materials (McGonigle, Jackson, and Davidson 2002; Jackson and McGonigle 2005).

Table 1.1. A comparison of the charges previously measured on insects in the literature

	Insect	Mean charge (pC)	SD (pC)	Context	N	Citation
Hymenoptera	*Bombus terrestris*	+32	35	Free flight into Faraday Pail	51	Clarke et al., 2013
	Apis melifera	+28.6 foraging +153.2 wintering	39.5 104.8	Measurement at hive entrance	339 352	Colin, Richard and Chauzy, 1991
	Apis melifera (flying)	+23.1	9.7	Tethered flight	11	Gan-Mor et al., 1995
	Apis mellifera (flying)	+7.04	5.62	Tethered flight	16	Lighthart et al., 2005
	Apis melifera (reconnoitring)	+45.2	4.3	Bees touched with probe or flew into measuring chamber	n/a	Es'kov and Sapozhnikov, 1976
	Apis mellifera (walking on materials)	+1.6 pC (Walking on tin plate) to +11.7 pC (Walking on wool)	0.1 to 1.3	Walking along 5cm of tin, silk, paper, glass, wool or bees wax.	n/a	Es'kov and Sapozhnikov, 1976
Diptera	*Calliphora vomitoria*	Positive (negative on asbestos)	n/a	Insect lightly rubbed on an asbestos, glass, Mica, wood or metal surface.	1	Edwards, 1962
	Uramyia halisidatae	Positive (negative on asbestos)	n/a	“	1	Edwards, 1962
	Rileymia americana	Positive (negative on asbestos)	n/a	Walking on dielectric materials	1	Edwards, 1962
	Musca domestica	-15 to +50	n/a		200	McGonigle et al., 2002
	Musca domestica	-12.7 on Acrylic -6.02 on Perspex +2.34 on Acetal +9.10 on Polypropylene +17.92 on PVC	0.71 1.29 0.32 0.75 1.46	Walking on dielectric materials	10	McGonigle and Jackson, 2002
	Musca domestica	Positive	n/a	Walking on dielectric materials	4	Jackson and McGonigle, 2005

Coleoptera	*Galerucella punctipennis*	Positive (negative on asbestos)	n/a	Insect lightly rubbed on a glass, Mica, wood or metal surface.	1	Edwards, 1962
Lepidoptera	*Lambdina somniaria*	Positive (negative on asbestos)	n/a	Insect lightly rubbed on a glass, Mica, wood or metal surface.	1	Edwards, 1962
	Fellia ducens	Positive	n/a	Insect lightly rubbed on a glass, Mica, wood or metal surface.	1	Edwards, 1962
	Halisidota m. angulifera	Positive (negative wings on asbestos)	n/a		1	Edwards, 1962
	Otographa californica	Positive (negative wings on asbestos)	n/a		1	Edwards, 1962

The electric charge on bumblebees has only recently been measured, with the use of calibrated Faraday pails allowing accurate measurements (Clarke et al. 2013). Bumblebees are required for the pollination of some plant species where the pollen can only be released by sonication (buzz pollination). This is a pollen release mechanism specific to some plant species whereby a bee grasps the flower and rapidly vibrates its indirect flight muscles, shaking pollen loose from the tip of the stamens. This has been theorised to cause charging in pollen (Corbet and Huang 2014), and Dr Erica Morley and I have found firstly that this pollen released is predominantly negatively charged and secondly that it is attracted to a nearby positively charged bee (Morley, Montgomery and Robert under review).

So far no measurements exist of the electric charge on solitary bee species. Electric charge may hold significant adaptive value for these bees as they are often highly pollinivorous and transport pollen via hairs on the underside of the abdomen rather than in corbiculae (Thorp 1979), so could vastly benefit from increased electrostatic adhesion of pollen. Some solitary bees, such as mason bees, are also noted as being much more efficient pollinators than honeybees and often bumblebees (Willmer, Cunnold and Ballantyne, 2017), and whilst their efficiency is likely to be related to their morphology and behaviour, it may also be facilitated by an elevated electric charge. Thus, one of the aims of this thesis is to expand the phylogenetic range of insect triboelectric behaviour and measure the electric charge on solitary pollinivorous bee species.

1.3 THE ELECTRICAL ENVIRONMENT

In order to understand the role of electrostatics in pollination it is necessary to understand the electrical environment in which these interactions take place. Electrostatics influences the relationship between bees and flower, but this relationship as well as the potential of both bees and flowers is influenced by electrical conditions such as the potential in the surrounding air. Bumblebees have been found to be able to detect the electric fields typical of those surrounding flowers (Clarke et al. 2013). These floral electric fields are dependent on the potential of the surrounding air as well as the charge on the bumblebee. The charge on the bumblebee may likewise be affected by potential of the air and the electrical and physical properties of the flowers they visit (Figure 1.1). Thus the plant-insect system cannot be considered in isolation but must be placed within the context of the electrical landscape.

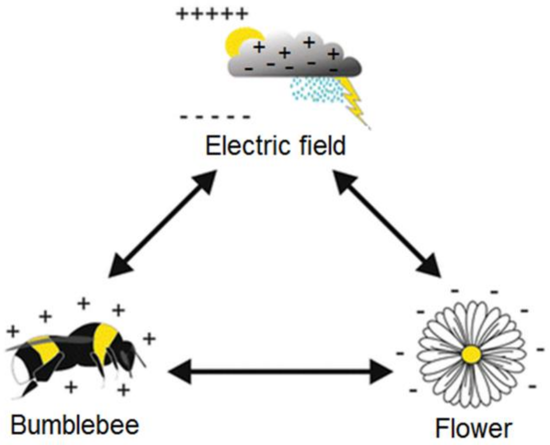

Figure 1.1. The electrical interactions between bees, flowers and the surrounding air cannot be studied in isolation as they all affect each other. From Clarke et al. (2017).

1.3.1 The Atmospheric Potential Gradient and the global electric circuit

The study of electroreception and the idea of an electric landscape has been historically concerned with marine environments. However, due to its mostly insulating nature, air can sustain huge electric potentials, and an electric field surrounds the earth due to the difference in potential between the earth and the conductive upper atmosphere. This potential difference is maintained by thunderstorms, which carry huge negative charges to the earth. During fair weather (dry, cloudless, low wind), the earth discharges via the vertical movement of charged ions, causing a current in the air. The movement of charge between the earth and the upper atmosphere is called the global electric circuit (Rycroft et al. 2000; 2008). The difference in potential between the earth and the ionosphere creates a vertical electric field (Figure 1.2). This is known as the atmospheric potential gradient (APG) and fluctuates according to local meteorological conditions. The APG also exhibits a curious daily fluctuation called the Carnegie curve, where the APG reaches its maximum at 19UTC and minimum at 03UTC regardless of global location. This is thought to correspond to the average daily frequency of global lightning strikes (Harrison 2013).

On a fair weather day, the APG is around 100 V/m (Bennett and Harrison 2008) with the potential in the air increasing by 100V with every 1m of altitude (Figure 1.2). Over a flat featureless landscape the APG will be a uniform electric field with parallel equipotential lines, as shown in Figure 1.2. The electric field is distorted by the presence of protruding objects. When a conductive object protrudes from the earth, an electric field occurs around the object due to the difference in potential between the structure and the surrounding air. For example, a 1.7m tall person standing in a fair-weather field would have a difference in potential between their head and the surrounding air of 170V. This resulting electric field is strongest around points of greatest charge, and is dependent on the shape and conductivity of the protruding object.

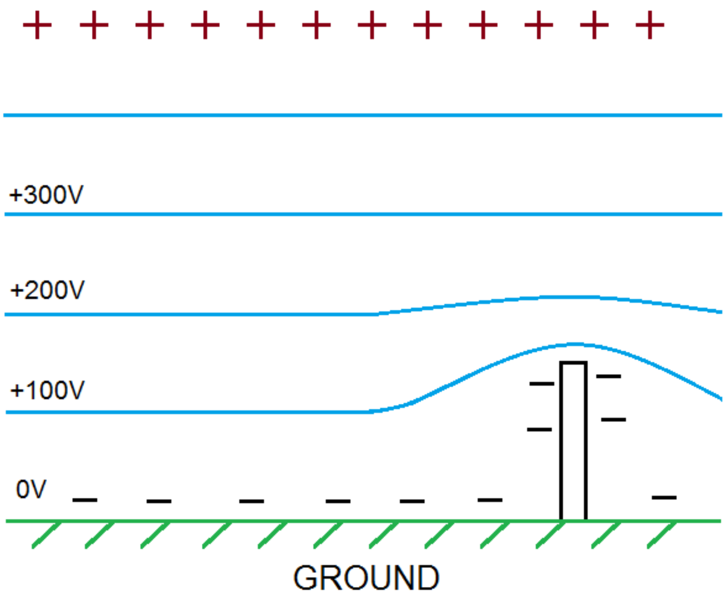

Figure 1.2 The APG results from the difference in potential between the earth and the upper atmosphere. Blue lines show equipotential field lines. On a fair-weather day on a flat landscape, the APG is around 100V/m. When a conductive object protrudes from the earth, the difference in potential between the top of the object and the surrounding air creates an electric field, shown by closer field lines.

1.3.2 The electric fields surrounding flowers

A flower protruding from the earth contains a conductive stem (Corbet et al. 1982; Baluška et al. 2006). As such, an electric field arises around the top of tall, conducting flowers due to the difference in potential between the top of the flower and the surrounding air. In 2013, Dominic Clarke and colleagues modelled the electric field surrounding a 30cm tall flower in a fair weather field (Figure 1.3B; Clarke et al. 2013). Assuming the flower is electrically grounded, the difference in potential between the top of the flower and the surrounding air is 30V. The positive potential in the air attracts negative charge to the surface of the flower, giving the top of the flower a relatively negative potential. The potential is not uniformly distributed, and the electric field strength will be strongest around protruding points (Figure 1.3B).

An electric field exerts a force on a charged object following Coulomb's law. Electrically charged paint powder sprayed on grounded flowers shows the shape of the floral electric field, where maximum deposition shows the greatest field strength (Figure 1.3A; Clarke et al. 2013). The greatest field strength is around the edges of petals, and around the reproductive structures in the centre. Positively charged pollen carried by an approaching pollinator would be attracted to these areas of greatest field strength.

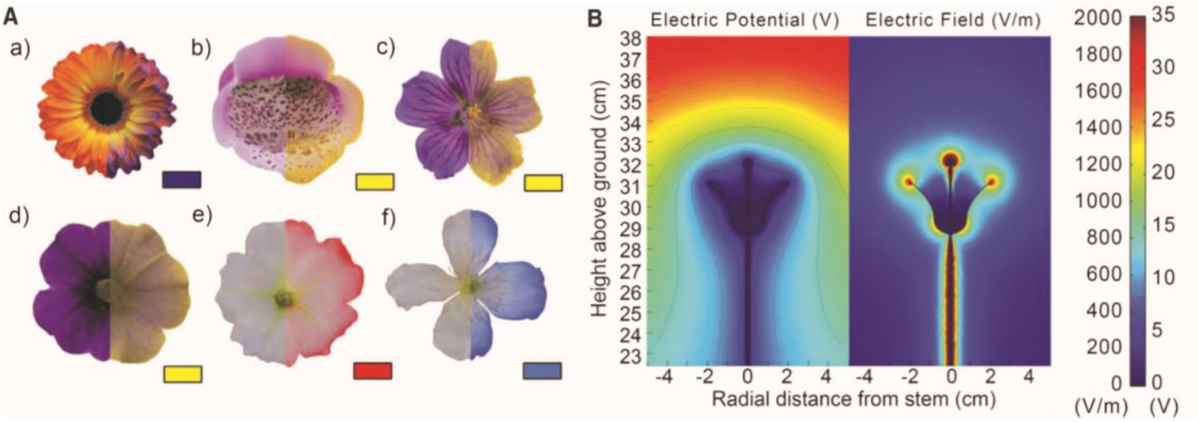

Figure 1.3. A) The shape of the floral electric field can be visualised by spraying positively charged paint powder at grounded flowers. High deposition indicates stronger field. (a) *Gerbera hybrida*, (b) *Digitalis purpurea*, (c) *Geranium magnificum*, (d) *Calibrachoa hybrida*, (e) *Petunia hybrida,* (f) *Clematis armandii*. B) A finite element model showing the potential in the air surrounding a conductive 30cm tall flower (left) and the electric field strength that results from this difference in potential (right). From Clarke et al. (2013).

The shape of the floral electric field has been suggested to have influenced the evolution of floral morphology (Armbruster 2001; Vaknin et al. 2001c; Vaknin 2009). Flowers with protruding stigma and anthers are readily found in nature (Figure 1.4). Protruding stigma have been found to readily attract airborne pollen with the extent of attraction increasing with style length (Vaknin et al. 2001c). Additionally stigma are more electrically conductive than surrounding floral structures (Corbet et al. 1982) and this low impedance pathway to the ground affects the electric field around the flower, strengthening the attraction of charged pollen towards the stigma. Exposed stigma and/or stamens are found across many plant genera of both anemophilous and zoophilous species, supporting the suggestion that some flower species have evolved a morphology to direct the pollen to and from sexual organs using Coulomb's force. This is evident in hummingbird pollinated hibiscus flowers (Figure 1.4 A, B) where it has been found that hummingbirds generate strong electric charges during flight (Badger et al. 2015), thus potentially facilitating the movement of pollen during pollination.

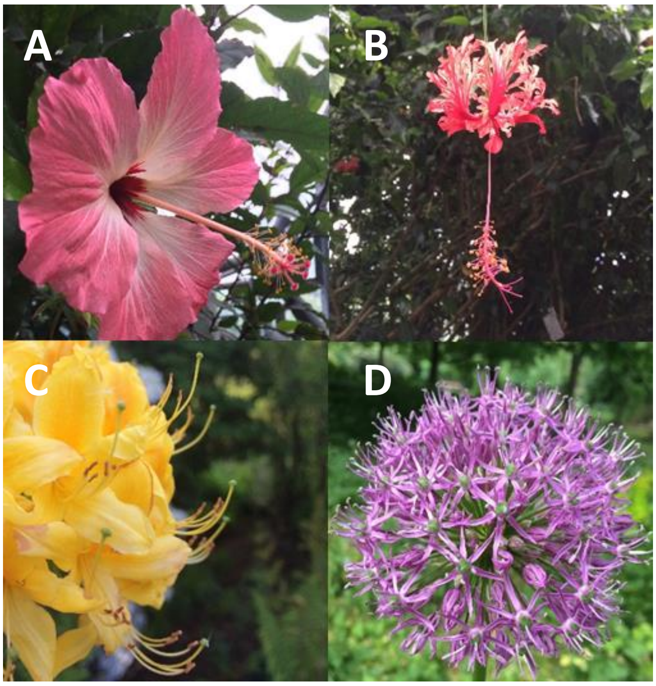

Figure 1.4. The protruding pistils and stamens of various floral species: A) *Hibiscus striatus*, B) *Hibiscus schizopetalus*. Hibiscus flowers are pollinated by insects and hummingbirds. C) *Rhododendron* 'Morris' hybrid, D) *Allium sp.*, Rhododendron and Allium are pollinated by insects, particularly bees.

1.3.3 The electric charge of pollen grains

Due to their tiny size and mass, small objects such as powders and pollen are disproportionately affected by electric fields as they can be propelled by relatively weak electric fields even if the charge upon each grain is very low. Bowker and Crenshaw (2007a) measured charges of a few femtocoulombs on pollen grains, but these relatively small charges were shown to be sufficient to move pollen grains in a biologically relevant electric field (Bowker and Crenshaw 2007a). As presented above, the morphology of a flower affects the shape and strength of the floral electric field, and in turn can increase the capture radius of airborne pollen. Bowker and Crenshaw showed that a charge of 1fC would be sufficient for a 10µm radius pollen grain to be captured by a plant over a 2mm distance (Bowker and Crenshaw 2007b). Working with Dr. E. Morley, I showed that pollen grains propelled from a vibrated tomato flower were attracted towards a nearby positively charged bee or charged metal plate. By filming the movement of pollen grains falling from a tomato flower in a horizontal electric field, we were able to reveal that the loss or gain of a single unitary charge is sufficient to affect the movement of a pollen grain in an electric field (Morley, Montgomery and Robert, under review). This demonstrates the very high sensitivity of pollen transfer behaviour to electric charge.

1.3.4 Bee charge and pollen transfer

After the initial discovery that pollen was negatively charged (McWilliam 1959), scientific attention turned to the electric charges on pollinators. Flying bees have been shown in a variety of laboratory and field experiments to be positively charged (Colin, Richard, and Chauzy 1991; Gan-Mor et al. 1995; Clarke et al. 2013; summarised in Table 1.1). An incoming positively charged bumblebee will induce a negative charge on the surface of the flower as it approaches, further strengthening the electric field surrounding the flower (Figure 1.5). This electric induction generates an electric field that facilitates the transfer of electrically charged pollen between bee and flower. As the bee approaches, negatively charged pollen on the flower will be attracted to the positively charged bee. Over short distances of a few millimetres this attraction can be strong enough to act against gravity, causing the pollen to jump from flower to bee. After visiting the flower, the bee flies on to forage on subsequent flowers. During flight, an interesting process takes place; the pollen attached to the bee equalises with the charge of the bee, giving the pollen a positive charge. When the bee approaches a subsequent flower the now positively charged pollen will be attracted towards the flower, specifically towards the parts of the flower with the strongest electric field such as the stigma. This can cause bidirectional movement of pollen when a bee approaches a flower, with pollen on the bee being attracted to the flower, and pollen already on the flower being attracted to the bee (Clarke et al. 2017).

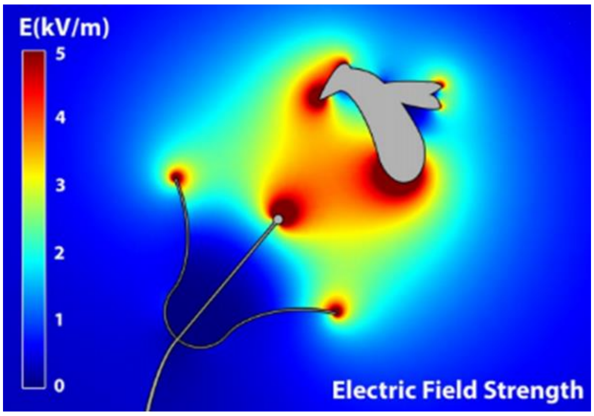

Figure 1.5. A modelled visualisation of the electric field between a positively charged bumblebee and a grounded flower. From Clarke et al. (2017).

The surface of the stigma is optimised for pollen capture and adhesion, often having a wet fluid secretion at the surface optimised to trap pollen upon contact (Heslop-Harrison and Shivanna 1977). Pollen of the same species readily produces pollen tubes furthering their adhesion to the stigma, whilst foreign pollen of a different species does not adhere as strongly and is more likely to be dislodged (Zinkl et al. 1999). The anthers are specialised to release pollen readily when mature, and insect pollinated flowers readily present pollen for dispersal by insects either from the stamens or from secondary structures (Howell, Slater, and Knox 1993). Presented pollen is easily dislodged, and flowers can stagger the release of this pollen to ensure it reaches as many flowers as possible. Due to the specialised structure of the stigma and the anthers, the forces required to dislodge pollen from the anthers are much lower than those required to dislodge pollen from the stigma. Thus, an incoming bee is likely to attract pollen from the anthers or secondary structures due to the weaker forces holding it in place and is less likely to attract pollen from the stigma where adhesion is stronger. In this way, the electric charge on a pollinator can vastly improve the reproductive success of a plant by facilitating the movement of pollen from the anthers of one plant to the stigma of another.

The bee also benefits from the electrostatic adhesion of pollen. For many species, pollen forms an integral part of the diet, providing vital protein for larvae and adults of honeybees (Brodschneider and Crailsheim 2010), bumblebees (Roger et al. 2017) and many solitary species (Cane 2016). In some social species such as bumblebees and honeybees, foragers have specialised baskets on their hind legs called corbiculae into which they groom the pollen (Thorp 1979). In solitary species pollen is often carried by specialised hairs on the ventral abdominal surface called scopa (Thorp 1979). Bee morphology is not uniform, and just as the floral electric field is strongest around protruding points, the distribution of pollen over the bee will be likewise heterogenous. On honeybees and bumblebees pollen adhering to the bee body can be groomed into corbiculae and carried to the colony for consumption. However, there are also safe spots predominantly on the dorsal surface where the bee cannot reach to groom the pollen (Willmer 2011; Koch, Lunau, and Wester 2017). These are of great importance in pollination, as the grains are not consumed but are transferred between flowers and are readily brushed off upon contact with floral structures. Pollen grains attach via exine spines or pollenkitt (Amador et al. 2017; Konzmann, Koethe, and Lunau 2019) and their transportation may be enhanced by electrostatic adhesion (Vaknin et al. 2000). The electrostatic attraction of pollen to the bee therefore has adaptive value to both plant and pollinator by increasing the pollen burden of the bee, hence increasing both the amount of pollen that reaches subsequent flowers culminating in successful pollination, and the amount of pollen that reaches the colony, providing sustenance for developing larvae.

1.4 ELECTRORECEPTION

In sensory ecology, we must discard our anthropogenic viewpoint and acknowledge that other organisms do not perceive the world as we do. Insects especially possess an astonishing variety of sensory modalities, allowing them to perceive stimuli completely undetectable to humans. Examples of sensory modalities alien to humans, but rife in insects include sensitivity to infra-red radiation (Evans 1966), ultraviolet light (Chittka et al. 1994), polarised light (Menzel and Snyder 1974), local humidity (Enjin 2017), and electric stimuli (Clarke et al. 2013; Greggers et al. 2013). It is hard to detect how anthropogenic activity affects modalities we cannot sense, yet these hidden effects are equally worthy of consideration in pollinator ecology. Consideration of the human impact on the electric landscape is important as insects have been shown to change their behaviour in the presence of a strong electric field, such as those surrounding high voltage power lines (Orlov 1990). Cockroaches avoid areas of intense field strengths (Newland et al. 2008; Jackson et al. 2011) while flies reduce their activity in the presence of an electric field (Edwards 1960). Matsuda and colleagues found a variety of insects avoided strong electric fields including 62 species from 13 different orders (Matsuda et al. 2015). Electric fields have hence been proposed as a method of pest control (Nonomura et al. 2012; Matsuda et al. 2015). Strong electric fields such as those surrounding power lines have been shown to affect honeybee behaviour (Bindokas et al. 1989) as well as the overall health of the colony (Greenberg et al. 1981; Bindokas et al. 1988).

The sensitivity of some insects to electrical stimuli was initially surprising as electroreception had historically been limited to marine and aquatic organisms, and electroreceptive mammals (some monotremes such as platypus and echidna) could only detect electrical stimuli underwater or in substrates with very high water content. The relatively insulating properties of air were perceived to be a barrier for aerial electroreception, as electroreception in marine organisms typically results from specialised conductive electroreceptive organs such as the ampullae of Lorenzini (Murray

1974). As conductive electroreceptive organs of this type were absent in insects, an alternative electroreceptive sensor had to be identified.

1.4.1 Electroreception in bumblebees

In 2013, Dominic Clarke and colleagues successfully trained bumblebees to discriminate between identical rewarding and unrewarding feeding disks that differed solely on their electric field. Rewarding disks containing sugar solution were given a 30V potential, whilst unrewarding disks containing quinine were given a 0V potential. The 30V potential was chosen to be representative of a 30cm tall flower in a fair-weather field. Over the course of 50 trials the bees learned to visit the 30V disks with an 80% success rate (Figure 1.6A; Clarke et al. 2013). Upon removal of the electric stimulus the bees reverted to random choice (Figure 1.6A). This learning can be observed with bees presented with the choice between 30V and 0V flowers, but not 10V and 0V, suggesting a threshold potential is required for electroreception. This study showed conclusively that bumblebees could make foraging decisions based purely on electrical stimuli. The authors went further and showed that the addition of an electric field allowed bees to more easily discriminate between two very similar colours. Bees were also shown to be able to distinguish between 30V flowers with different electric field shapes (bullseye vs uniform). These experiments suggest that the floral electric field acts as a floral cue, potentially facilitating the identification of rewarding flowers by bumblebees.

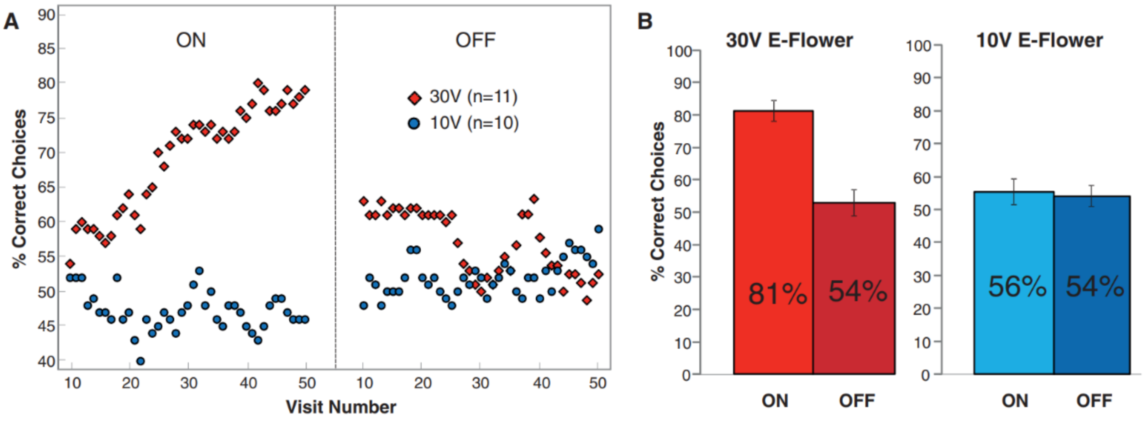

Figure 1.6. A) The learning curves of bumblebees trained to visit 30V disks (red diamonds) or 10V disks (blue circles) during training (voltage on) and control (voltage off). B) The mean correct choices to 30V and 10V disks of the last 10 bee visits. Error bars show SEM. From Clarke et al. (2013).

1.4.2 Proposed sensory mechanism – mechanosensory hairs

Several candidate mechanoreceptors were proposed as potential electroreceptors in bumblebees including the antennae and the mechanoreceptive hairs on the head of the bee. Gregory Sutton and colleagues showed that the hairs were deflected by a biologically relevant electric field, and that the deflection of these hairs resulted in electrical impulses in nerves at the base of the hairs (Sutton et al. 2016). This finding was not replicated with the antennae, which required much higher electric field strengths for deflection. Thus, the hairs on the back of the head were postulated to be both mechanoreceptive and electroreceptive. Coulomb's law dictates a charged body in an electric field will experience an electrical force. In the bee hair system, the hair is deflected in an electric field due to the charge on the hair, and the extent of deflection will increase as the charge on the hair increases (Sutton et al. 2016). Thus, the more highly charged the bee, the more sensitive the bee will be to electric stimuli. The extent that bumblebees rely on electrical information to make foraging decisions in nature is currently unknown. However, it can be proposed that bee charge holds adaptive value by increasing the bee’s sensitivity to otherwise undetectable stimuli, giving electroreceptive bees an advantage by facilitating the identification of rewarding flowers.

1.4.3 Electroreception in honeybees

Honeybees have also been repeatedly shown to be positively charged (Es'kov and Sapozhnikov 1976; Colin, Richard, and Chauzy 1991; Gan-Mor et al. 1995). Honeybees communicate the location and distance of potential foraging resources to each other using a series of movements known as a waggle dance (Von Frisch 1974). In this, they move in a figure of eight, where the direction of the dance indicates direction from the sun, and the duration of the dance indicates distance. Dancing bees emit physical, audible and airborne signals via the vibration of the thorax and wings (Wenner 1962). Greggers et al (2013) showed that a dancing honeybee also emitted an electrical signal, which corresponded to its movement and its charge, suggesting that bees may use electrical signals for communication within the colony (Es’kov and Sapozhnikov 1976; Greggers et al. 2013).

1.4.4 Proposed receptors – antennae

The air vibrations and the electric field produced by the dancing bee both induced a response in nearby listening bees (Greggers et al. 2013). Movement of the antennae were found to stimulate the Johnston’s organ and honeybees were found to also respond to biologically relevant electrical stimuli via the proboscis extension reflex. In these honeybees, the mechanical deflection of the antennae is responsible for the electric sensitivity. Thus, a higher charge on the bee will allow greater sensitivity to electro-mechanical stimulation, potentially allowing the bee to be more receptive to learning the location of new foraging areas. The benefits of electric charge are therefore not limited to foraging bumblebees but may affect communication in honeybees within the hive. Honeybees within the hive have been previously found to be more positively charged than those out foraging (Colin, Richard, and Chauzy 1991), suggesting that charging mechanisms may differ between bee species and be tailored to the situation, where bumblebees may benefit more from charge during foraging and honeybees may benefit more within the hive.

1.5 OTHER POTENTIAL ROLES OF ELECTRIC CHARGE AND ELECTRORECEPTION

Both electromechanical sensors (antennae in honeybees, hairs in bumblebees) are deflected in an electric field due to Coulomb force. The sensitivity of these insects to electrical stimuli is therefore affected by their electric charge. As both honeybees and bumblebees are pollinivorous, they also benefit from being electrically charged via the increased electrostatic attraction and adhesion of pollen. In addition to this, there are a number of potential effects of bumblebee charge, both advantageous and disadvantageous to both bee and flower that merit consideration.

Increased bee charge potentially allows increased sensitivity to electric fields due to increased Coulomb force acting on electromechanical sensors. This may benefit foraging bumblebees who are thought to use electroreception to identify rewarding flowers when foraging but it may have other functions. This could include sensing incoming bad weather, as honeybee activity is known to increase the day before rain (He et al. 2016). Electroreception may also affect predator-prey insect interactions. Electroreceptive predators may readily detect charged prey and electroreceptive prey may more easily detect charged predators. A greater electric charge increases sensitivity to electrical stimuli but also increases conspicuousness, leading to a greater risk of detection by electrosensitive insects. Despite the potential functions of electroreception and electric charge in insect trophic interactions, the extent of electroreception in different insects remains largely unexplored.

Insect charge may affect insects independently of electroreception. Whilst increasing pollen attraction and adhesion is of use to bees, a charged insect will also attract any other small, charged particles. This can include dust, soot and other particulates, which may come with an associated cost of increased cleaning. Charged insects can attract and accumulate airborne pathogenic fungal spores and bacteria leading to a fitness cost. Honeybees flying in a wind tunnel were found to attract and adsorb airborne bacterial spores and viruses with the rate of adsorption increasing with the charge on the bee (Lighthart et al. 2000; Prier, Lighthart, and Bromenshenk 2001; 2005). There may therefore be an evolutionary trade-off to being electrically charged, where the extents of the benefits and risks are specific to the ecology of individual species.

When considering the electrical interaction between bees and flowers we must also consider the effect on the flower. As sessile organisms, plants must sense and respond to their environment in order to survive. Insect pollinated plants need to attract insects to reproduce. They do this using a variety of cues that can be used to indicate nectar availability, including colour and scent, with the period of scent emissions corresponding with the peak activity of their pollinators. In some plants this is due to an internal circadian rhythm e.g. snapdragons (Kolosova et al. 2001), but in others it is a response to environmental factors e.g. *Petunia integrifolia* (Hoballah et al. 2005) such as light intensity and temperature which are indicative of good foraging conditions and hence correlate with an abundance of pollinators. Recently evidence has surfaced that plants may be able to sense the presence of their pollinators directly, rather than having to rely on environmental or temporal cues. Flowers were shown to increase nectar sweetness in response to vibrations produced by flying insects, potentially rewarding floral constancy and further attracting subsequent pollinators (Veits et al. 2019). This direct sensing raises the question of whether bumblebee charge acts as an indicator of pollinator presence, providing a cue for flowers to correlate their emissions with pollinator abundance.

Physiological changes in plants can be mediated and stimulated by electrical potentials within the stem in response to stimulation such as touch, rain, wounding and bending amongst other stimuli including electrical stimulation (Stahlberg, Cleland, and Van Volkenburgh 2006; Volkov 2017). The application of external electric fields to various plant species has resulted in a variety of effects such as increased growth rate (Mizuguchi et al. 1994), increased synthesis of compounds (Inaba et al. 1995) and physical movements in carnivorous species (Volkov et al. 2007; reviewed in Volkov 2017). A charged bee landing on a flower causes an increase in potential within the stem (Clarke et al. 2013). For the plant, this could indicate the presence of a charged pollinator. This information may be useful to a plant by allowing it to sense the presence of its pollinators and to more strongly correlate its volatile emission period with the foraging activity of relevant pollinators.

A second potential interaction between insect charge and floral scent was proposed by Erickson (1982) who found that dust accumulated around the edges of placoid sensilla on the antennae of dead bumblebees. Erickson theorised this was due to the presence of polar lipids around the sensilla acting as a charged electret and attracting airborne particulates. This charge would attract polarised or charged odour molecules, increasing the sensitivity of the sensilla. A charged insect therefore attracts oppositely charged pollen from a flower but may also attract odorant volatiles, thereby benefitting from enhanced chemosensitivity, facilitating the identification and localisation of rewarding flowers. These two proposed interactions between charge and scent may both occur: A charged bee may induce an increase in the emissions of floral volatiles to which it has increased sensitivity due to its charged sensilla. Thus the electrostatically mediated release of floral volatiles would be subsequently rewarded by an increase in visits from charged, chemoreceptive pollinators.

1.6 IDENTIFICATION OF KNOWLEDGE GAPS (AIMS OF THE THESIS)

Despite the perceived advantages of being electrically charged, very little is known about how bees gain their charge. Bees are said to gain charge during flight, although there is conflicting evidence to support this. Bees may also gain charge by triboelectrification due to friction between themselves and their surroundings. The investigations by Clarke and colleagues into the sensitivity of bees to electrical stimuli and the movement of pollen between bee and flower are all based on laboratory measurements of bumblebee charge. It is not currently known whether these charges are found on bees in nature, or to what extent these models are representative of the forces acting on pollen grains during pollination. Throughout this thesis, I fill some of these knowledge gaps. The questions addressed in this thesis are as follows:

1. How do bumblebees gain and maintain an electric charge? Do they charge during flight? Do their triboelectric properties make them gain charge whilst foraging? How well do they retain their charge?
2. Are laboratory measurements of bumblebee charge representative of the charges on bumblebees in nature? Are the charges on bumblebees flying outdoors influenced by weather conditions?
3. Are the triboelectric properties and the electric charges measured on bumblebees representative of bees as a whole? Is electric charging a unique feature of pollinating or pollinivorous insect species? How ubiquitous is electric charging in insects?
4. Does electrostatics affect plant-pollinator interaction and communication? Specifically, does bumblebee charge affect floral volatile emissions?

The ultimate aim of this thesis is to investigate the role of insect charge in pollination ecology using a range of novel and established techniques to measure the charge on flying bees under different conditions. Using these techniques I will identify potential mechanisms of charging in bumblebees and investigate the charge of bumblebees under natural conditions. For the first time, I will extend charge measurements to a species of solitary bee and demonstrate that electric charge may play a wider role in the ecology of pollinators and the process of pollination than previously thought. Finally I will investigate how the electric charge on bumblebees could affect the emission of bee-attractive volatiles from flowering plants, providing a dynamic role for electric charge in plant-pollinator communication. Together, these investigations will further current knowledge of the generation of electric charge in pollinators, and explore its potential ecological functions.

2 THE PHYSICS AND BACKGROUND OF ELECTROSTATIC CHARGING AND CHARGE MEASUREMENT

2.1 BACKGROUND TO STATIC ELECTRICITY

Electrostatics is the study of the electric charge of electrically insulating materials. The study of electrostatics has largely been driven by the desire to understand and often reduce the accumulation of electric charge in materials. In industry, the static build-up of electric charge can have catastrophic consequences, resulting in fires and explosions (Glor, 2013). However, electrostatics also has useful applications and is used in technology such as spray painting and photocopying. Although much of the study of electrics is based around the study of flow of charge and exploitation of electrically conductive materials (commonly known as electrodynamics), the earliest studies were much more based around insulating materials.

The very first historical mentions of electrostatics are from Thales in 600BC who rubbed an amber rod with silk and found that small particles of straw were attracted to the rod (Cross 1987). In the 1500s William Gilbert (1544-1603) recorded this phenomenon from other materials and coined the term 'electrical' from the Greek word electron for amber. Gilbert found that some materials would become electrically charged by friction. Benjamin Franklin's recognition in 1752 of the link between electricity and lightning later sparked renewed interest and 30 years later the study of charge became quantitative with the formation of Coulomb's Law (1785). Charles-Augustin de Coulomb (1736-1806) observed that two charged balls suspended on a thread repelled each other, thus forming the first electroscope. The deflection between the balls could be used to measure the charge on each via Coulomb's Law. Coulomb's law states that the force *F* between two charged bodies obeys an inverse square law:

$$F = \frac{kq_1q_2}{d^2} \tag{2.1}$$

Where q_1 and q_2 are the charges on the balls, k is a constant and d is the distance between the balls.

The field changed in 1799 when Volta invented the battery and a flow of continuous current could be observed. Electricity then had applications, and the study of electromagnetism shortly followed. One problem that still remained unsolved was the question of how electrically charged objects could influence each other at distance without a conducting substance between them. Michael Faraday (1791-1867) conceptualized the idea of field lines acting in the space between charged bodies. These lines were defined as points where the direction of the line at any point is the same as the direction of the electrical force acting on a unit of charge in that space. Faraday also introduced the concept of the induction of electric charge (Faraday 1843). He proposed that insulating materials were comprised of many small, isolated conductors each containing a net neutral charge. He proposed that charge was able to move within these conductors but not between them. An external electric field would then separate the positive and negative charges within these conductors, polarising them. We now know this to be true, where the neutral conductors envisaged by Faraday are atoms containing positive protons and mobile, negative electrons (Thomson, 1897).

2.2 MECHANISMS OF CHARGING

Materials can gain or lose charge by a variety of mechanisms. A key distinction in material charging is whether a material has a net change in charge (i.e. that it has gained or lost charge) or whether its net charge is the same but that the charge distribution within the material has changed. These two methods are not mutually exclusive and a material may have a net change in charge as well as having a difference in charge distribution. Often a change in charge distribution is key to a change in net charge. An example of this would be where an insulating material has been polarized so one end is positive and the other negative. If an external conductor is brought against the negative end, negative charge will be conducted away and the material will be left with a net positive charge.

One of the main ways a material will gain a net charge is by contact charging and frictional charging (Cross 1987; Diaz and Felix-Navarro 2004). These are similar but distinct. Contact charging is the process where two materials are briefly touched together with no friction between the two. Frictional charging involves rubbing materials together and is commonly known as triboelectrification (Cross 1987).

2.3 TRIBOELECTRIFICATION

2.3.1 The triboelectric series

When two insulating materials are rubbed together, charge is exchanged and one material gains a more positive charge whilst the other becomes more negatively charged. Despite this phenomenon being first observed over 2000 years ago, the exact mechanism of this charge transfer is still unclear. The transfer of electric charge is likely to be due to an exchange of electrons, ions or nanoparticles between materials (Matsusaka et al. 2010; Williams 2012; reviewed in Pan and Zhang 2018). Whilst the charge carriers are unclear, so too are the reasons why a material is more likely to gain a positive or a negative charge. Nevertheless, materials can be ranked on their likelihood of gaining a positive or negative charge upon contact. This material ranking system is called the triboelectric series. Comparisons between triboelectric series show some consistencies and inconsistencies (reviewed by Diaz and Felix-Navarro 2004). These inconsistencies can often be attributed to the difference in methodologies between experiments, as well as different surface structures, as two identical materials with different surface structures may have very different triboelectric properties.

The charge carriers involved in triboelectric charging are still widely debated. One model is that charge transfer is due to an exchange of electrons between materials upon contact. This holds true for metals, where electrons are highly mobile and electrons will readily move from the metal with the lower work function to the one with the higher work function (Lacks and Sankaran 2011).
The triboelectric series shown in Figure 2.1 is an abbreviated series from McCarty and Whitesides (2008) showing the amalgamation of the series reviewed by Diaz and Felix-Navarro (2004). Assuming the electron transfer model, the tendency to attract electrons (and hence gain a negative charge) is expressed as material’s electron affinity. The inverse is the work function, which

is the energy required to remove an electron. Thus, materials at the top of the series will have a very low work function and a low electron affinity, and readily lose electrons and gain a positive charge. However, cyclic triboelectric series exist, showing that the phenomena of triboelectrification cannot be purely due to material electron affinity (Figure 2.1).

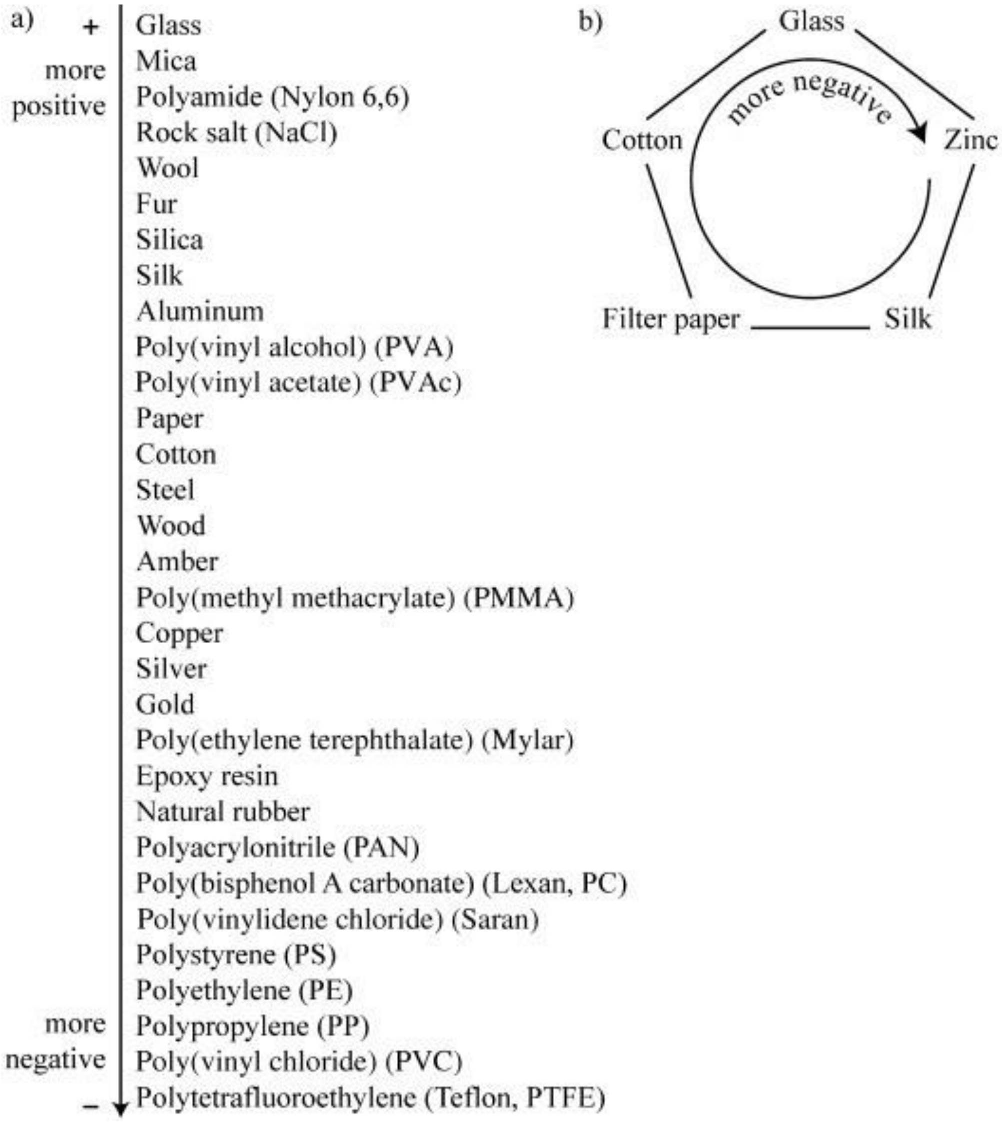

Figure 2.1. A) An example triboelectric series constructed by combining several different triboelectric series from Diaz and Felix-Navarro (2004). B) An example of a cyclic triboelectric series. From McCarty and Whitesides (2008).

Interestingly, identical materials can still experience charge separation when rubbed together, even if it arises from a block of one material being split in half. When this occurs, if the material is rubbed together in a symmetric fashion the surfaces acquire positive and negative charges apparently randomly. If they are rubbed asymmetrically, the material with the smaller contacting area usually becomes negative and the surface with the larger contacting area becomes positive (Lowell and Truscott 1986; Lacks and Sankaran 2011). Thus, triboelectrification is unlikely to simply be a function of electron affinity but will be affected by surface properties of the materials and charge transfer is ultimately likely to be due to a combination of a variety of mechanisms.

2.3.2 The role of humidity on triboelectric charging and charge dissipation

Relative humidity hugely affects triboelectric charging, so that the charges gained by materials are typically reduced in higher humidity (Greason 2000). This is because water provides a conductive layer on the surface, preventing local build-ups of charges and creating a uniform, conductive layer. However, studies have found that at low humidity, object charging actually increases with humidity (Pence, Novotny, and Diaz 1994; Wiles et al. 2004) potentially as water allowed greater conductivity between dielectric surfaces and therefore better charge transfer during contact electrification.

Charged objects typically lose charge to the surrounding air over time, due to conduction along the material surface and adsorption of ions from the surrounding air (Xu, Zhang, and Chen 2007; Kindersberger and Lederle 2008a; 2008b). This rate of decay is greater when the object is more highly charged (Xu, Zhang, and Chen 2007) and when the concentration of charged ions in the surrounding air is elevated (Kumara et al. 2011). The rate of charge decay also increases with relative humidity of the air, as the air conducts charge away more efficiently and the material surface becomes wetter and more conductive (Nomura, Satoh, and Masuda 2003; Elajnaf et al. 2007; Lutz and Kindersberger 2009).

Onogi and colleagues found charge decay curves from charge leaking off fabrics both to a grounded surface via contact but also to the air (Onogi, Sugiura, and Nakaoka 1996). They theorised that this decay was due to evaporation of water off the material carrying charges away. Charge dissipation increased with increased humidity suggesting water assists the dissipation of charges into the air (Onogi, Sugiura, and Nakaoka 1996; de Lima Burgo et al. 2011). Static dissipation can be quantified by the decay half-life; the time taken for the charge on an object to reach half its original value. The decay curve will often not decay to zero but will instead end at an equilibrium potential that is dependent on the material properties and the interaction between the material and its surroundings (de Lima Burgo et al. 2011). For bee charge to facilitate the movement of pollen, charge must be retained by the bee during flight or be constantly generated at a rate greater than or equal to its dissipation, so that the bee is electrically charged when it approaches a flower. In parts of this thesis I analyse not only how bees generate a charge, but how well they keep it relative to other materials and insects, and how relative humidity affects the bees ability to gain and retain charge.

2.3.3 The triboelectric interaction with air

The triboelectric series exclusively compare the relative charges of solid materials. However, some series have air listed as the most positive material (Crutcher, Warner, and Crutcher 2007; Bera 2016; Spicer 2017), although the basis of this placement is unclear. Being a composition of gases, frictional charging with air is not triboelectrification as air is not a solid material. In addition, although air features at the top of some series there is often no experimental basis for its position. Frictional charging of materials using air was likely achieved using triboelectrification with solid particulates present in the air source rather than with the air itself. This uncertainty in the physics of triboelectrification introduces questions that are key to our understanding of the charging of bees and other insects as they interact with their environment.

A basis for air-mediated triboelectrification stems from the observation that aircraft generate a negative charge during flight (Illingworth and Marsh 1986). This is a severe problem in aerospace engineering as an accumulation of charge can affect instruments, disrupt adequate communication between aircraft and ground control and cause explosions when refuelling. The accumulation of this charge happens largely under two conditions. The first is during ascent and descent through a cloud layer. In these instances, the ice particles (as well as fine dust particles) in the clouds are likely to be causing triboelectrification upon friction with the aircraft due to the high speeds and therefore strong friction rate. The second cause of large charge build-ups occurs in rotary winged aircraft flying low over desert or dusty ground. The updraft of the dust and the subsequent contact between the dust and the composite blades of the aircraft can cause large generation of charges due to triboelectrification (Grosshans, Szász and Papalexandris 2017).

Whilst bees in flight are likely to encounter many small particulates in the air, they are less likely to contact ice particles and do not venture near clouds. Airborne particulates originate from both natural and anthropogenic sources and include dust, pollen, soot, aerosols and ions. Bees flying outdoors could therefore gain charge through triboelectrification with these airborne particulates in a similar manner to aircraft.

2.3.4 Triboelectrification of bees and insects

The electric charges measured on insects were first proposed to be due to triboelectrification between the walking insect and the surface it was on (Edwards 1962). To test this, a number of experiments were completed over the latter half of the 20th century to establish the triboelectric properties of insects. Insects were usually positively charged, with the charge magnitude depending on the material they were compared against (Table 1.1). Honeybees were found to become positive when walking on any material including glass (Es'kov and Sapozhnikov 1976). This is interesting as glass is often placed near the top of the triboelectric series (Diaz and Felix-Navarro 2004) so this result suggests honeybees are triboelectrically very positive. The electric charge on houseflies varied depending on the material, with flies becoming positive when walking over lowly ranked materials such as PVC and Acetal, but becoming negatively charged when walking on Acrylic (Jackson and McGonigle 2005). Edwards (1962) rubbed insects on different surfaces and found that a selection of insects from different orders became positively charged when rubbed against any material other than asbestos (Edwards 1962). Thus with a few exceptions, insects are likely to place near the top of the triboelectric series.

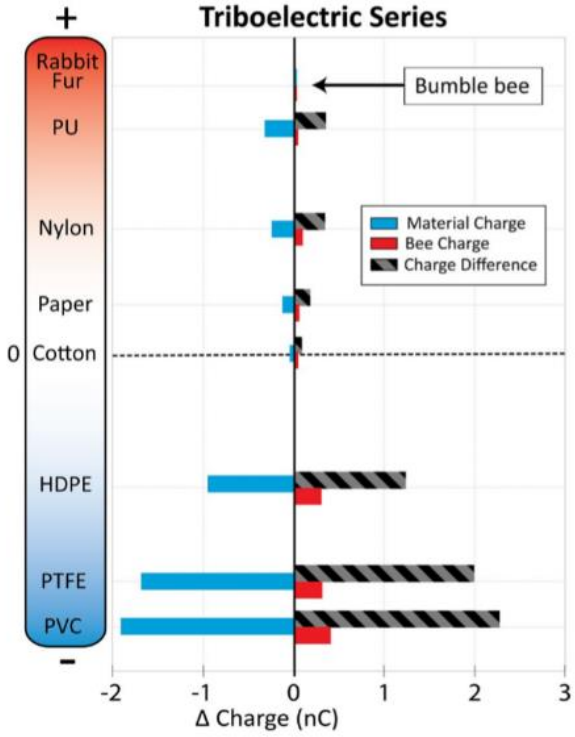

Figure 2.2. A triboelectric series established by rubbing freshly euthanised bumblebees against dielectric materials. Y axis shows the relative triboelectric position of the material from Diaz and Felix-Navarro (2004). The X axis shows the difference in charge of the material (light blue) and the bumblebee (red) after rubbing the material against the dorsal surface of the bee. The grey and black striped bars show the charge transferred between bee and material during rubbing. Each datum is the average of ten repetitions. From Clarke et al. (2017).

Manually rubbing dead insects allows comparison of material properties of insects relative to other materials, whilst allowing insects to walk over substrates shows that this charge generation happens organically and may occur in nature. However, none of these experiments address the potential triboelectrification of insects with materials they are likely to encounter in their natural environment. Triboelectric series are often composed of plastics and dielectrics, as these tend to generate the most charge and are often of interest due to their extensive industrial applications. However, these are arguably the materials least likely to be encountered by foraging insects. To this end, one aim of this thesis is to explore the concept of triboelectrification in the natural environment. Here, I focus on the foraging trips of bumblebees. Bumblebees have been previously placed on the triboelectric series and found to be at the top, becoming positive against any material tested (Clarke, Morley, and Robert 2017; Figure 2.2). However, these materials are typically dielectric polymers, not materials commonly encountered by bumblebees. To investigate whether foraging bees would gain charge due to triboelectrification it is necessary to compare them with a substrate they commonly encounter. A foraging bumblebee will often encounter many flowers in a single foraging trip. Thus the triboelectric interaction between bees and flowers could significantly affect the charge gained by foraging bumblebees.

Upon foraging on flowers, bees will often rub up against petals, stamens and stigma in the process of trying to access the nectary. This is beneficial for the plant as it increases the likelihood of pollen transfer between flowers and even between reproductive organs of the same flower. Some flowers (such as snapdragons) even require the bee to rub up against its petals in order to access the nectar and pollen. This frictional contact provides a potential source of charging. A difference in charge between the bee and the flower benefits the flower as well as the bee by attracting pollen onto the bee, increasing the amount transported between flowers and thereby increasing reproductive fitness. Triboelectric charging on flowers would therefore have adaptive value as a charging mechanism by enhancing the electric field between bee and flower upon separation, increasing the likelihood of waste pollen being attracted to either flower or bee.

To understand the triboelectric interaction between bees and flowers, the relative triboelectric properties of both bees and flowers are explored in Chapter 3. A floral triboelectric series is established comparing the mechanical and triboelectric properties of bees and flower using freshly euthanized insects. The charges on foraging bumblebees are then measured before and after visiting flowers, to detect whether friction between the two could cause triboelectric charging that may contribute to the overall charge on the bee.

2.4 ELECTRICAL PROPERTIES OF BEES

2.4.1 The bee cuticle

The bumblebee cuticle is made of chitin, which is chemically similar to alpha-chitin found in shellfish (Majtán et al. 2007). The structure of the cuticle in bees is much more uniform and regular than that of shellfish chitin, with bee chitin having a hexagonal surface pattern (Majtán et al. 2007). Chitin is an interesting material in terms of its tribology and its potential piezoelectric properties. It is considered for use as a biodegradable natural-material-based triboelectric nanogenerator (BN-TENG) (Jiang et al. 2018). This is a device which generates electricity on a small scale based on movement rubbing two dielectric materials with separate triboelectric properties together (Wang, Yang, and Wang 2017). Chitin was placed in the middle of a triboelectric series established by Jiang, with the series being Egg White > Silk Fibrosin > Chitin > Cellulose > Rice paper (Jiang et al. 2018). All materials gained a positive charge when rubbed against Kapton film (polyimide). Polyimide is low on the triboelectric series but not at the bottom (Diaz and Felix-Navarro 2004). From this we can deduce that chitin, along with the other natural materials, is tribo-positive to an extent but we do not know its exact placement.

The polarisability of a material can be quantified by its relative permittivity, also known as its dielectric constant Ɛ. Whilst resistivity is a measure of how well charge flows through a material, permittivity is a measure of how well that material itself charges up. The permittivity of a vacuum $Ɛ_o$ is 1. A perfect conductor will have infinite permittivity, whilst most insulators will have a relative permittivity of between 1-10. The higher the polarisability of a material, the greater its relative permittivity and the more easily it will be polarised in an electric field.

Chitin has a relative permittivity of between 5.2 and 7.0 and an electrical conductivity of between 5 x10^{-7} and 2.8x10^{-5} S/cm depending on temperature (Seoudi et al. 2005). It can be therefore classed as semiconductive, having a higher conductivity than glass but lower than silicon. Interestingly, chitin, which makes up the majority of the bumblebee cuticle and wing, is also a piezoelectric material. Chitin harvested from shellfish has been investigated for its use as a bio-inspired sensor and chitin from butterfly wings has also been shown to have piezoelectric properties (Binetti et al. 2009). The bending movement of the cuticle and wings could therefore contribute to bee charging during flight, though this has not been investigated.

Experiments have been conducted with cockroach cuticle to investigate the electrical properties of the insect exoskeleton. Scheie and Smyth excised the pronotum of a cockroach and floated it in an electrolyte bath connected to an electrode (Scheie and Smyth 1967). They put a drop of electrolyte on the top side of the floating cuticle and measured the potential difference across the cuticle. They found no evidence of generation of electric polarisation, but when they put a potential across the cuticle they found that the ducts within the cuticle allowed ion transfer across the cuticle providing a conductive pathway. The grease or surface wax on the cockroach was assumed to be the major dielectric barrier (Scheie and Smyth 1967).

2.4.2 Cuticular waxes

The charging of hydrocarbons is largely dependent on their chemistry. Cuticular hydrocarbons on *B. terrestris* are predominantly long-chain alkane hydrocarbons with Tricosane being the most abundant (Oldham, Billen, and Morgan 1994). Tricosane is a non-polar C-23 hydrocarbon and should therefore not charge easily, as changes in the distribution of molecules are unlikely to result in net changes of charge. Other abundant hydrocarbons in the cuticular wax are Pentacosane, Nonacosane, Hentriacontene, Eicosyl octadic-9-eonate and docosyl octadic-9-enoate although there are also more (Sramkova et al. 2008). From a different study, heptacosane was found to be a cuticle wax as well as hexacosyl octadecenoate (Rottler-Hoermann, Schulz, and Ayasse 2016). Little is known about the electrical properties of these waxes, and a full assessment of the electrical properties of each hydrocarbon is beyond the scope of this thesis.

2.5 MEASUREMENTS OF ELECTRIC CHARGE

Throughout this thesis I use three main methods of measuring the electric charge on bees: A Faraday pail, a copper tube sensor and a ring charge sensor (RCS; Figure 2.3). The use of each sensor is dependent on the context, the bee species used and whether the bees could be trained to fly into or through the sensor. The Faraday pail (Faraday 1843) is a well-established instrument for the measurement of electrostatic charge on small objects. The ring charge sensor (RCS) is more experimental, although induction rings working on this principle have been used for charge measurements in previous experiments in the literature (Colin, Richard, and Chauzy 1991). Where the RCS is used, it is calibrated with the Faraday pail. This ensures comparable measurements from both instruments and allows the evaluation of measurement accuracy and systematic errors before use.

Figure 2.3. The charge measurement sensors used to measure the electric charge on bees and small objects, showing a copper tube sensor (left), a ring charge sensor (middle) and a Faraday pail (right).

2.5.1 The Faraday pail

A Faraday pail is a bucket-shaped device used to measure electric charge. It originates from Faraday's ice pail experiment in 1843, demonstrating induction, where a charged object is introduced inside a metal pail (Faraday 1843). The charge on the object induces an opposite charge on the cup interior, which can be measured by an external electrometer. In Faraday's original experiment, this was a gold-leaf electrometer.

The Faraday pail used in the present experiments was a JCI 147 Faraday pail coupled with a JCI 140 static monitor (John Chubb Instrumentation Ltd. Cheltenham, UK). The measurement sensor of the pail is a small cup, which is electrically floating and is enclosed by a grounded shield to prevent external electric fields affecting the potential of the cup. The cup sits atop a JCI static monitor which detects the potential difference induced in the cup by the charged object. When an object enters the cup, the electric charge on that object induces an equal but opposite charge in the cup. The change in potential of this cup is registered as a voltage from the field mill. Bumblebees can be trained to fly into a Faraday pail (Clarke et al. 2013), which provides useful measurements of bumblebee charge in laboratory bees. This method can therefore be used to measure the charges on bees under different laboratory conditions. However, once the bees are in the pail they must be manually removed before subsequent measurements can take place. This prevents repeated measures on individual bees, such as measuring the bee before and after visiting a flower or being exposed to an environment. For measurement of mobile bees, other inductive techniques are more practical. Here, I present such techniques, such as the copper tube or the ring charge sensor (RCS), which I developed to address my specific questions.

2.5.2 Copper tube sensor

The copper tube sensor is based on the bee charge sensor used by Colin and colleagues in 1991. In their study, the sensor was used to measure the charge on honeybees entering and leaving a hive. The sensor consists of two concentric copper tubes that are isolated from each other by an insulating material (Colin, Richard, and Chauzy 1991). The inner tube is connected to an electrometer to measure an increase in potential caused by a charged bee entering the tube. The electric charge on the bee (Q_{bee}) is related to the electric charge induced in the cylinder (Q_{tube}) by

$$Q_{tube} = aQ_{bee} \qquad (2.2)$$

where *a* is the reduction coefficient. The reduction coefficient is dependent on the geometric properties of the tube and is calculated:

$$a = \frac{h}{2\sqrt{\frac{h^2}{4+r^2}}} \qquad (2.3)$$

Where *h* is the length of the cylinder and *r* is its radius (Colin, Richard, and Chauzy 1991).

For the copper tube sensors used here, each sensor consists of two concentric copper tubes separated by insulating polycarbonate, with the inner tube connected to a custom made ammeter fitted with a 10GΩ resistor. This amperometric device measures the current in picoamps and has a gain which can be altered to fit the range of the incoming inductive signal. The picoammeter outputs a voltage signal that is proportional to the inductive current. The outer copper tube is grounded to act as an electrical shield. The time signal of the current induced in the inner tube by a walking charged bumblebee is integrated to calculate the charge on the bumblebee and scaled according to equations 2.2 and 2.3. The copper tube sensors were calibrated for charge measurement by dropping cubes (1cm X 1cm X 1cm) of polyurethane foam through both tubes into a Faraday pail.

The integral of the current registered by the copper tube sensor was always proportional to the charge registered by the Faraday pail, and the gain corresponded with the amplification factor on the picoammeter and the dimensions of the copper tube sensor.

2.5.3 Ring charge sensor (RCS)

The RCS works with a similar induction principle but does not rely on contact between the bee and the sensor, ensuring that the bee charge is unaltered by its measurement, and allowing measurement of the charge on flying bees. The RCS comprises 2 concentric copper rings. These are insulated from each other by a layer of insulating material, usually polycarbonate. As with the copper tube sensor, the outer ring is grounded and acts as an electrical shield, whilst the inner ring is connected to a picoammeter.

When a charged object moves through the ring, it induces a current in the copper. Current *I* is a measurement of the flow of charge so that

$$\frac{dq}{dt} = I(t) \tag{2.4}$$

The unit of current is defined so that one Ampere of current is equivalent to the flow of 1 Coulomb of charge per second (1A = $1Cs^{-1}$). The charge on a single electron is 1.602×10^{-19} C so that 1A of current is equivalent to 1.602×10^{19} electrons flowing past that point each second.

As current is the flow of charge over time (Equation 2.4), the current in the ring can be integrated to find the charge on the object. A positively charged object approaching the ring will induce electrons to flow toward the ring, causing a positive current (Figure 2.4B) in the picoammeter. As the object passes through the ring and starts moving away from the ring, the direction of the current reverses and becomes negative. The current then returns to zero, thus the integral of the positive section of the graph is the inverse of the integral of the negative section. The root mean squared of the section of the graph could therefore be used as a measure of charge of a passing object. However, bee movement was rarely linear, and as the current could take a while to return to zero it was disproportionally affected by noise. Due to the noisy signal and the bees often decelerating rapidly after passing through the ring, only the approach section of the graph was used as a charge measurement of flying bees. During calibration, the integral of this current is directly proportional to the charge on the object (Figure 2.4C).

The RCS is a modified version of the copper tube sensor, but where the tube sensor measures the charge on walking bees, the geometry of the RCS is adapted to measure the charge on bees during flight. For the fieldwork in Chapter 4 as well as several other experiments, the RCS is used as a sensor to make non-contact measurements of the charge on flying bees. This is beneficial as unlike walking sensors the bee is not touching the ring and therefore will not lose charge by conduction. As such, the charge on the bee flying through the RCS can be measured multiple times and remains unaltered.

The charge on the object passing through the RCS can be calculated from its geometry. It is more robust however to calibrate the RCS with a Faraday pail before doing an experiment. This then accounts for differences in sensitivity between different ammeters and any environmental changes that may affect the RCS or Faraday pail sensitivity. For each experiment, the RCS is calibrated by dropping small cubes of charged polyurethane foam through the RCS into a Faraday pail (Figure 2.4A). For each cube dropped, the current in the ring is compared to the increase in charge registered by the Faraday pail (Figure 2.4B). The integral of the current caused by the cube approaching is calculated and compared to the charge in the Faraday pail. When the integrals are compared to the charge in the Faraday pail there is a linear relationship between the charge measured by the RCS and the pail (Figure 2.4C).

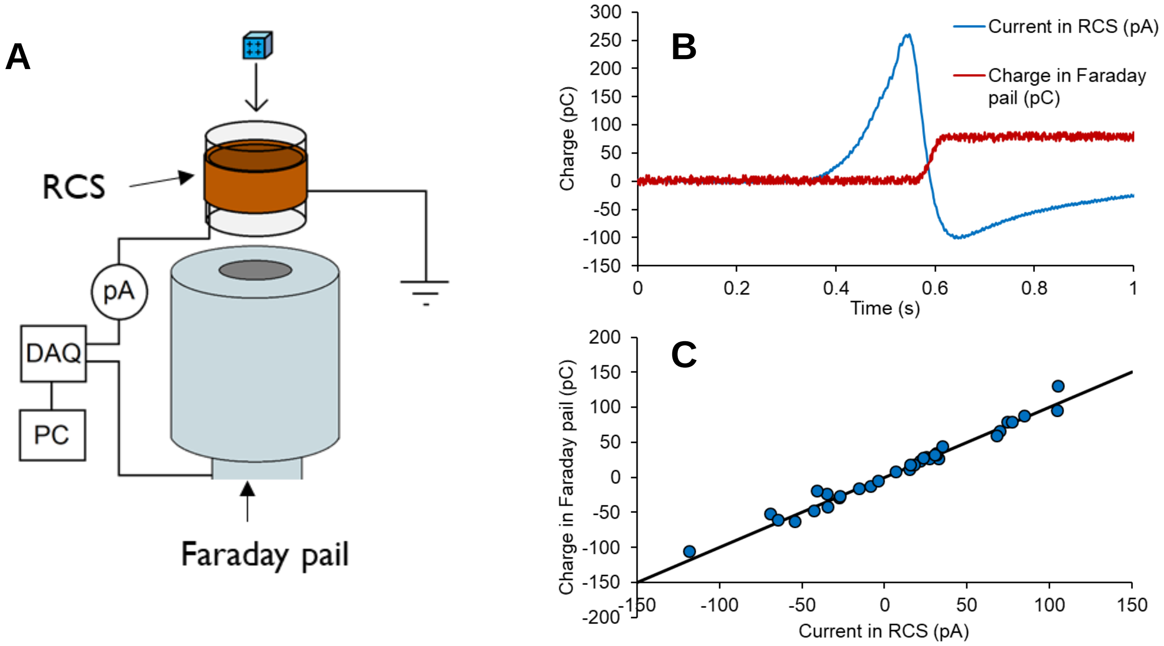

Figure 2.4. A) A diagrammatic representation of the calibration of the RCS with the Faraday pail. Triboelectrically charged cubes of polyurethane foam are dropped through the RCS into the pail. B) The current in the RCS is compared with the increase in charge measured by the Faraday pail for each cube drop. C) The integral of the current in the RCS is directly proportional to the charge measured in the Faraday pail. Black line shows 1:1 relationship.

The RCS and the Faraday pail both provide useful methods for measuring the charge on bees and other insects. Bumblebees can be trained to fly into the Faraday pail and through the RCS to access food, allowing measurements of their charge. For other insects, the RCS and copper tube sensors can be placed over the entrance of a colony, allowing measurement of insects entering and leaving. This method was used to measure the charges on bumblebees, wasps and mason bees under natural conditions. In this way, the RCS allows measurement of electric charge on insects in their natural environment.

3 THE TRIBOELECTRIFICATION OF BUMBLEBEES

3.1 CONTRIBUTIONS

For three of the experiments documented in this chapter, some of the data was collected by undergraduate students under my supervision. For the measurement of bumblebee charge before walking down a tunnel containing dielectric materials, I was assisted by Danielle Damon. The measurements of bumblebee charge after different durations of flight were performed by myself, James Hepworth and Christina Vivian. The establishment of a floral triboelectric series was performed by myself, James Hepworth, Christina Vivian and Matthew Bond. The measurement of the electric charge of bees before and after visiting flowers was performed by myself, Siwan Davies, Matt Lynch and Lucy Collingwood.

In all of these experiments I personally designed the experiment, collected part of the data, wrote the methodology and analysed the data. Any data not collected by myself was done under my direct supervision or filmed for my subsequent analysis and quality control. I independently manipulated the data, calibrated the equipment and analysed the results of each experiment.

3.2 THE TRIBOELECTRIC PROPERTIES OF BUMBLEBEES

3.2.1 The literature surrounding insect triboelectrification

Triboelectrification is the process whereby an object gains an electrical charge upon friction with a different material. A charge separation between two materials arises, with one material gaining a positive charge and the other becoming more negatively charged upon contact. Materials can be ordered by their tendency to gain a positive charge to create a triboelectric series.

Most historic measurements of insect charge are from insects that have been charged triboelectrically, either by allowing the insect to walk over a dielectric surface (Es'kov and Sapozhnikov 1976; McGonigle and Jackson 2002; McGonigle, Jackson, and Davidson 2002; Jackson and McGonigle 2005) or by rubbing a dead insect against a dielectric material (Edwards 1962; Clarke, Morley, and Robert 2017). Using both techniques, scientists have shown that bees, and insects in general, can be considered triboelectrically positive, gaining a positive charge upon contact with most materials. In the most recent study of bumblebee electric ecology it was found that bumblebees became positively charged when rubbed against any material, placing them at the very top of the triboelectric series with an equivalent triboelectric position to rabbit fur (Clarke, Morley, and Robert 2017). Although bees are unlikely to encounter many of these dielectric materials in nature (e.g. nylon, polystyrene), their position at the top of the triboelectric series makes it likely that they will gain a positive charge upon landing on most surfaces. Triboelectric interactions with various materials in their surrounding environment may therefore contribute to the positive electric charges measured on flying bees in the laboratory (Clarke et al. 2013) and in the field (Colin, Richard, and Chauzy 1991).

Previous measurements of electric charge on flying bumblebees were done in a laboratory with bumblebees flying in a wooden flight arena with a Perspex ceiling and a floor covered in gaffer tape (Stage Electric, UK). Wood is triboelectrically neutral, consistently placed towards the centre of most triboelectric series (Diaz and Felix-Navarro 2004) with minor differences between different types of wood (Greason 2012; Greason 2013). Perspex (otherwise known as acrylic, lucite, or poly-methyl methacrylate (PMMA)) is also placed near the middle of the triboelectric series (Diaz and Felix-Navarro 2004). The top layer of gaffer tape is comprised of polyethylene, a polymer consistently placed towards the bottom of the triboelectric series (Diaz and Felix-Navarro 2004). The dimensions of the flight arena (72cm x 104cm x 30 cm) allow flight but prolonged flight will result in collisions with the walls and ceiling of the arena, and most bees walk on the floor for a duration before taking flight. Bees are likely to gain a positive charge when walking over the polyethylene flooring due to the relative triboelectric properties of bumblebees (highly positive; Clarke et al. 2013) and polyethylene (highly negative; Diaz and Felix-Navarro, 2004). It is possible that the positive charges measured on flying bumblebees in the laboratory by Clarke et al. (2013) are due to triboelectric charging. If this is the case, the charges measured in the laboratory will not be representative of bumblebees flying outdoors, as outdoor bees will not be walking on polyethylene. Field measurements of bumblebee charge address this problem in Chapter 4.

The triboelectric position of bumblebees was established by rubbing euthanised bumblebees against a variety of materials (Clarke, Morley, and Robert 2017; Figure 2.2). Most studies of insect triboelectric properties have measured the charges on live insects after walking over surfaces constructed of different materials, but this is yet to be done with bumblebees. To address this gap, and to establish whether live bumblebees can also be placed at the top of the triboelectric series, I measured the electric charge of bumblebees walking over various surfaces. I then investigated the potential for a triboelectric interaction between bees and flowers, firstly by establishing a floral triboelectric series, and then by measuring the electric charge on bumblebees before and after visiting flowers. Additionally, I measured the electric charge on bumblebees before and after flight in different conditions. These experiments aimed to determine whether the positive electric charge previously measured on flying bumblebees was a product of flight (as has been suggested in the literature) or is due in part to triboelectric interactions between the bee and its surroundings.

Table 3.1. Summary of the experiments in Chapter 3.

Experiment	Research question	Method
Triboelectric charging in bumblebees	Do live bumblebees gain charge by triboelectrification?	Bumblebee charge measured before and after walking on dielectric materials.
Triboelectric charging in flowers	Do flowers differ in their triboelectric positions?	Pairwise comparisons of flowers to crease a floral triboelectric series.
Bee-flower triboelectrification	Do bees gain charge by triboelectrification when visiting flowers?	Compare bee charge before and after visiting flowers from different positions in the triboelectric series.
Bumblebee charge before and after flight	Do bumblebees have a higher charge after flight?	Compare bee charge before and after flight.
Bumblebee charge during flight	Do bumblebees gain charge during flight?	Bumblebee charge and flight duration recorded after flight into a Faraday pail.
Charge retention and relative humidity	How well do bumblebees retain their charge?	Measure the charge dissipation on bumblebees.

3.2.2 Triboelectric charging in bumblebees

3.2.2.1 Methods

Bumblebees (*Bombus terrestris audax*, Koppert UK) were encouraged to walk down a tunnel with equilateral triangular cross-section (length 100mm, height 13mm) made of different dielectric materials (Figure 3.1). This shape enabled constant physical contact between bee and material and was still large enough for the bee to walk through. At either end of the tunnel were two copper tube sensors, based on the measurement sensor used by Colin et al. (Colin, Richard, and Chauzy 1991). Each sensor consists of two concentric copper tubes separated by insulating material, with the inner tube connected to a custom made picoammeter. The outer copper tube is earthed to act as an electrical shield. The time signal of the current induced in the inner tube by a charged walking bumblebee was integrated to calculate the charge on the bumblebee (Colin, Richard, and Chauzy 1991). The charge sensors were calibrated for charge measurement by dropping cubes (1cm x 1cm x 1cm) of polyurethane foam through both tubes into a Faraday pail. The charge measured by both copper tubes was directly proportional to that measured by the Faraday pail with R^2 values of 0.936 and 0.921. Bumblebees were collected whilst foraging within a laboratory flight arena and placed in individual 50ml falcon tubes. During each trial, the falcon tube was placed at one end of the dielectric tunnel. The bees would leave the tube and explore the tunnel. Once a bee had walked down the tunnel in both directions without pausing, the bee was marked with a small amount of paint on the thorax and returned to the arena. Bees that did not leave the falcon tubes were returned to the arena. 10 bees were used for each material. Each bee walked through the corridor in both directions to account for differences in the charge measuring tubes, and the mean charge of bees walking in both directions was measured before and after each bee had walked along the material. The charge sensors were used to measure the charge on the bee before and after it had walked through the dielectric tunnel in both directions (Figure 3.1). The materials used were selected to compare against the triboelectric series presented by Clarke, Morley, and Robert (2017). The change in bee charge due to walking through the dielectric tunnel was compared for different dielectric materials to establish the triboelectric position of live bumblebees.

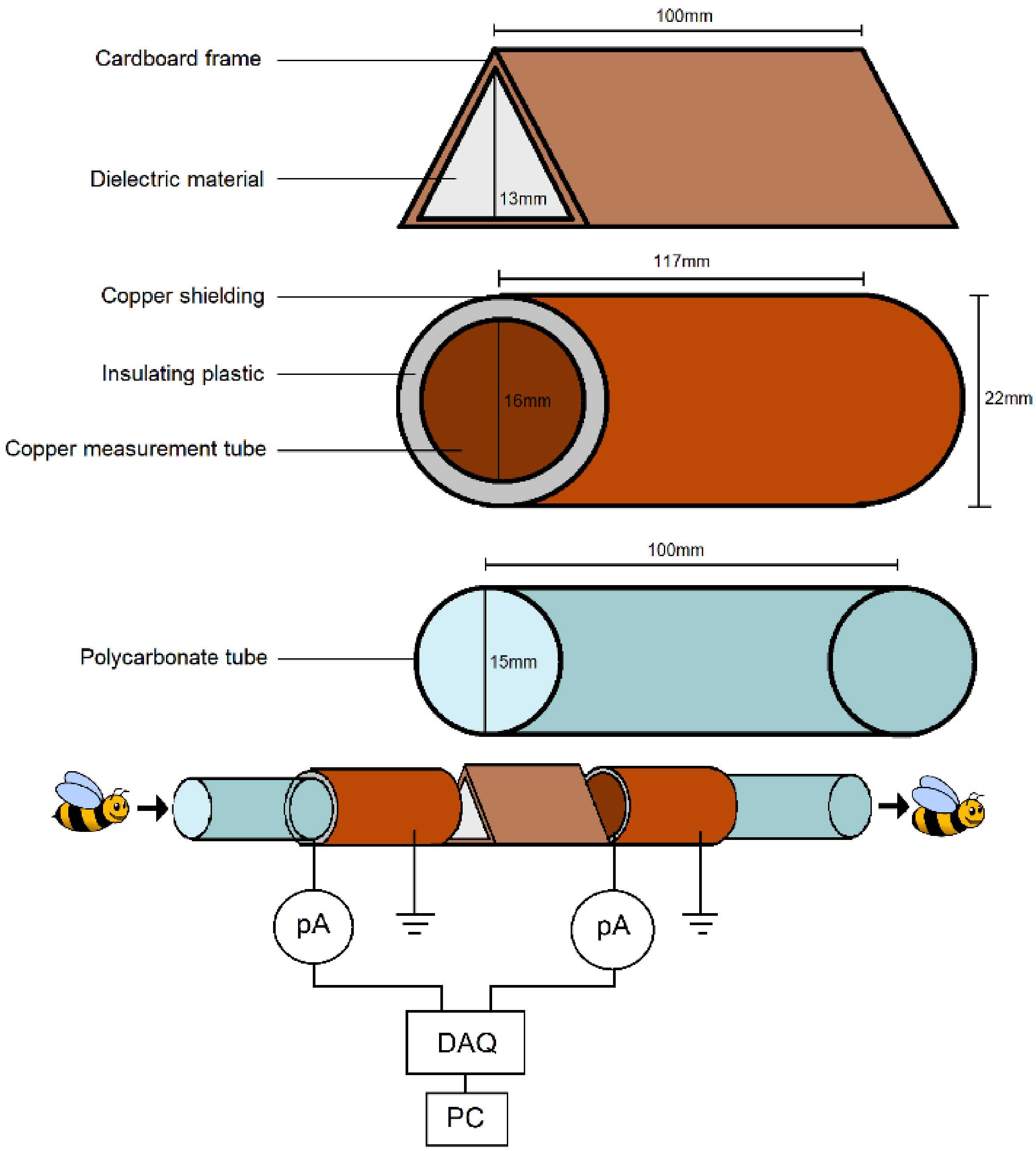

Figure 3.1. Diagrammatic representation of the triboelectric tunnel. This instrument consisted of a triangular tunnel with an inner lining of dielectric test material and an outer case of cardboard (top). This tunnel was flanked by two copper tubes acting as charge sensors. For each copper tube, the inner tube is connected to a picoammeter and the outer tube is electrically earthed. The data are collected via a data acquisition board (DAQ) and analysed in MATLAB. The copper tubes are flanked by transparent polycarbonate tubes allowing visual confirmation of bees entering and leaving the sensors. Thus a bee walking down the tunnel encounters the following: Polycarbonate tube - Copper tube sensor – Dielectric material – Copper tube sensor – Polycarbonate tube. This allows comparison of bee charge before and after walking on the material.

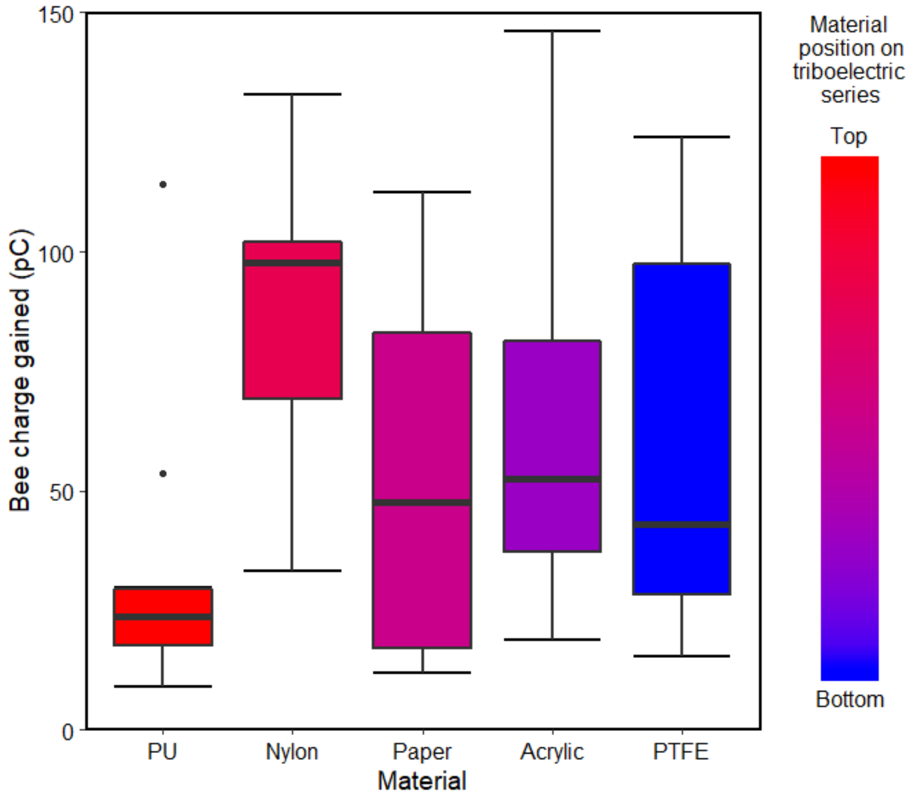

Figure 3.2. The gain in charge of 10 bumblebees walking through the dielectric tunnel over different materials, showing median, IQR and range excluding outliers. Colour box indicates the test material position on the triboelectric series established by Clarke, Morley, and Robert (2017).

3.2.2.2 Results

Bees always gained positive charge when walking over the dielectric materials (Figure 3.2). When walking on materials usually placed at the top of the triboelectric series (such as polyurethane foam (PU; Clarke, Morley, and Robert 2017), the bees gained less charge than when walking over materials at the bottom of the triboelectric series such as PTFE. The charge gained by the bee was significantly affected by the material's triboelectric rank (ANOVA, P=0.025, $F_{4,45}$ = 3.086). Bees walking over the material with the highest triboelectric rank (PU) gained significantly less charge than those walking over the material with the lowest triboelectric rank (PTFE; Mann-Whitney U test, P=0.045, W=27). With the exception of nylon, the charge gain of the bees largely corresponds to the triboelectric position shown by Clarke *et al*. (2017) (Figure 2.2). The bees gained the greatest charge when walking on nylon (mean charge gain 89 ± 33 pC) and the lowest charge when walking over PU

(33 ± 31pC). The differences in the series may be due to slight differences in the material properties, although the polyurethane foam used in this study is identical to that used in (Clarke, Morley, and Robert 2017). This study, alongside the data presented in (Clarke, Morley, and Robert 2017), suggests that bumblebees gain a positive charge triboelectrically, both when being rubbed against materials and when walking over materials of their own volition.

3.2.3 Triboelectric charging in flowers

A triboelectric series of bee-pollinated flowers was established using a similar method to that used by Clarke and colleagues in their 2017 study (Clarke, Morley, and Robert 2017). Bumblebees were taken from a laboratory colony and euthanised by freezing. Flowers were picked fresh from the University of Bristol Royal Fort gardens and the stem was cut 5mm below the flower. A Milty electrostatic eliminator (Zerostat 3 anti-static gun, Milty, Herts, UK) was used to neutralise the positive and negative charges on both flower and bee prior to triboelectrification. This procedure serves to normalise both plant and bee with as little charge as possible from the start of the test. Bee and flower were lightly rubbed together for 10 seconds using metal forceps before being individually dropped into a Faraday pail. Metal forceps were used to minimise triboelectric charging between the flower and the forceps. The forceps were not grounded to minimise charge conduction away from the flower. The order of the bee and flower being dropped was alternated to account for charge dissipation. Each bee and each flower were used only once. 10 bees and 10 flowers were compared for each flower species to establish a floral triboelectric series and identify whether bumblebees gain a positive or negative charge upon triboelectrification with flowers.

When bees and flowers were rubbed together in all cases the charge gained by the bee was more positive than the charge gained by the flower (Figure 3.3). When the flowers were ranked by the mean charge difference between bee and flower to create a floral triboelectric series, the most positive flower was borage (*Borago officinalis*) and the most negative was Agapanthus (*Agapanthus sp.)*. The majority of bees (69%) were positively charged after being rubbed against flowers whilst the flowers were generally negatively charged after contact (70%). These results suggest that bees landing on flowers are likely to gain a positive charge through triboelectrification with the flower, and that charge gain is likely to be influenced by the flowers triboelectric material properties. To test this hypothesis, the charge on flying bumblebees was measured before and after visiting flowers from each end of the floral triboelectric series to establish whether bees gained charge when visiting flowers, and whether this was affected by the flowers' position in the triboelectric series.

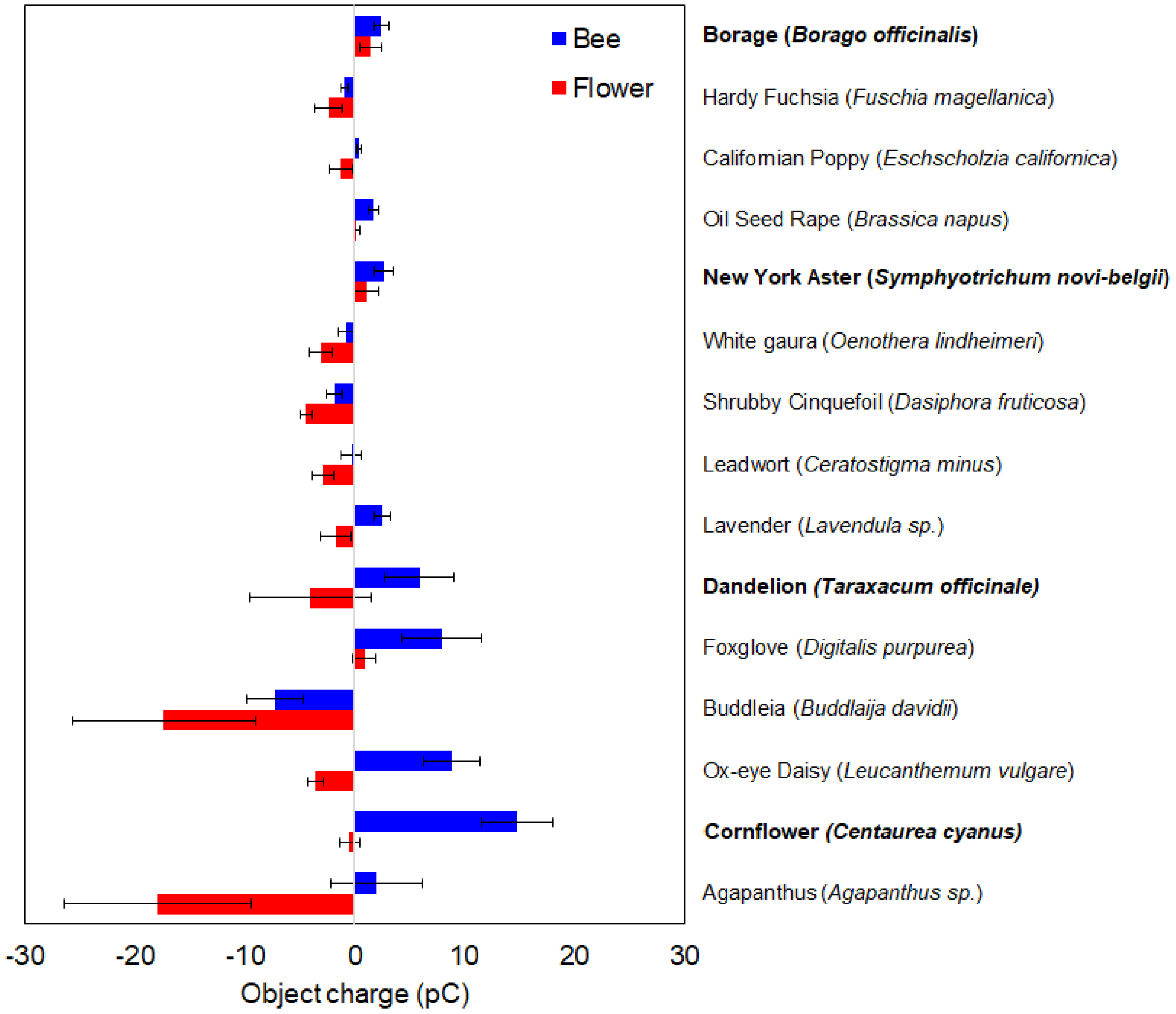

Figure 3.3. The floral triboelectric series. Object charge was obtained by rubbing euthanised bees against flowers and measuring the resulting charge gain or loss using a Faraday pail. The series reveals the tendency of flower species to acquire a negative charge and the bee to gain a positive charge. Object charge is shown as the mean value measured on the bee (blue) and the flower (red) in each pair after rubbing. Error bars show standard deviation (N=10 bees and flowers for each combination). Flower species in bold were selected for use in subsequent experiments with free-flying foraging bees.

3.2.4 The triboelectric interaction between bees and flowers

3.2.4.1 Methods

To identify whether foraging bumblebees gain charge when visiting flowers, bumblebees were trained to visit flowers in a foraging arena. A cut flower was held in electrically grounded floral foam, with the head of the flower protruding into a plastic cup (Figure 3.4). To access the flower, the bees had to fly through a copper ring around the rim of the cup (80mm diameter, 26mm width), acting as a ring charge sensor (RCS). The inner ring was connected to a picoammeter and calibrated with a Faraday pail, enabling the measurement of the charge on individual bees before and after they had visited the flower. Four flower species were presented that had been previously placed at different positions in the floral triboelectric series: Borage (*Borago officinalis*), Dandelion (*Taraxacum officinale*), New York Aster (*Symphyotrichum novi-belgii*) and Cornflower (*Centurea cyanus*). The change in charge of the bee after visiting the flower was compared to the flowers position on the triboelectric series. All statistical analyses were done in R (version 3.5.1).

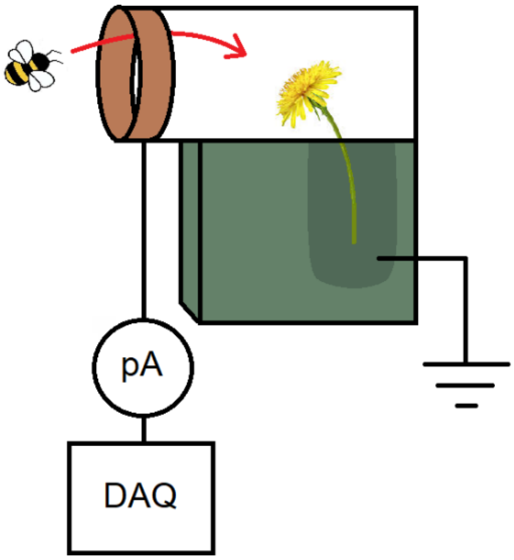

Figure 3.4. The apparatus for measuring the charge on a bumblebee before and after visiting a flower. To access the flower the bee had to fly through the RCS, allowing measurements of bee charge before and after visiting the flower. The flower was held in a block of dampened floral foam which was electrically grounded to simulate a flower in nature. Different flowers were used from different positions on the floral triboelectric series.

3.2.4.2 Results

Flower species significantly affected the amount of charge gained by an actively foraging bee (ANOVA, P = 0.036, $F_{3,63}$ = 3.017), with bees visiting cornflowers gaining significantly higher charges than those visiting borage (Tukey's post-hoc test, P = 0.036) and those visiting New York Aster (P =

0.044, Figure 3.5). The charge gained by the bee was directly related to the triboelectric position of the flower it visited (shown in Figure 3.3), with bees visiting flowers at the top of the triboelectric series (e.g. cornflower, position 1) gaining significantly higher charges than when visiting flowers at the bottom of the triboelectric series (e.g. borage, position 14).

Altogether, these three experiments show that bumblebees gain charge triboelectrically when walking on surfaces and when visiting flowers, suggesting that triboelectrification may contribute to the electric charges measured on bumblebees whilst foraging.

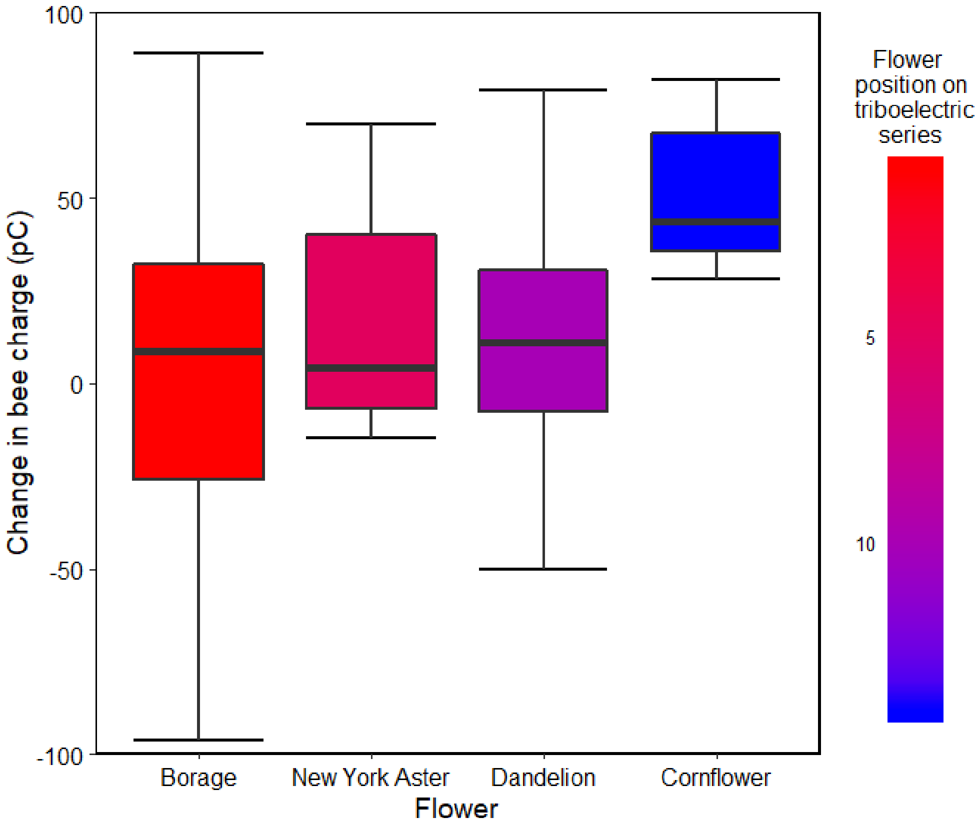

Figure 3.5. Triboelectric interaction between free flying bumblebee and freshly cut flower shown as the charge on bumblebees before and after visiting different flowers. Electric charge is shown as median, IQR and range. The colour bar shows the flowers position on the triboelectric series established in Figure 3.4. Bees visiting cornflower (N = 7) gained significantly higher charge than bees visiting borage (P = 0.036, N = 24) and New York Aster (P = 0.044, N = 9) but not dandelion (P = 0.703, N = 27).

3.3 DO BEES GAIN CHARGE DURING FLIGHT?

3.3.1 Theory and hypotheses

It is commonly stated that bees gain charge during flight but there is conflicting evidence to support this statement. Gan-Mor et al. (1995) showed that honeybees had a greater charge after tethered flight than euthanised honeybees that were passively swung on a motorised carousel (Gan-Mor et al. 1995). This indicated the presence of charge generation during flight, but no mechanisms were proposed. Early measurements of surface potentials of honeybees entering and exiting a hive found that bees were more positively charged when entering the hive (ie after flight) and that this charge varied over the course of the day (Erickson 1975). Contrastingly, Colin and colleagues (1991) found that honeybees had a greater charge when in the hive than when flying outdoors (Colin, Richard, and Chauzy 1991), which suggests generation of charge within the colony rather than during flight. Es’kov and Sapozhnikov (1976) found that bees flying long distances (200m) did not have significantly higher charges than those flying short distances (5m), suggesting bees do not generate charge during long-distance flight (Es’kov and Sapozhnikov 1976). These studies were all conducted with honeybees, which have been proposed to use electroreception for communication within the hive (Greggers et al. 2013). This has not yet been shown for bumblebees, who have been shown to use electroreception within the laboratory to make foraging decisions during flight using mechanosensory hairs (Clarke et al. 2013; Sutton et al. 2016).

In bumblebees, the extent of deflection of the hairs and therefore the sensitivity to electrical stimuli is dependent on the charge of the bee (Sutton et al. 2016). Bumblebees therefore benefit from being charged in flight as they are proposed to use electroreception to distinguish rewarding flowers, whilst honeybees are proposed to use electroreception for within-hive communication and may therefore benefit more from being electrically charged within the hive rather than on the wing. Correspondingly, charge generation in bumblebees and honeybees may therefore be different due to the different environments in which charge is beneficial, with an increased adaptive value to in-flight charging in bumblebees. The literature surrounding bumblebee charge is not nearly as extensive as that of honeybees. The electric charges measured on dead bumblebees after triboelectrification (Clarke, Morley, and Robert 2017; mean bee charge ± SD: 263 ± 348 pC) are much greater and more variable than those measured on live bumblebees after flight (Clarke et al. 2013; 32 ± 35 pC). Beyond that, we have very little information about bumblebee charge in different environments and the mechanisms of charge generation. Whether bumblebees gain charge during flight, or whether their charge is solely a product of a triboelectric interaction with flowers and their environment is measured here.

3.3.2 Bee charge before and after flight

3.3.2.1 Methods

To establish the role of flight in charging, the charges on bumblebees were measured before and after flight. A colony of *Bombus terrestris audax* (Koppert, UK) was connected to a flight arena (112cm x 77cm x 30cm) made of wood with a transparent perspex ceiling, on a day:night cycle of 16:8 hrs. The humidity in the arena was kept between 52-64% and the temperature was 19-24°C. The arena had a hole (8 cm diameter) cut in the floor below which was a cup containing a cotton wick with 30% sugar solution. The bumblebees were initially trained to fly into the cup, obtaining a sucrose reward. The cup was then substituted for a JCI Faraday pail (JCI 141, Chilworth Global, Southampton, UK) connected to a PC via a data acquisition board (CompactDAQ Chassis, National Instruments, Austin, Texas). After flying into the pail, the bees were marked with paint on their thorax to prevent pseudoreplication through subsequent release into the arena. A copper tube sensor (117mm length, 22mm outer diameter, 16mm inner diameter) was used to measure the charge on bees exiting the colony. The charge on each bee was measured by the copper tube sensor as it left the hive, and by the Faraday pail after a period of flight (Figure 3.6A).

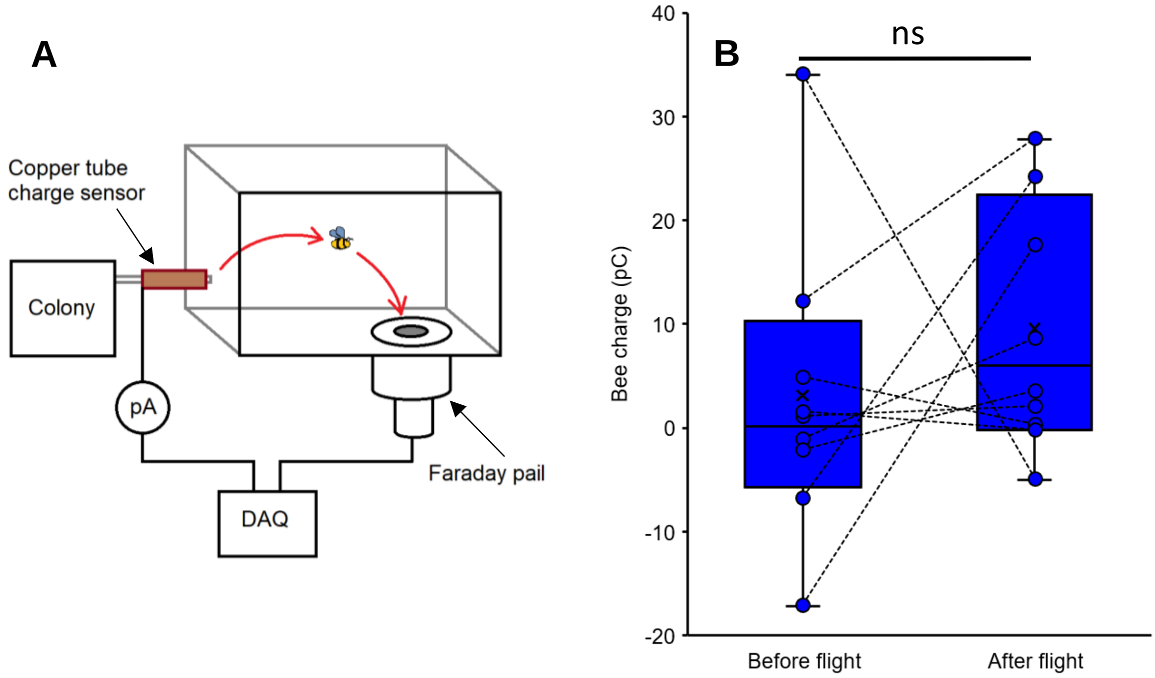

Figure 3.6. A) The charge on the bees was measured as they exited the colony via a copper tube connected to a picoammeter, and once they had flown into a Faraday pail. The output of both picoammeter and Faraday pail was recorded via a data acquisition board (DAQ) connected to a PC. B) The electric charge measured on individual bumblebees leaving the colony in the laboratory and the charges on the same individual bees after flight into the Faraday pail, showing mean (X), median and IQR.

3.3.2.2 Results

The charge on each bee was greater after flight into the pail (Figure 3.6B) but not significantly (Wilcoxon, N = 8, P = 0.3828, V = 25). It was very difficult to get the bees to take flight immediately upon exiting the colony and to fly into the Faraday pail without contacting the walls or ceiling or walking again. The low sample size reflects this, and a greater sample size would allow a better comparison of the charge of bees before and after flight.

3.3.3 Bee charge and flight duration

3.3.3.1 Methods

As the bumblebees very rarely took flight immediately upon exiting the colony, the relationship between flight time and bee charge was further investigated by training bumblebees to fly into a Faraday pail as before, and recording the flight duration from the moment each bee became airborne to the moment it landed in the Faraday pail. The charges of bees that flew into the pail were compared to those that walked into it, and differences in charge were analysed using an independent-measures T-test.

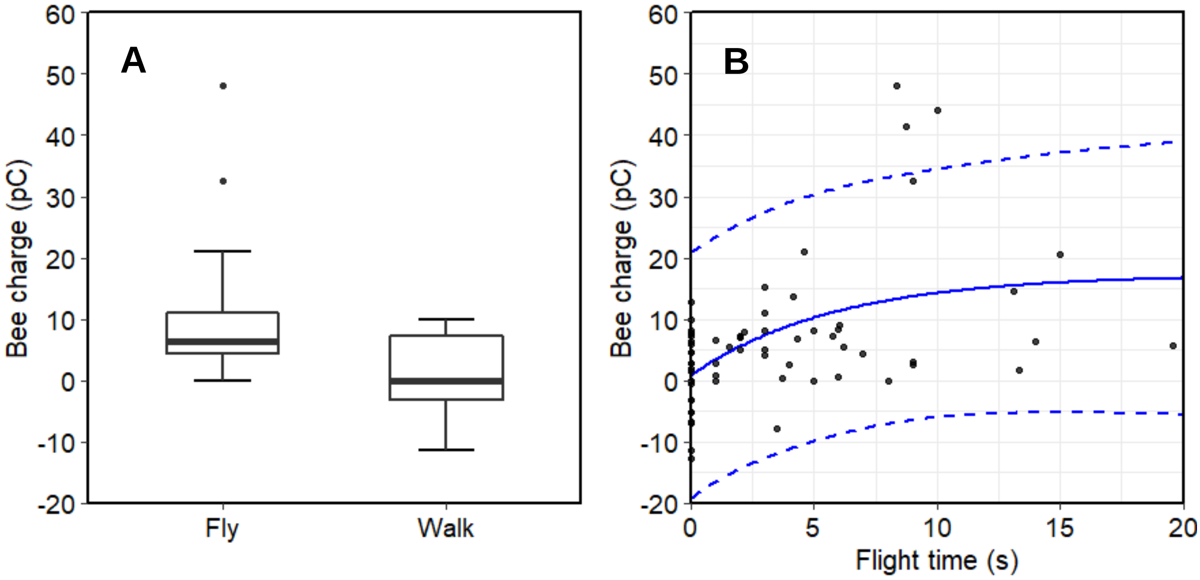

Figure 3.7. A) Electric charge measured on walking (N = 25) and flying (N = 41) bumblebees, showing median, IQR and range excluding outliers. Charge was significantly higher in flying bees than walking bees (P = 0.002). B) Charge measured on flying bees after different flight durations (N = 41). The best fitting model was a logarithmic model showing an increase in charge over flight time (solid blue line). Dashed lines show 95% confidence intervals of the model.

3.3.3.2 Results

Bumblebees that flew into the Faraday pail had significantly higher charges than those that walked into the pail (Figure 3.7A; P = 0.002, Mann-Whitney, N = 25 walking, 41 flying). Bee charge increased with flight time with the bees with the most positive charges having flown for around 10s (Figure 3.7B).

Assuming the electron transfer theory of triboelectrification, the charge on the bee will become more positive upon contact with most materials due to the difference in electron affinities between bee and material. In most cases the material will have a higher electron affinity than the bee, and the bee will lose electrons upon contact. Over flight, the electron affinity of the bee will increase as it becomes more positively charged and it will lose electrons at a reduced rate as it nears its saturation charge. At saturation, the electron affinities of the bee and its surroundings are approximately equal, so there is no net movement of charge. If the bee charge is greater than the saturation charge, the charge will leak away to its surroundings. Assuming a flying bee is in constant contact with particulates of uniform distribution and material composition, the rate of charge gain of the flying bee can be modelled as:

$$\frac{dq}{dt} = \alpha(q_s - q_i) \tag{3.1}$$

Where α is a constant, q_s is the saturation charge and q_i is the bee charge at a point in time (McGonigle, Jackson, and Davidson 2002). Assuming an initial bee charge of 0pC (Figure 3.7B), the charge on the bee q is related to the saturation level by:

$$q = q_s(1 - e^{-\propto i}) \tag{3.2}$$

A log model was fitted to the bee flight data using ordinary least squares method in MATLAB to model the in-flight charging of a bee based on the triboelectric charge generation of a spherical insulator (Greason 2000). This choice of a log model is based on the work on the triboelectric charging of houseflies walking on dielectric surfaces (McGonigle, Jackson, and Davidson 2002), which suggested that an insect encountering friction at a constant rate will charge up at a decreasing rate until it nears a saturation charge (Equation 3.1). In this instance the friction is between the bee and particulates in the air rather than a solid surface. The rate of charge gain depends on the relative triboelectric position of the particulates and the bee which can be expressed as electron affinities (Yu et al. 2015). The triboelectric position of the particulates is not known without being able to quantify specifically the material composition of the air in the laboratory. The triboelectric position of the bee is however well established, as bees have been shown to gain a positive charge upon friction with almost any material (Clarke, Morley, and Robert 2017), so it can be assumed that bees would gain a positive charge upon friction with most untested materials, as further explained below. The best fitting log-model to the bee flight data was

$$q = 17.3 - 16.4e^{-0.17t} \tag{3.3}$$

The modelled relationship and the 95% confidence intervals are shown in Figure 3.7B. The best fitting curve supports triboelectric charging during flight, with the bee gaining charge at a decreasing rate. This suggests that bees that are already charged are unlikely to gain further charge during flight, but that bees that are negligibly charged will gain charge during flight. The positive charge measured on free-flying bees is therefore unlikely to be entirely a product of flight, but also of triboelectrification with surfaces, flowers and the surrounding environment.

3.4 CHARGE RETENTION IN BUMBLEBEES

3.4.1 Charge retention compared to dielectric materials

Triboelectrification with dielectric materials and with flowers results in bumblebees gaining a positive electric charge. For this charge to facilitate pollen retention and electroreception, the charge must be retained during flight.

To evaluate the charge retention on bumblebees a euthanised bumblebee was attached to the end of a wooden rod (29.6cm long, 0.25cm diameter) by a small amount of ethyl cyanoacrylate glue (Loctite, Henkel, Ohio) on the underside of the thorax. The bee was gently rubbed against a 10cm length of polystyrene until it gained a charge greater than 1nC. The bee was held in a Faraday pail for 15 seconds and the decay in charge on the bee was measured over this time. For comparison, a nylon ball (13mm diameter) was affixed to an identical wooden rod and triboelectrically charged in the same way. Nylon was chosen as it is consistently near the top of the triboelectric series (Diaz and Felix-Navarro 2004) and the balls were similar in size and dimensions to the bumblebees. The charge decline was measured for 5 bees and 5 nylon balls in this way in a room with 45% RH at 20.8°C. It was found that bumblebees retained their charge very well, losing only a negligible amount of charge over the 12s period, whilst the charge on the nylon balls declined much more rapidly, with a decay curve with a half-life of 2.54 s (Figure 3.8).

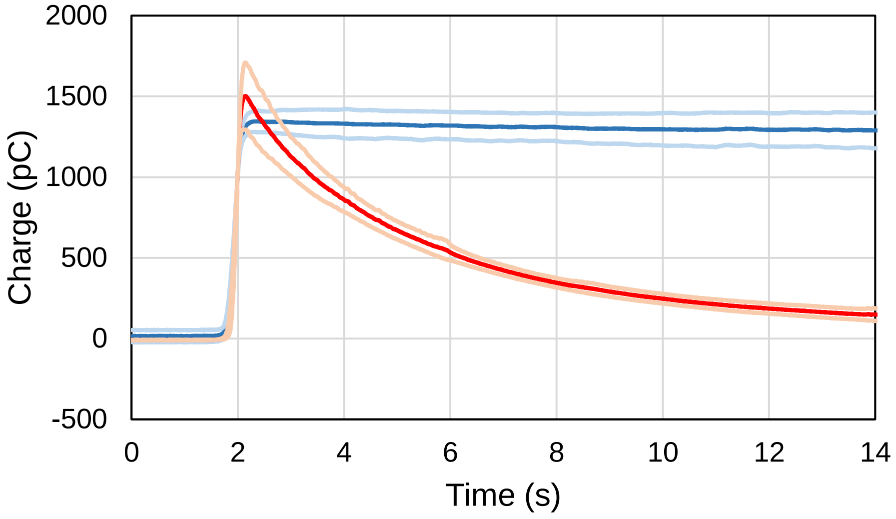

Figure 3.8. The mean decay of charge from a nylon ball (red) and a bumblebee (blue) charged positively by triboelectrification, then held in a Faraday pail. Faint lines show ±SD, N=5 for each.

3.4.2 The effect of relative humidity

The rate of charge decay on a material increases with increasing relative humidity. To further investigate the charge decay on bumblebees in varying humidity, an experiment was conducted using the same 5 bees to measure the rate of triboelectric charge gain and loss whilst changing the relative humidity in the room. The bumblebee was charged by gently rubbing it along a 10cm long length of polystyrene 10 times and held in the Faraday pail as before. The relative humidity in the room was manipulated using a humidifier (A28LW, Maplin, UK) and the initial charge on the bee, and the charge lost over the 30s period was compared for bees in each humidity (Figure 3.9). The charge decay on 5 bees was measured in 7 different relative humidities, and the mean charge decay in each humidity was compared (Figure 3.9).

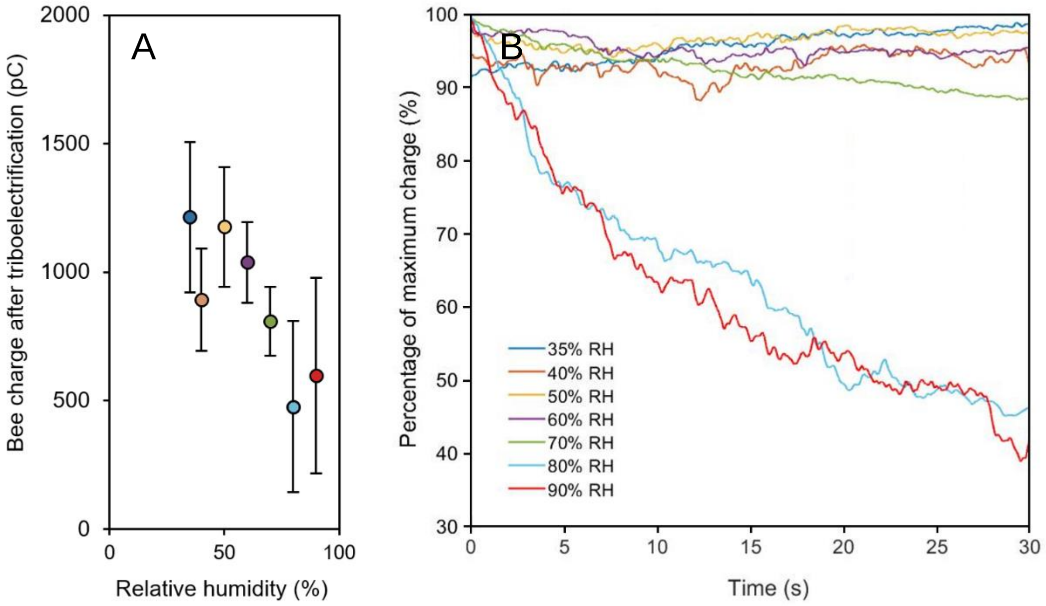

Figure 3.9. A) The mean charge gained by bumblebees by triboelectrification and B) the charge subsequently lost to the air over time in different relative humidity. The mean charge gained by the bee after rubbing against polystyrene decreased as the relative humidity increased. The charge on the bees dissipated more rapidly in higher humidity. After 30s of being held in the pail, the bees in 80-90% RH had lost over half their charge whilst the bees that were in < 70% RH still retained over 90% of their original charge. Error bars show SD.

The charge retention of bumblebees was significantly affected by the relative humidity of the air, with bees gaining charge much less effectively in higher humidity (Figure 3.9A) and the charge being lost much more rapidly when the humidity was over 70% (Figure 3.9B). This result is consistent with the literature, where triboelectric charging is shown to be much less effective in higher relative humidity (Németh et al. 2003; Nomura, Satoh, and Masuda 2003; Schella, Herminghaus, and Schröter 2017) and where charge is lost much more rapidly when the surrounding air is more humid (Nomura, Satoh, and Masuda 2003; Elajnaf et al. 2007; Lutz and Kindersberger 2009). The retention of charge in bees raises several questions for bumblebee ecology. Has the adaptive value of bumblebee electric charge driven morphological adaptations causing charge retention such as hairs and waxes? Do bees gain and retain charge better than other, non-pollinating insects? Is insect pollination less efficient in humid weather? The extent that relative humidity affects the charge on bumblebees flying outdoors is further explored in Chapter 4, and the generation and retention of electric charge in bumblebees is compared to that of other insects in Chapter 5.

3.5 SUMMARY OF RESULTS

The position of bumblebees at the top of the triboelectric series has now been firmly established, with both live and dead bees gaining a positive charge upon contact with any tested material. This includes flowers, with bees always gaining a more positive charge than flowers upon contact. Flowers can be ordered to create a floral triboelectric series, and the charge gained by a foraging bumblebee is affected by the triboelectric position of the flower. It appears therefore that the positive charge measured on bees is at least partly a product of triboelectrification. Bees also gain charge during flight, with bee charge increasing during flight until it nears saturation. The charge gained by bees is retained well, especially in low humidity where charge loss is minimal. These results suggest that bumblebee physiology and perhaps cuticular structure is optimised for the triboelectric gain and retention of charge, which would provide an adaptive advantage for pollen retention and electroreception.

3.6 DISCUSSION

In this chapter I have demonstrated that bumblebees can gain a positive charge through a variety of methods. Charge transfer during triboelectrification between bees, dielectric materials and flowers demonstrates that bees readily become positively charged upon contact with most materials. Bees gain a positive charge when foraging on flowers, which is likely to increase the attraction of pollen to the bee, thus increasing the foraging efficiency of the bee and the reproductive success of the flower. Bees also gain a positive charge during flight although this is likely to only occur when the bee has a low initial charge. Above 17pC it is likely the bee will lose charge to its environment, though bees retain their charge very well in comparison to materials with similar triboelectric properties. This 17pC saturation is greater than the charge frequently measured on many insects

(Table 1) including honeybees (Lighthart et al., 2005) and flies (McGonigle and Jackson, 2002) but lower than the mean charge previously measured on bumblebees (Clarke et al., 2013). This suggests that this charge is boosted by triboelectric interactions between the bee and its environment. The triboelectric gain and retention of charge shown in this chapter conveys ecological benefits to the bee by increasing the bee's ability to attract and carry pollen and increasing sensitivity to electrical stimuli. Further questions arise, including whether these charges are found on bumblebees foraging in nature and whether these charging mechanisms apply to other insects.

Flying bees have higher charges than walking bees (Figure 3.7A), suggesting charge generation appears to be at least partially a product of flight. Bee charge increases with flight duration over short flights, reaching peak charge after 10s of flight (Figure 3.7B) but subsequently does not increase further. The pattern of charge gain is similar to that found in walking houseflies by McGonigle and colleagues (McGonigle, Jackson, and Davidson 2002) where the rate of charge gain decreased as flies neared a saturation charge. The charges on bees flying for the longest durations are not as high and are generally more variable, suggesting bees charge up initially and that the charge leaks away over time. Measurements over longer flight durations will inform this process, but are impractical under laboratory conditions, as the dimensions of the flight arena discourage prolonged flight and bees airborne for long periods of time often collided with the walls and roof of the arena. Es'kov and Sapozhnikov (1976) noted that honeybees that had been flying long distances (200m) did not have higher charges than bees that had flown short distances (5m) and noted that flight duration had no apparent effect on their electrification (Es'kov and Sapozhnikov 1976). The results presented here on charge and flight duration in bumblebees corroborate these findings. I propose that bumblebees generate a charge during flight, but this charge reaches saturation quickly, after which time further flight does not cause increased charging. A bee flying 5m will therefore have a similar charge to a bee that has flown 200m, although differences may be observed between bees flying very short distances.

The saturation charge on flying bumblebees can be exceeded via triboelectrification once the bee has landed on a surface. The saturation charge is not the maximum charge that a bee can possibly have but is the charge at which the electron affinity of the bee is equal to that of the surrounding air. Above this point the charge will leak away, potentially via adsorption of ions present in the air or via conduction with any surface it contacts. However, as shown, bees are also very good at keeping their charge. The modelled saturation charge on a flying bumblebee is 17pC. A bee with an initial charge lower than 17pC will gain charge during flight. A bee with a charge of higher than this is likely to slowly lose charge over time. The charge on a foraging bumblebee is likely to temporary increase upon each subsequent flower visit.

Charge generation and retention during flight ensures a bee has a positive charge as it approaches a flower. As pollen is predominantly negatively charged, this promotes initial attraction and adhesion of pollen onto the bee. The positive charge of the bee is then elevated as the bee rummages around on the flower, with the extent of triboelectrification depending on the flower triboelectric position and the degree of friction between bee and flower. This charge separation during triboelectrification between bee and flower promotes the attraction of loose pollen present on the flower to the oppositely charged bee and promotes the subsequent continued adhesion of pollen to the bee during flight. The charge gain during flight and triboelectrification with flowers coupled with the slow rate of charge decline on bumblebees is likely to contribute to the positive charge measured on flying bumblebees (Figure 3.10).

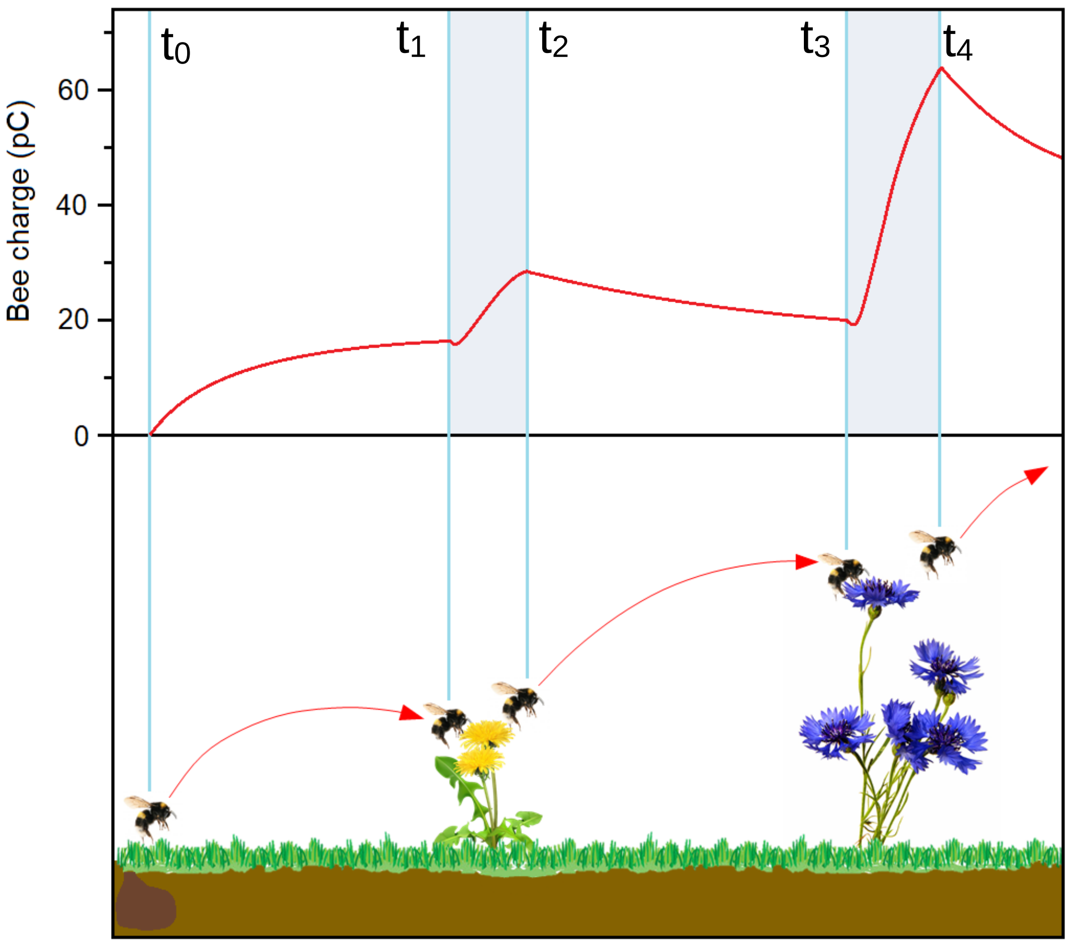

Figure 3.10. Diagrammatic representation of the theoretical electric charge on a bumblebee during a foraging trip. As the bee exits the colony (t_0) it will have a very weak electric charge (from charges measured on bees exiting a colony in Figure 3.6). As it flies, it will gain charge at a decreasing rate until it reaches a saturation charge of around 17pC (from Figure 3.7B). When it approaches and lands on a dandelion flower (t_1), the positive charge on the bee will attract negatively charged pollen from the flower. Initially the bee may lose charge to the flower by conduction, but as it moves over the flower, the bee will gain a positive charge from the dandelion flower by triboelectrification (median charge gain 8pC from Figure 3.5). When the bee leaves the dandelion flower (t_2) the charge on the bee is greater than its saturation charge, so the bee will slowly lose charge to the surrounding air. As the bee approaches a cornflower (t_3) the positive charge on the bee will attract negatively charged pollen from the cornflower. The bee will gain charge triboelectrically whilst foraging on the cornflower. The cornflower is low on the triboelectric series so the bee will gain a highly positive charge (median charge gain 44pC from Figure 3.5). The bee will leave the cornflower (t_4) with a highly positive charge (64pC) which will then slowly decline as it flies.

The amount of charge gained by the bee is likely to be affected by the floral morphology affecting the contact area between bee and flower. Surface contact area affects bee charging, as contact area between materials as well as direction and strength of friction affects the direction and extent of charge transfer (Lowell and Truscott 1986; Lacks and Sankaran 2011), especially on hairs (Martin 1941). Flowers that are relatively flat (such as dandelions and cornflowers) that will mostly only touch the ventral surface of the bee, may cause bees to charge up less than flowers that ensure the bees must rub all of their body against petals to access nectar (e.g. vipers bugloss, snapdragons). Contact between the top of the bee and the petals of flowers is likely to increase the extent of triboelectric charging, due to the increased contact area between bee and flower, with the hairy dorsal surface perhaps charging better than the legs and the head upon contact. Hair and fur have historically been placed at the top of many triboelectric series (Diaz and Felix-Navarro 2004), and bees have been shown to be no exception (Clarke, Morley and Robert 2017). Thus, a floral morphology encouraging contact between the bees hairy dorsal surface and the petals may cause greater charging in bees, and may have adaptive value to the plant by promoting adhesion of negatively charged pollen to the bee, and hence increasing the chances of that pollen reaching subsequent flowers and resulting in successful pollination. The charge transferred by triboelectrification is likely to increase with time spent on the flower and increasing contact time between bee and petals. As floral morphology is highly variable and often specialised, the evolution of floral structures may have been influenced by the adaptive value of bee-flower triboelectrification. This may have played a role in the evolution of physical barriers between the bee and the nectary (such as in snapdragons), which ensure the bee remains on the flower for longer and rubs against it as much as possible.

The floral triboelectric series established in this chapter does not show any obvious link between flower morphology and bee charge, but the series was established by rubbing the whole of the bee body against the flower, and as such is not biologically representative of the surface contact area between bees and flowers in nature. The measurements of bee charge before and after visiting flowers is more representative of the contact between bees and flowers, yet this also does not suggest that floral morphology is the only factor affecting bee charge gained. Two flower species with similar morphology (cornflower and dandelion) cause bees to charge to different extents. Measurements of charge on bumblebees visiting flowers with vastly different morphologies would inform the extent of triboelectric charging during foraging.

To better understand the tribology of bees, material surface properties should also be considered. Bee cuticle and hairs contain chitin, an insulating piezoelectric and dielectric material (Majtán et al. 2007; Ghosh and Mandal 2017). Bumblebee cuticle is also covered by a layer of wax. This waxy layer may play a role in the charge transfer between the bee and its surroundings by affecting the tribology of the bee, the conductivity of the cuticle and the adsorption of ions. The flower also has a complex heterogenous surface, with the macroscopic and microscopic surface structures varying vastly between flower species. Petals also contain a surface layer of wax (Buschhaus, Hager, and Jetter 2015; Guo et al. 2017), the chemistry of which may affect the interaction between flowers and bees. Rose petals have been proposed for use in triboelectric nano-generators, as they become positively charged when contacted with PMMA, a highly triboelectric plastic, producing small currents sufficient to light an LED (Chen et al. 2018). Chitin has also been investigated for the same mechanism with the potential for use in green electronics such as implantable medical devices (Jin et al. 2016; Jiang et al. 2018). Thus, the investigation into the triboelectric properties of natural materials such as insect cuticle and plant surfaces is not just vital for understanding pollination, but could reveal previously overlooked materials for use in bio-inspired energy harvesting technology.

4 THE ELECTRIC CHARGES OF BUMBLEBEES FLYING OUTDOORS

Some of the work in this chapter is accepted for publication in the Journal of Physics, Conference Series.

4.1 INTRODUCTION

In this thesis I have shown that bumblebees flying in the laboratory are typically positively charged, and that this charge is likely to be a function of their triboelectric properties. Laboratory experiments are useful for isolating and identifying the charging mechanisms of bumblebees and form the basis for investigating the role of bee charge in pollination. Models of pollen transfer have been previously constructed showing that the Coulomb force acting on pollen are sufficient to propel grains between bee and flower (Clarke, Morley, and Robert 2017) assuming the bumblebee has a 32pC charge based on laboratory measurements (Clarke et al. 2013). These models are likely to be realistic only if the charges on bees flying in nature are similar in polarity and magnitude to those charges measured in the laboratory. Whilst some literature exists concerning the electric charge of honeybees (Erickson 1975; Es'kov and Sapozhnikov 1976; Colin, Richard, and Chauzy 1991), the charges carried by bumblebees foraging in nature are currently unknown.

The generation of electric charge can be affected by variation in the local environment. In triboelectric charging of powders and polymers, relative humidity often has the greatest effect on charge generation, as higher humidity increases air conductance and prevents local build-ups of charge. Studies have shown that powders accumulate much lower charges in higher humidity, which has been verified with triboelectric charging of powders (Eilbeck et al. 2000; Németh et al. 2003; Nomura, Satoh, and Masuda 2003; Young et al. 2007) as well as pollen (Banerjee and Law 1998). This is largely due to the increased conductance of wetter surfaces allowing charges to spread more evenly across a surface, preventing local build-up of charge. Bumblebees have been shown in the laboratory to gain less charge in higher humidity and for any charge gained to dissipate more quickly (Chapter 3).

Lu et al. (2017) showed that temperature can affect charge generation, with triboelectric nanogenerators (TENGs) being less efficient in higher temperatures due to a temperature-induced change in electron affinity of the triboelectric material (Lu et al. 2017). Zimmer (1970) found that the tendency of polymers to become negatively charged was reversed at high temperatures but these temperatures (>90°C) by far exceed those experienced by bumblebees (Cross 1987; Bailey 1993).

Pressure and humidity have both been found to affect charge in triboelectric charge-generation devices (Nguyen and Yang 2013), with increased humidity causing a reduction in charge gain, and increased atmospheric pressure causing an increase in charging. However, it is currently unknown to what extent environmental factors affect charging in insects. To address this knowledge gap, an experiment was conducted to measure the electric charge on bumblebees flying outdoors in natural conditions. The aim of this experiment was firstly to ascertain whether laboratory measurements of bee charge were representative of those of free flying bumblebees, and hence to evaluate the predictive value of previous models of pollen transfer and electroreception. Secondly, because the triboelectric properties of materials are known to be affected by relative humidity, the charges on bees flying in different electrical and meteorological conditions were compared to better understand the mechanisms affecting charge in outdoor bees.

4.2 METHODS

4.2.1 The field site

The field site was situated at the University of Bristol School of Veterinary Medicine in Langford, North Somerset, UK (51° 20' 47.58'' N 2° 46' 44.3928'' W, Figure 4.1). The site consists of a L-shaped laboratory building housing the bumblebee colonies, and an adjacent area roughly 40m^2. The patch of land contains 3 honeybee hives, the field mill and a weather station to provide continuous measurements of the local weather conditions alongside measurements of the atmospheric potential gradient (APG). An urban pollinator seed mix (Habitat Aid, UK) of bumblebee-friendly wildflowers was planted on this land to provide nourishment for the bumblebee colonies.

Figure 4.1. The field site at the University of Bristol School of Veterinary Medicine in Langford, North Somerset, UK. The location of the field mill, the weather station and the room containing the bumblebee colonies are indicated. Image credit: Google maps.

4.2.2 The bumblebee colonies

Over the duration of the experiment, three colonies of *Bombus terrestris audax* (Koppert, UK) were established inside a bee observation room at the field site. Each colony was connected to the outside via a polycarbonate tube which fed through a hole in the wall and allowed the bees to access the colony (Figure 4.2A). The external entrance to these tubes was via a plastic cup, rimmed by two concentric copper rings forming a ring charge sensor (RCS; inner diameter 90mm, outer diameter 92mm, width 28mm, Figure 4.2B). The inner ring was connected to a picoammeter and used to measure the charge on the flying bees. The outer ring acted as an electric shield to prevent the inner ring being influenced by the movement of charge outside the ring. To enter the colony the bees had to fly through the ring, and land on the plastic cup before crawling through the tube. Bees typically landed as close to the tube entrance as possible to minimise walking distance, so rarely landed on the ring. Likewise, bees leaving the colony usually took flight as soon as they were able. Bees rarely landed or walked on the ring, but any measurements taken when bees were in physical contact with the rings were discarded. The ring was partially shielded from wind and rain by a metal box situated around the entrance to the colony. A metal awning at the top of this box prevented rain wetting the measurement ring which could affect conductance and therefore reliability of charge measurements (Figure 4.2B).

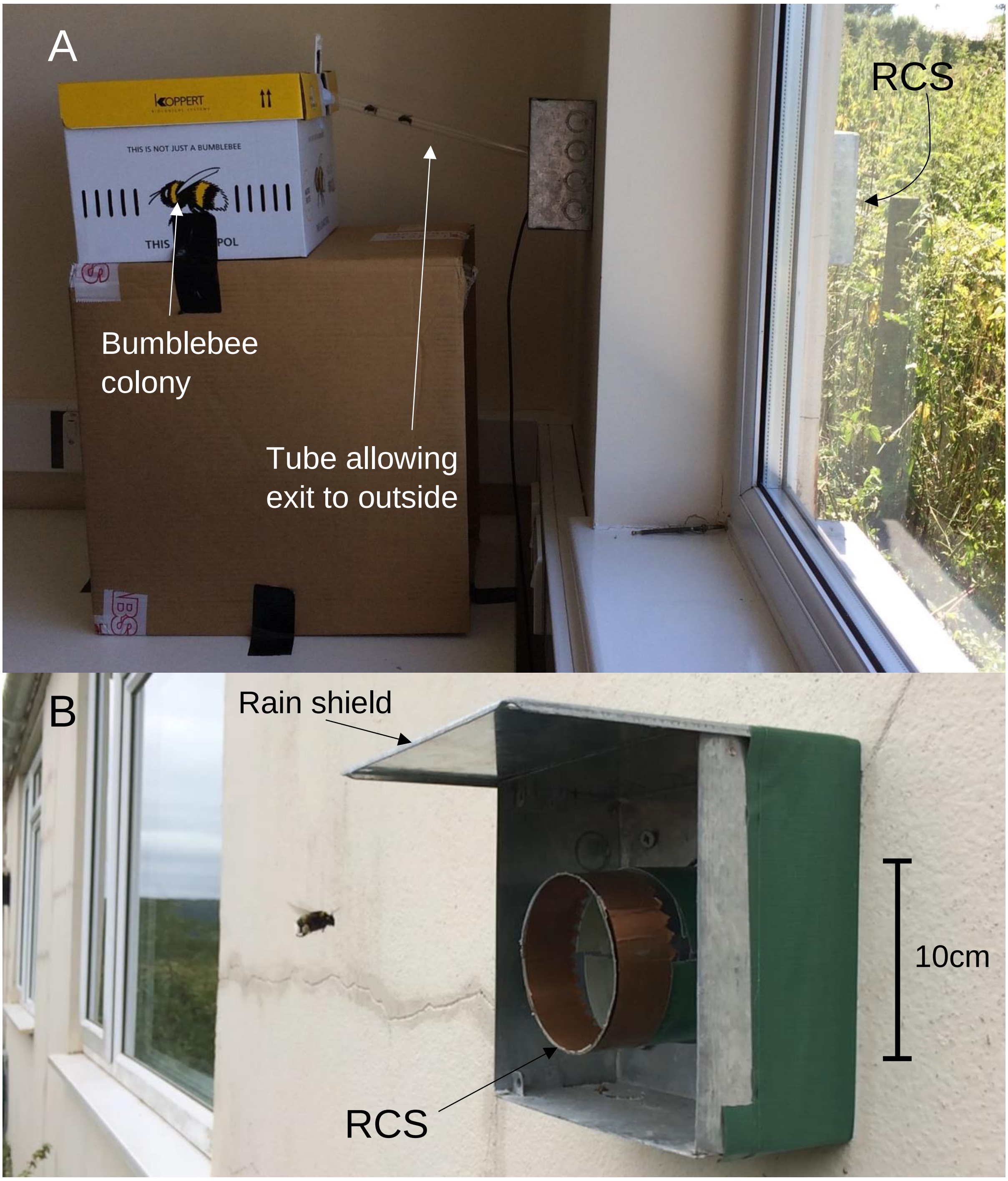

Figure 4.2. Measuring bumblebee charge in natural flight conditions. A) The position of the bumblebee colony relative to the RCS. A transparent polycarbonate tube connected the colony to the RCS allowed bees to enter and exit the colony through the wall of the laboratory. B) A bumblebee approaching the RCS after a foraging flight outdoors. The metal box surrounding the RCS provided electrical shielding and shelter from wind and rain.

4.2.3 Bee charge measurements

Measurements of bee charge were collected by connecting each ring to a picoammeter via a shielded BNC cable. The data were read continuously from the picoammeter at a sampling rate of 1kHz via a NIDAQ data acquisition chassis (CompactDAQ Chassis National Instruments) containing a voltage input module (NI-9215, National Instruments) and analysed in MATLAB 2014. A low pass filter was applied to the signal to filter out 50Hz electrical noise.

During the autumn, there were two bumblebee colonies housed in the laboratory simultaneously. These colonies used separate entrances with separate RCSs, labelled 2B and 4A. The inducted current measured from both picoammeters was recorded simultaneously using separate channels of the data acquisition system. The picoammeters were calibrated in the laboratory using a 10GΩ resistor before and after the field measurement period, ensuring accurate recording of induced currents. Both RCSs were also calibrated *in situ* with a Faraday pail at several points throughout the data collection periods in varying conditions (see RCS Calibration).

A camera (C12SB Omega, Taiwan) was used to visually confirm the flight of the bumblebees into each colony through the RCS. Each camera was placed 60cm in front of each RCS, facing the colony and mounted on wooden poles to be the same height as the RCS. The cameras allowed confirmation of the direction of flight and whether the bee was carrying pollen. It also allowed qualitative identification of the picoammeter current and confirmation that a change in current was caused by the movement of a bee passing through the ring. Only flights where the bee approached the ring in a straight line and flew at an approximately constant speed were included. Flights where the bee touched the ring were discarded, as were flights with multiple bees, and flights where the bee hovers around the ring.

The identification of individual bees was not feasible, as identifying markers (such as RFID tags or paint on the thorax) would be likely to affect the charge of the bees. Because of this it is likely that multiple measurements were made on the same bees. However, this is mitigated by the prolonged study period and the use of three distinct bumblebee colonies. Each colony contained 200-500 bees, most of which left the hive to forage. Whilst a small amount of pseudoreplication likely exists due to a few repeated measurements on individual bees, the large sample size and the long study period ensure that the measurements collected are representative of the charges carried by *B. terrestris* under natural conditions.

4.2.4 Data collection periods

The charges on bees were measured from three colonies over two different time periods over the summer and autumn of 2018. The summer data collection period ran for five days from 28/06/2018 to 06/07/2018 measuring from a single bumblebee colony (Colony 1). The autumn data collection period ran from 24/08/2018 to 15/10/2018 and spanned ten days of data collection spread throughout this period. Over this time the charges were measured on bees from two colonies (Colonies 2 and 3).

During the summer period, Colony 1 was established in June during a period of very fine weather and the charges were measured on individual foraging bumblebees over this period. During this time, the temperature and relative humidity were measured using a thermometer-hygrometer (RT811C, Compact Hygro-Thermometer Clock, Radiance Instruments Limited, Guangdong, China).

The bulk of the data collection was during the autumn, from 24/08/2018-10/10/2018. This was done with two fresh bumblebee colonies with accurate measurements of meteorological conditions from the weather station and electric field measurements from the field mill. The autumn dataset therefore has more complete and extensive weather measurements as well as data for the electric field strength and the concentration of ions present in the air. For the meteorological measurements over the autumn period, precipitation data was collected using a Maximet GMX100 optical rain gauge (Gill Instruments Limited, Hampshire, UK). Temperature, relative humidity, pressure, solar radiation and wind speed and direction were measured with a Maximet GMX501 compact weather station (Gill Instruments Limited, Hampshire, UK).

Measurement of the electric field was collected via a field mill (Boltek EFM-100), on a 2m high pole using an EFA-21 combined power module and RS-485 to USB adaptor. The sampling frequency of the field mill was 1Hz, and the range measured up to 20kV/m. Ion concentration and polarity was measured using two air ion counters (AlphaLab, Utah, USA), placed adjacently on a shelf between the two bumblebee colonies. One counter was set to measure positive ions whilst the other set to measure negative ions. The measurement polarity of the counters was alternated periodically to account for any bias in measurements from either counter. The casing of the ion counters was electrically grounded and the output from each counter was measured continuously in MATLAB using the NIDAQ data acquisition hardware.

The summer collection period has lower precision for its meteorological measurements and does not contain electrical data for ion count or electric field. However, the summer period has much higher temperatures and recorded a greater range of bee charges. The variables measured are summarised in Table 4.1. The datasets from the summer and autumn collection periods are analysed separately due to the difference in measurement equipment available during those periods.

Table 4.1. The data collection periods and the meteorological parameters available for each day of measurement. The ion count data was dependent on both ion counters functioning reliably for continuous measurement of both positive and negative ions. On days where only one ion counter was working, the working ion counter measured the concentrations of positive and negative ions alternately. During these periods the measurements of positive and negative ions are therefore not simultaneous. On Day 12, one of the counters malfunctioned part way through the day, so there are partial measurements for that day.

Date	Day of measurement	Data collection period	Colony	Weather station active?	Field mill active?	Measurement of positive and negative ions	Weather	N bees total	N bees inbound	N bees outbound	Start time	End time
28/06/2018	1	Summer	1	No	No	No	Sunny	11	5	6	15:11:07	15:32:50
29/06/2018	2	Summer	1	No	No	No	Sunny	26	17	9	10:57:59	11:44:46
02/07/2018	3	Summer	1	No	No	No	Sunny	55	26	29	16:00:26	17:04:08
04/07/2018	4	Summer	1	No	No	No	Rain	42	19	23	12:01:05	13:35:58
06/07/2018	5	Summer	1	No	No	No	Sunny	51	28	23	10:38:09	11:33:28
24/08/2018	6	Autumn	2	Yes	Yes	Not simultaneous	Unsettled	33	24	9	11:21:27	14:10:00
28/08/2018	7	Autumn	2 and 3	Yes	Yes	Not simultaneous	Sunny/Cloudy	289	161	128	10:47:52	16:17:00
30/08/2018	8	Autumn	2 and 3	Yes	Yes	Not simultaneous	Sunny/Cloudy	119	71	48	14:00:10	17:08:00
10/09/2018	9	Autumn	2	Yes	Yes	Yes	Sunny	34	23	11	16:14:00	16:54:00
13/09/2018	10	Autumn	2 and 3	Yes	Yes	Yes	Sunny	148	77	71	14:42:00	15:57:00
24/09/2018	11	Autumn	2 and 3	Yes	Yes	Yes	Sunny	164	95	69	13:38:38	14:39:00
25/09/2018	12	Autumn	2 and 3	Yes	Yes	Partial	Sunny/Cloudy	86	41	45	13:06:47	13:58:00
28/09/2018	13	Autumn	2 and 3	Yes	Yes	Yes	Sunny/Cloudy	238	127	111	11:54:13	15:00:00
06/10/2018	14	Autumn	2 and 3	Yes	Yes	Yes	Rain	31	15	16	12:59:52	13:53:00
15/10/2018	15	Autumn	3	Yes	Yes	Not simultaneous	Rain	33	18	15	10:23:34	11:48:00

4.3 RCS CALIBRATION

The rings were calibrated at 4 different times during the experiment, at the start and end of both the summer and autumn data collection periods. The calibration process involved removing each ring from the wall (whilst ensuring it remained connected to the picoammeter) and placing it over the rim of a Faraday pail (JCI 141, Chilworth Global, Southampton, UK). Cubes of polyurethane foam (10mm x 10mm x 10mm) were charged by firing charged ions at the cubes using an anti-static gun (Milty Zerostat 3). The cubes were given a range of positive and negative charges (-100 to +200 pC) and dropped through the ring into the pail (Figure 4.3). The increase in charge registered by the pail is compared to the integral of the current measured by the ring to calibrate the ring with the pail (Figure 4.4). The temperature and relative humidity was recorded during each calibration.

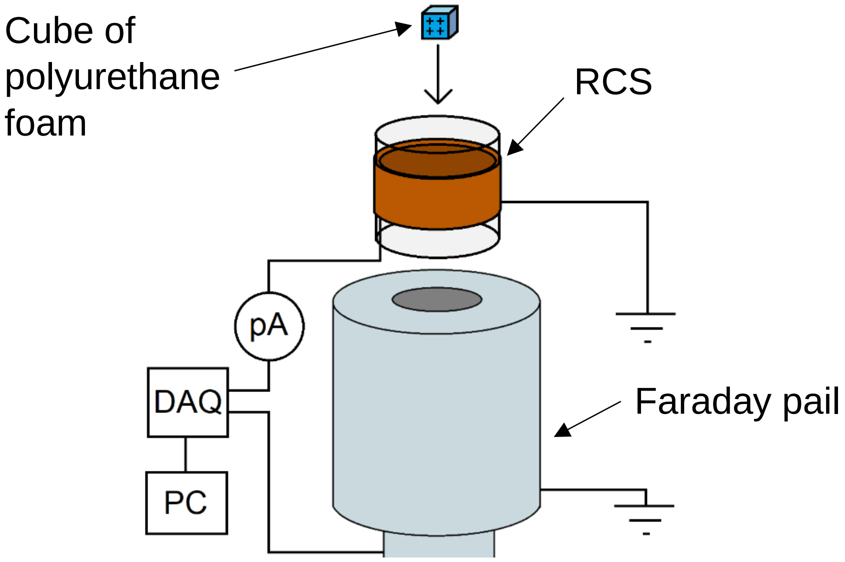

Figure 4.3. Calibration of the RCS with the Faraday pail. The RCS was removed from the wall and placed over a calibrated Faraday pail. Cubes of polyurethane foam were electrically charged using a Milty static eliminator gun and dropped through the RCS into the pail. This allows direct comparison of the charge measured by the RCS with the charge measured by the Faraday pail.

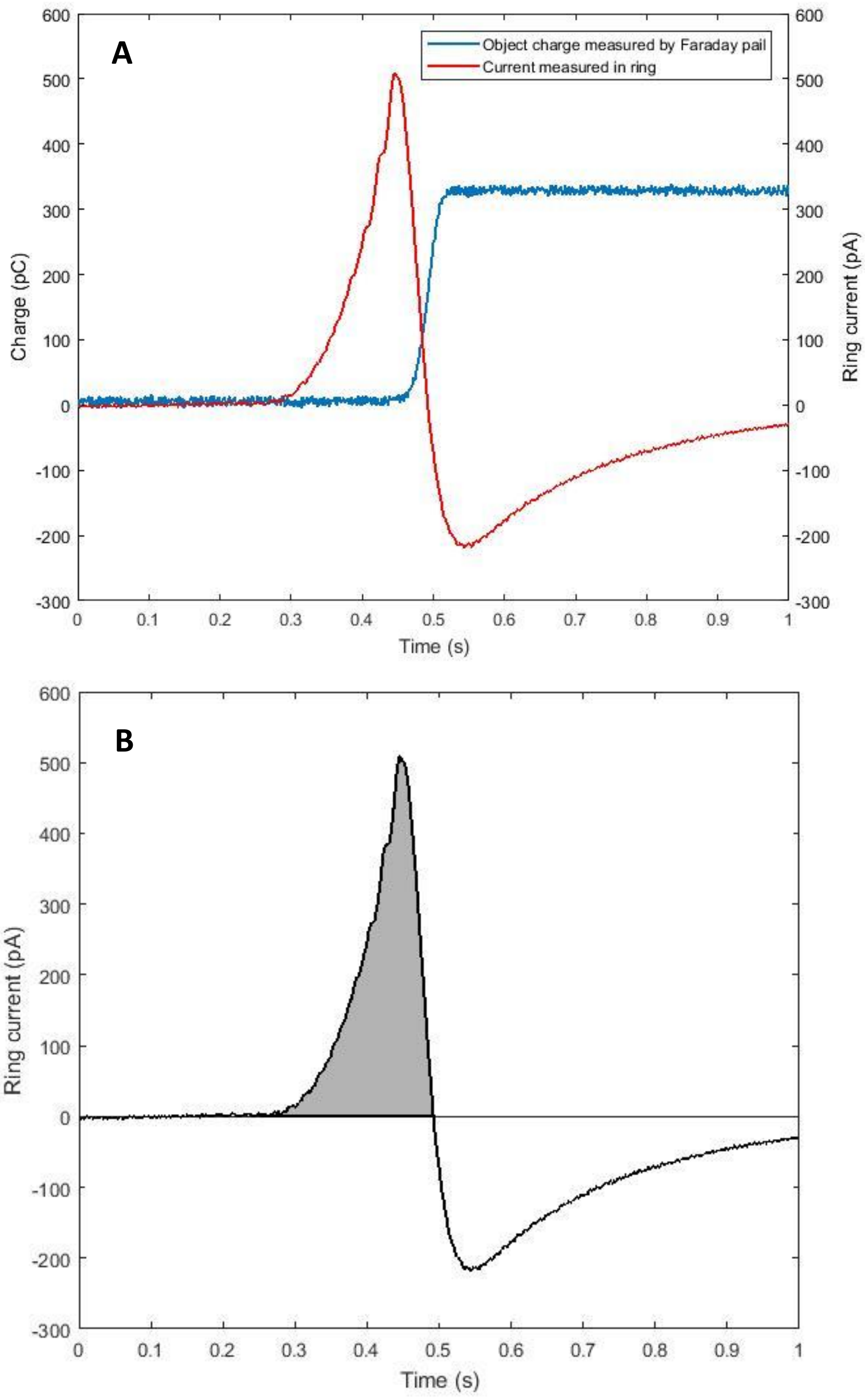

Figure 4.4. Time-resolved electrical charge measurements. A) Example calibration signal showing the charge in the pail and the current in the RCS ring induced by dropping a charged polyurethane cube through the ring into the pail. B) The section of the current curve integrated during measurements. Shaded area shows the section integrated for charge.

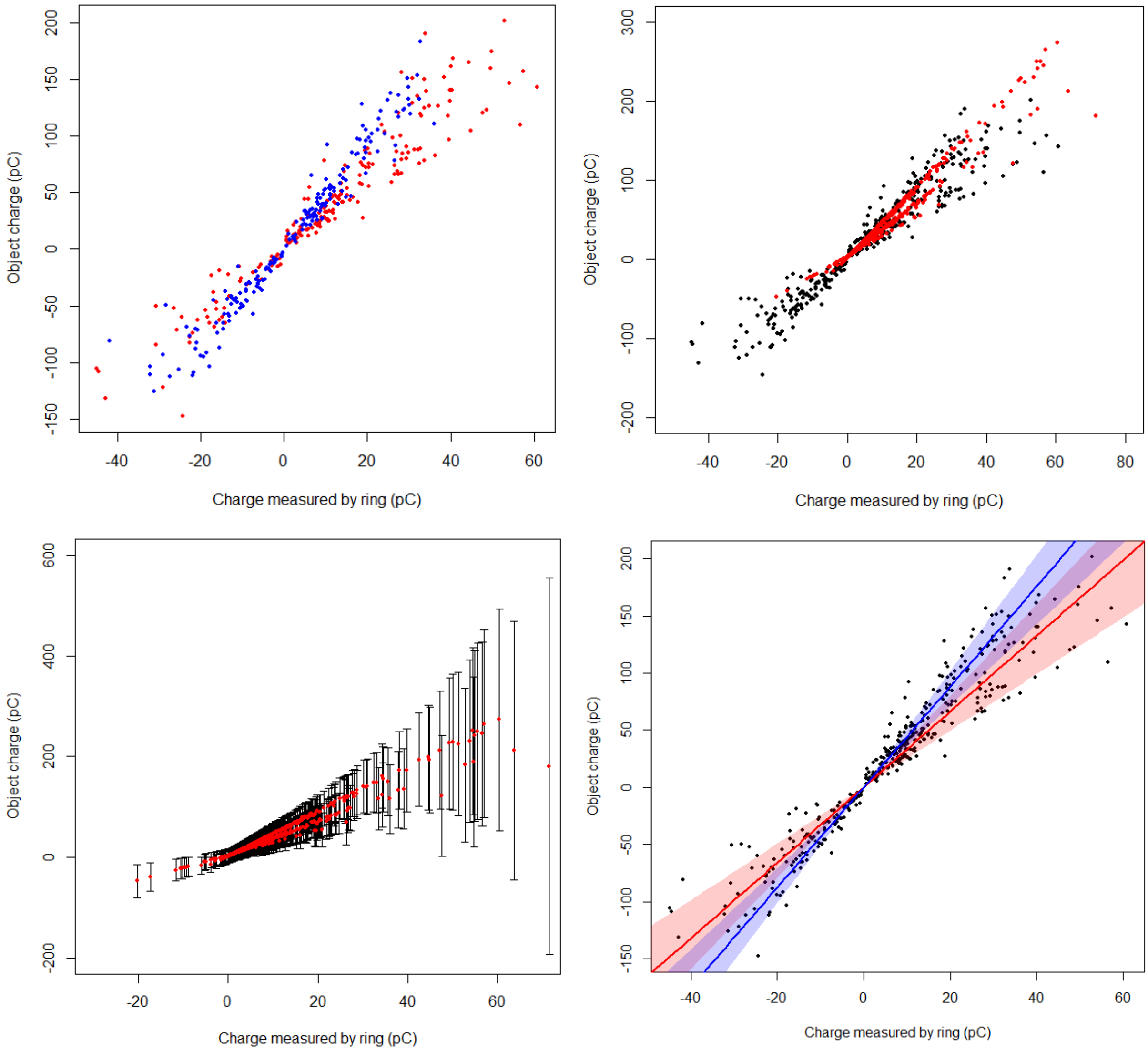

Figure 4.5. Quantification of RCS calibration with Faraday pail measurements. A) The calibration data for cubes of polyurethane foam dropped into a Faraday pail through ring 4A (blue) and ring 2B (red) showing the object charge of the foam as measured by the Faraday pail and the charge induced in the rings. B) Calibration data (black) and the predicted bee charges (red) calculated using the model. The slightly striated nature of the predicted bee data is due to the calculations using the two different RCS rings. C) Bee charges predicted by the charge induced in the rings ± 95% prediction intervals. D) Calibration data (black points) and the predicted charge values for bees flying through ring 2B (red) and ring 4A (blue). Charges are predicted for bees flying in low humidity (30%RH, upper shaded boundary) average humidity (61%RH, solid line) and high humidity (99%RH, lower shaded boundary).

The ring was not calibrated during rain to prevent damage to the Faraday pail. The calibrations were conducted in conditions representative of those experienced by foraging bees, from 45-80% relative humidity, representative of the conditions of 95% of the bee charge measurements. The other 5% were taken during rain when it would have been impractical to calibrate the ring and the pail. The integral of the current measured by the ring is linearly correlated with the charge measured with the Faraday pail (Figure 4.5). This is because current is the flow of charge from equation 2.3, so the total charge transferred over time can be calculated from the current by integrating:

$$q(t) = \int_0^t i \tag{4.1}$$

The charge on bees was measured in different flight conditions. To ensure that any differences in charge measured under different conditions were due to changes in the bees' charge and not simply differences in the rings' sensitivity, the RCS were also calibrated in different conditions. This accounts for differences in sensitivity between the RCS rings (one on each colony entrance) as well as the differences in sensitivity caused by varying humidity or other meteorological conditions.

A linear model was created to investigate the effects of humidity, pressure, electric field and wind speed on the relationship between the ring charge of both rings and the Faraday pail charge. Of these variables, only humidity was found to affect the relationship. The model showed differences in sensitivity between the two RCSs (Figure 4.5A, D). This was to be expected given that both picoammeters had slightly different internal resistances and that different lengths of BNC cables were used with different connectors (although all of these variables were set up to be as uniform as possible and did not differ over the experimental period).

The best performing model was a General Additive Model that included the RCS identity and the relative humidity. This model showed that there was a very strong correlation between ring current integral and object charge (Figure 4.5), explaining 93% of the deviation. The modelled relationship shown here is similar to that found by Peltonen et al. (2015) who developed a similar probe to measure charge remotely and calibrated it with a Faraday pail with similar results (Peltonen, Murtomaa, and Salonen 2015). The GAM accounts for the increasing variation in the data as the charge gets higher, thus predictions made using the model account for the increasing variance and increase the associated error of the predicted values accordingly. As both ring identity and relative humidity were known at all points throughout the experiment these could be incorporated into the model to produce accurate bee measurements in all conditions.

4.4 RESULTS

4.4.1 Overall bee charges

4.4.1.1 Field bumblebee charges

Bumblebees flying into the colony over the summer and autumn data collection periods were typically positively charged with the vast majority (97.3%) of the bees measured carrying positive charges and 75.3% of bees having charges between 0-50pC (Figure 4.6). Previous studies with honeybees have shown that foraging bees are predominantly positively charged (90.3 % positively charged (Colin, Richard, and Chauzy 1991)) as are bumblebees foraging in the laboratory (94% positively charged (Clarke et al. 2013)). The charge magnitude measured on outdoor foraging bumblebees here is also similar to that previously measured on bumblebees in the laboratory (mean charge 32 pC (Clarke et al. 2013)) and on honeybees flying outdoors (mean charge 28.6pC (Colin, Richard, and Chauzy 1991)). The electrical charges carried by bees entering the colony were significantly higher than those on bees leaving the colony (Mann-Whitney, $P < 0.0001$, $W = 273296$, $N = 1175$, Figure 4.7, Table 4.2).

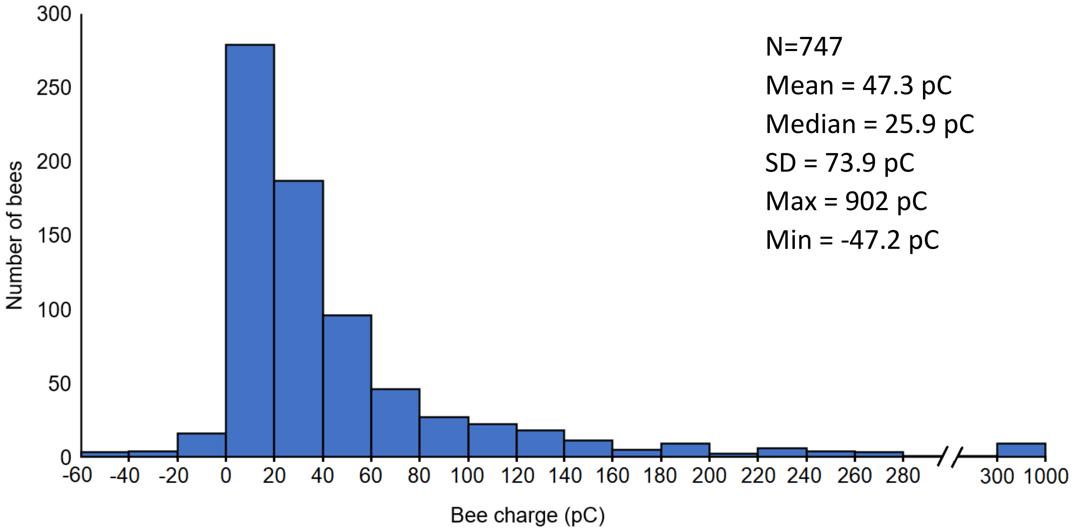

Figure 4.6. The electric charges measured on bumblebees after flight outdoors.

4.4.1.2 Differences in charge on incoming and outgoing bees.

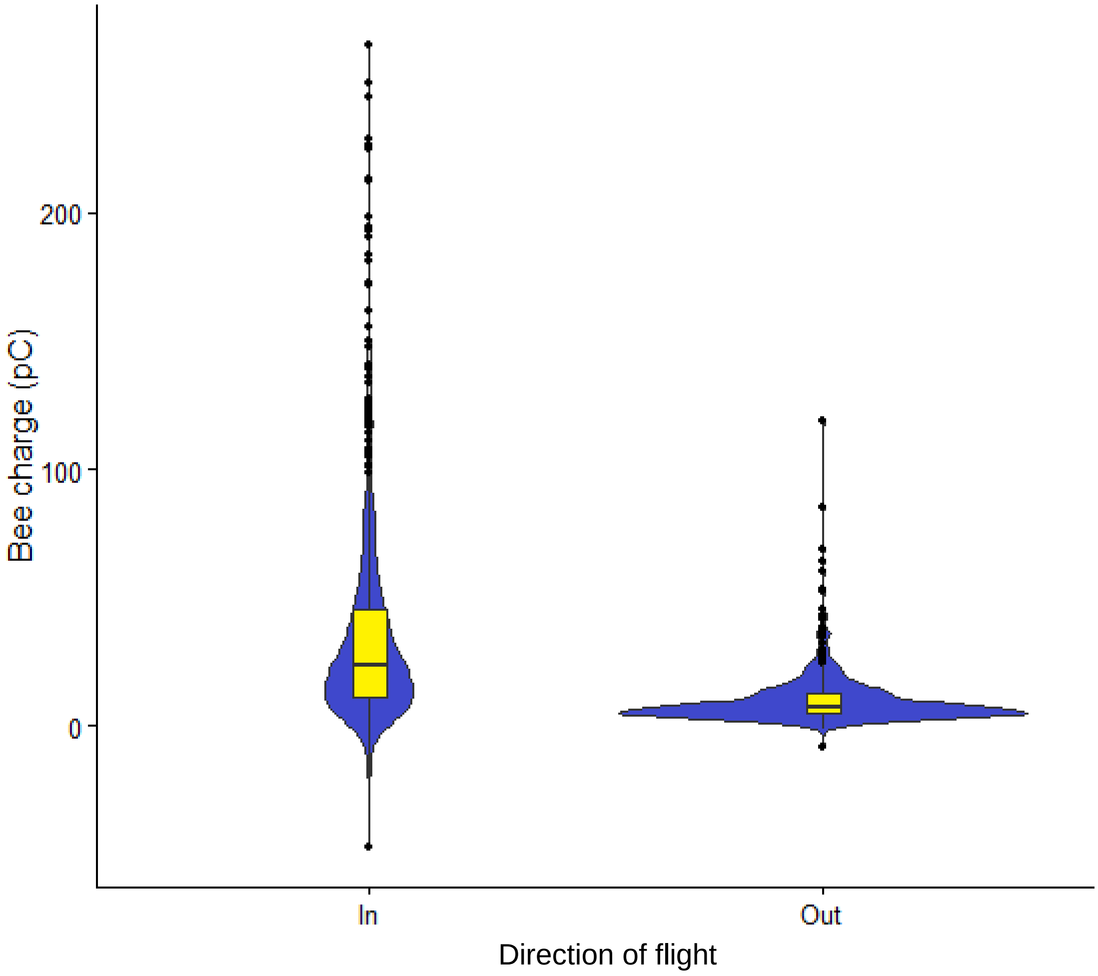

Figure 4.7. Violin plot showing the distribution of bee charges on bees flying in and out of the colony including data from the summer and autumn period. The boxplots show the median, IQR and range. Black points show data more than 1.5x the IQR.

Table 4.2. Descriptive statistics of the charge measured on bumblebees flying into and out of the colony.

	In	**Out**
N	747	613
Mean charge (pC)	47.2	13.8
Median charge (pC)	25.8	7.6
SD (pC)	73.9	20.6
Maximum charge (pC)	902.0	208.0
Minimum charge (pC)	-47.3	-7.9

4.4.1.3 Pollen baskets

During the autumn data collection period the bees were filmed when approaching the colony, and the presence or absence of pollen baskets on the legs of the bees was recorded.

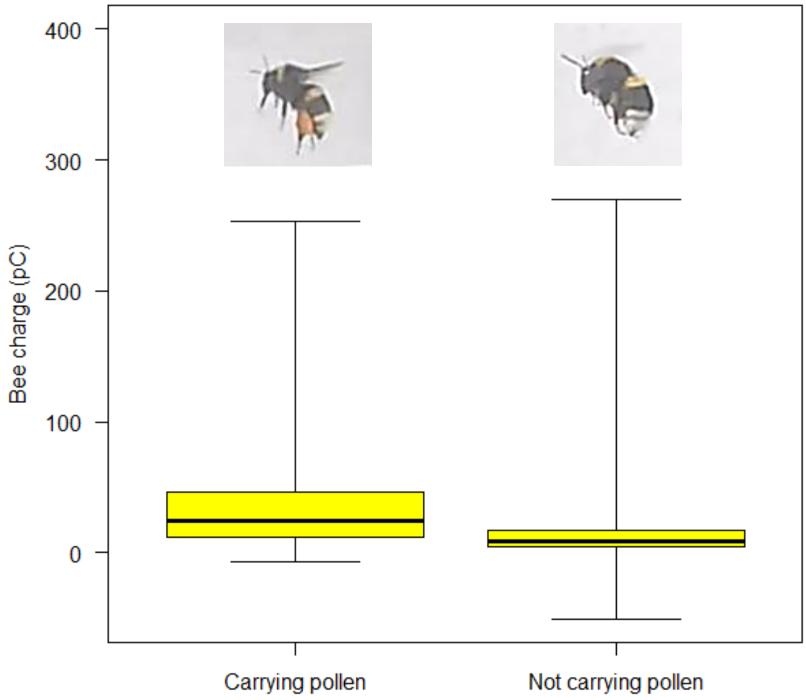

Figure 4.8. The charges on bees flying back to the colony with or without pollen. Photos above each box show the typical view of a bee returning to the colony with and without pollen.

Bees carrying pollen had significantly higher charges than bees without (bees carrying pollen N = 455, bees not carrying pollen N = 197, Mann-Whitney, W = 40086, P = 0.0322, Figure 4.8). Pollen acquisition declined over time throughout the autumn period. This is probably due to the needs of the colony changing over time, the weather declining towards the end of the measurement period, and also likely reflects the availability of pollen towards the end of the data collection period. On the last day of measurement, the majority of the bees flying into and out of the colony were queens and no bees were foraging for pollen.

4.4.2 The effect of weather and electrical conditions on bee charge

Over the summer data collection period (Days 1-5), the weather was predominantly warm and sunny, with one day of rain (Day 4; Table 4.1; Figure 4.9). During the autumn period (days 6-15) it was cooler and wetter, and the bumblebees had lower charges.

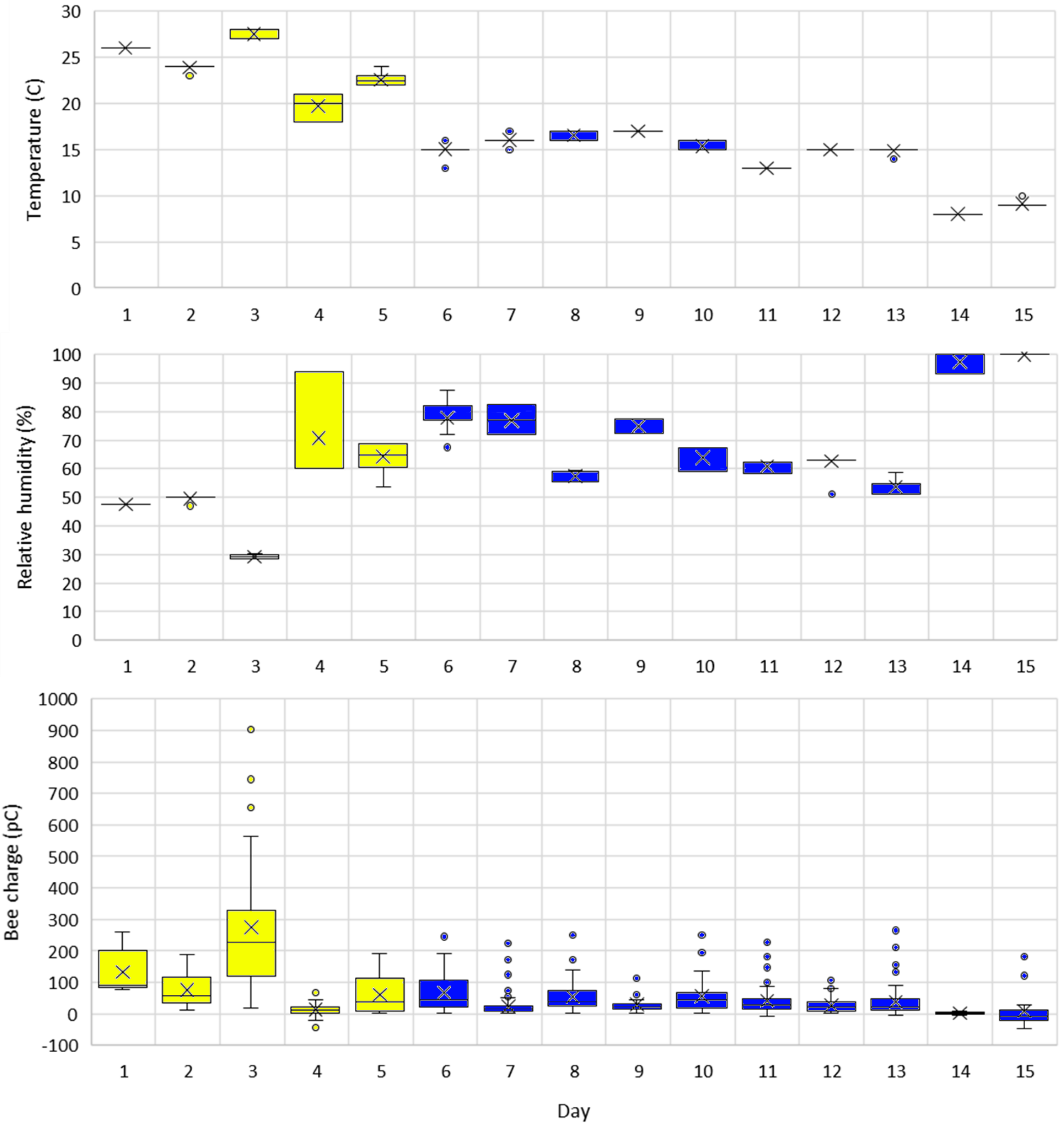

Figure 4.9. The temperature and humidity over the summer (yellow, days 1-5) and autumn (blue, days 6-15) and the charges measured on incoming bees on these measurement days. Bees had the highest charges on day 3, which was the warmest day with the lowest humidity. The lowest charges were found on days 4, 14 and 15, which were the wettest days. Day 14 and 15 were also the coldest days.

4.4.2.1 Summer

The charges carried by inbound bees during the summer period were significantly affected by weather (Kruskall Wallis, P < 0.0001, X = 22.3, DF = 93, Figure 4.10), with bees having significantly lower charges during rain than in any other weather (Wilcoxon pairwise comparison with corrections for multiple testing (Sunny-Cloudy P = 0.005; Cloudy-Raining P<0.001; Sunny-Raining, P<0.001).

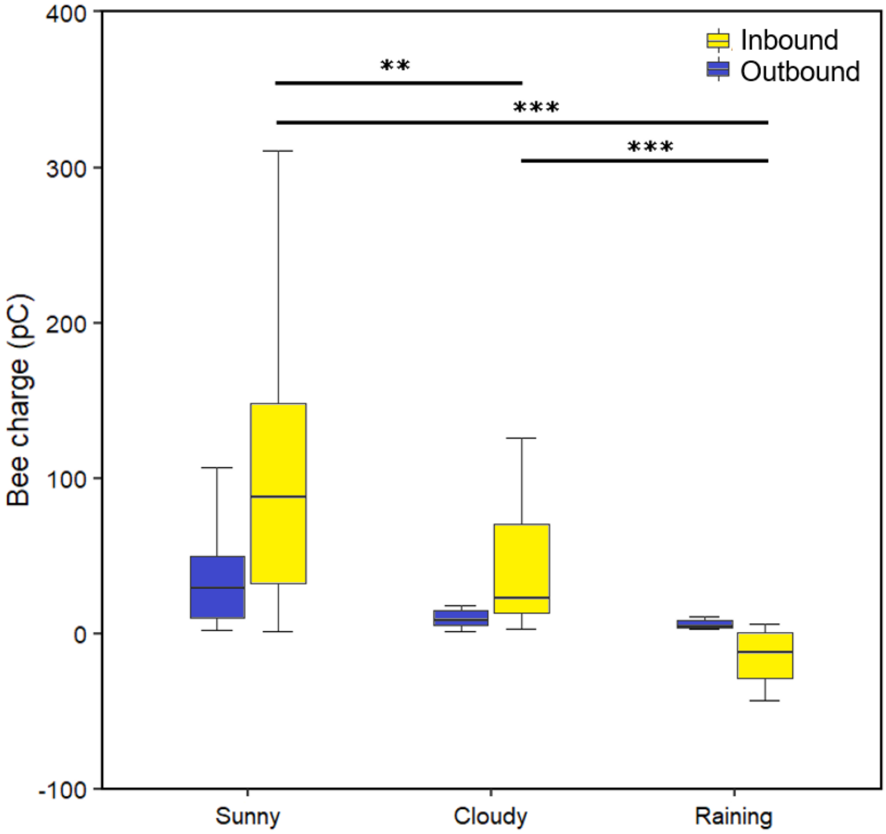

Figure 4.10. The charge on inbound and outbound bees in different weather conditions over the summer measurement period. Bees flying in sunny and cloudy conditions were significantly more positive than bees flying in rain. Bees flying in sunny weather were significantly more positively charged than those flying when cloudy.

The most positive charges were measured from bees flying in sunny conditions (Figure 4.10). Bees flying in rain were negatively charged. The charge data was transformed to make all values positive the log-linear relationship between the positive charge values and the meteorological conditions.

Both temperature and relative humidity significantly affected bee charge, but the two were also highly correlated. Relative humidity explained more of the variance, and relative humidity is known to significantly affect charging in triboelectric studies (Eilbeck et al. 2000; Nomura, Satoh, and Masuda 2003; Németh et al. 2003; Young et al. 2007; Nguyen and Yang 2013; Schella, Herminghaus, and Schröter 2017) so temperature was removed from the model to investigate the relationship between relative humidity and bee charge. The most parsimonious model showed a negative exponential relationship between bee charge and relative humidity:

$$Q_{bee} = e^{7.15-0.054RH} + Q_{min} - 1 \tag{4.2}$$

Where Q_{bee} is the bee charge, Q_{min} is the most negative charge measured on all the bees and RH is relative humidity.

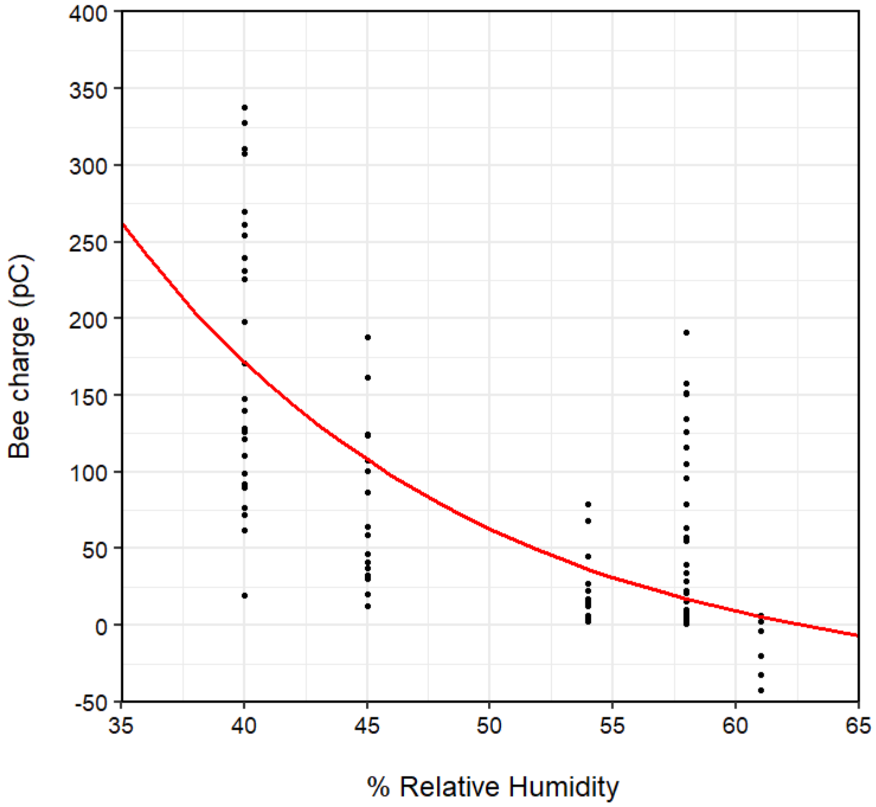

Figure 4.11. The relationship between relative humidity and the charge on bumblebees flying back to the colony over the summer period. The modelled relationship from equation 4.3. is shown as a red line.

4.4.2.2 Autumn

The autumn period was cooler and wetter than the summer period, with the last two days of measurement being the coldest and wettest, and having the lowest bee charge (Figure 4.9). Relative humidity, pressure and temperature significantly affected bee charge. There is an interaction between pressure and humidity, where the relationship between charge and humidity is affected by pressure. Air pressure and relative humidity both have been found to negatively correlate with air conductivity (Pawar, Murugavel, and Lal 2009), but air conductivity (measured as the concentration of ions in the air) did not affect bee charge.

All other variables which exhibited no collinearity were removed from the model, but many of the environmental variables were correlated such as relative humidity and temperature ($|r| = -0.49$), relative humidity and pressure ($|r| = -0.6$) and relative humidity and ion concentration ($|r| = 0.8$). The degree of collinearity between these variables means we cannot exclude them from the model without due consideration about how likely they are to affect the charge on the bee.

Of the significant variables, temperature is the least likely to affect bee charge. Tribology studies have shown changes in triboelectric properties of materials at very high temperatures, but the temperature range within this study (8-22°C) is very unlikely to have caused this effect. Pressure has been shown to affect charge generation in TENGs, but in the opposite way of the results shown here. In this study, bees flying in low pressure had lower charges, whilst bees flying in high pressure had higher charges. In the TENG study the charge generation increased as the pressure decreased, which is the opposite of the effect found here. The mechanisms affecting charge generation in the TENG studies are likely to differ from the generation of charge by bumblebees as the pressure affects charge in the opposite direction.

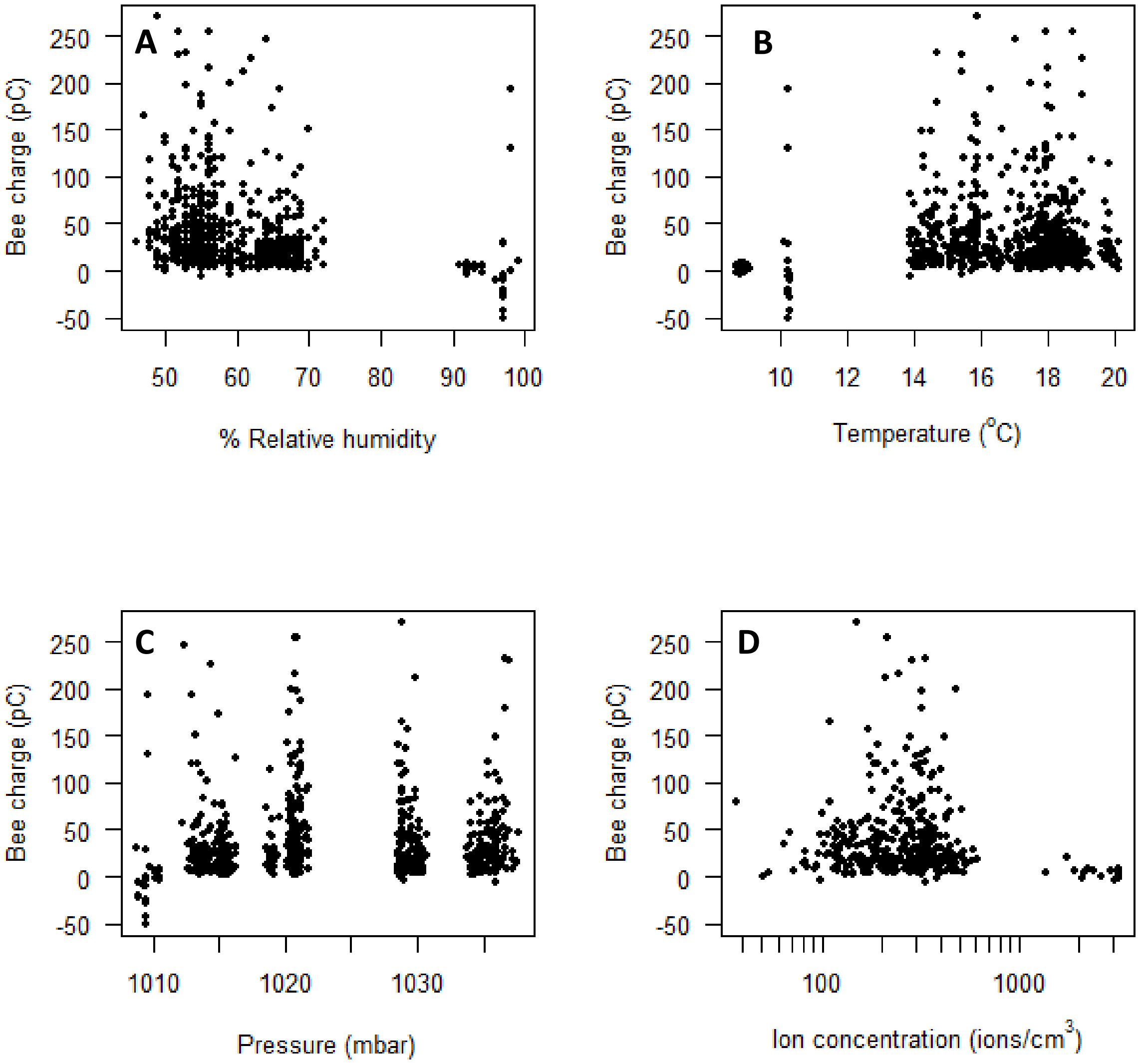

Figure 4.12. The charge on the bees entering the colony alongside the meteorological and electrical conditions of interest. A) The charge measured on bumblebees in different relative humidity. B) The charge measured on bumblebees and measured air temperature. C) The charge on bumblebees and the air pressure at the time of measurement. D) The concentration of positive and negative ions and the charges measured on bumblebees during the period that both ion counters were fully functioning.

A model including all significant variables (pressure, relative humidity and temperature) was compared with that just including pressure and relative humidity, and one simply measuring the effect of RH on bee charge. The model containing all variables explained most of the variation in the bee charge data (R^2=0.079) with the model omitting temperature having an R^2 = 0.073, and the humidity model R^2=0.064. The amount of variation in the bee charge data explained by the models is extremely low in all cases, with the model containing all the variables explaining 1.5% more of the variation than the model containing just humidity. As relative humidity is well known to affect triboelectric charging, and since relative humidity correlates strongly with temperature and pressure, and accounts for the majority of the variation explained by the model, the relationship between relative humidity and bee charge was further investigated.

Humidity

A generalised linear model was used to investigate the effect of humidity on the bee charges measured on worker bees flying into the colony.

The equation for the model is:

$$\ln(Q_{bee} - Q_{min} + 1) = -0.026RH + 5.56 \tag{4.3}$$

Where Q is bee charge and RH is relative humidity. This then can be rearranged for bee charge Q:

$$Q_{bee} = e^{5.56-0.026RH} + Q_{min} - 1 \tag{4.4}$$

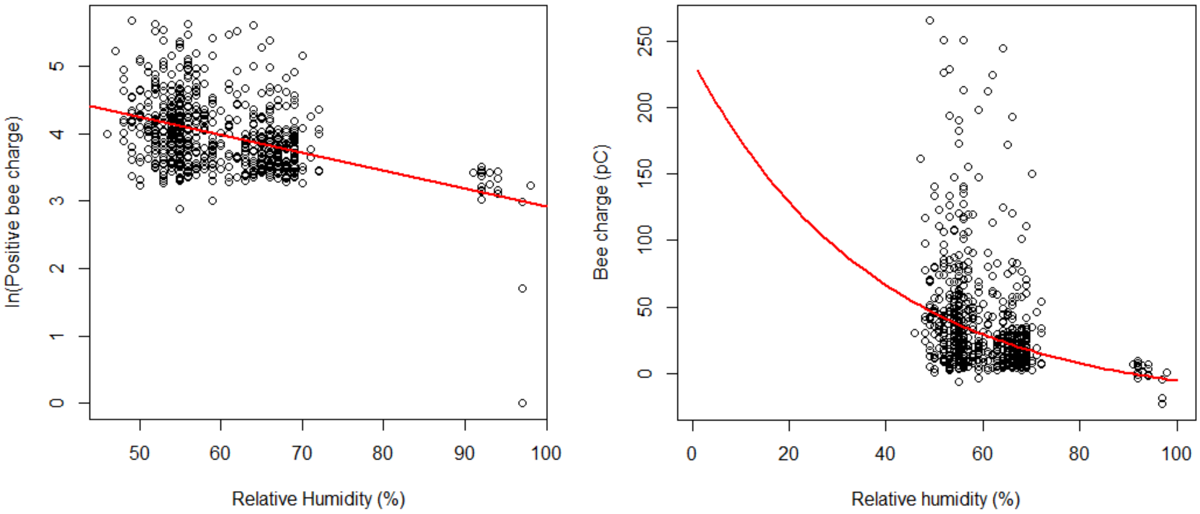

Figure 4.13. The modelled relationship between relative humidity and bumblebee charge.

The modelled relationship between relative humidity and bee charge using the autumn dataset (Figure 4.13) follows the same negative exponential relationship as that seen with the summer bee charge data (Figure 4.11) and supports the relationship shown in the triboelectric literature where relative humidity negatively correlates with triboelectric charge gain.

4.4.3 Results summary

Bumblebees flying outdoors are predominantly positively charged, carrying charges of a similar magnitude to that measured on bees in the laboratory. Bees flying back to the colony had stronger charges than bees leaving the colony, suggesting bees gain charge during flight or foraging. The strength and direction of the electric field did not affect bee charge, nor did the concentration and polarity of ions present in the air outside the bee colonies. However, the charge measured on bees is strongly dependent on the weather conditions, with bees flying in dry, warm, sunny weather having the most strongly positive charges, exceeding those measured on bees in flight arenas. Relative humidity is the strongest predictor of bee charge, with there being a negative exponential relationship between humidity and bee charge.

4.5 DISCUSSION

The charges measured on bumblebees in the field were similar in polarity and magnitude to those measured in the laboratory. In general this is encouraging, as it suggests the models of pollen transfer during pollination done by Clarke et al. (2017) are largely accurate, where it is shown that bee charges of 32pC are sufficient to propel pollen grains through the air (Clarke, Morley and Robert 2017). The charge measurements in this chapter show that these bee charges required to provide sufficient Coulomb force to propel pollen through the air between bee and flower do indeed exist in nature. It is therefore likely that electrostatics does enhance pollen transfer in natural conditions, and that as bumblebee charge is strongest in dry, sunny conditions, pollination is likely to be most efficient in fair weather. Dry, warm conditions are when bee charge is highest, but also when bee foraging activity is highest so are likely to be the conditions where pollination is most likely to occur.

The bees returning to the colony after flight were more positively charged than those leaving the colony, supporting a charge generation mechanism during flight or foraging. Both proposed mechanisms are supported by evidence shown in Chapter 3, where it is shown that bees generate a charge during flight but that bees also gain charge when visiting flowers due to a proposed triboelectric exchange of charge. As we have limited information about where the bees have been during their flight, how far they have flown or what they have encountered, we cannot say at this point which of these mechanisms is contributing more towards charge generation.

This difference in charge between the incoming and outgoing bees is especially interesting as it differs from the results found by Colin et al. (1991) which showed that honeybees that remained in the colony were more positively charged than bees that were returning from foraging (Colin, Richard, and Chauzy 1991). Charge is essential for electroreception in both species, and bumblebee electroreception and honeybee electroreception have been proposed to have different purposes, with bumblebees using electroreception to detect differences in floral resources (Clarke et al. 2013) and honeybees using it for communication within the hive (Greggers et al. 2013). Bumblebees therefore benefit from being charged during flight whilst honeybees benefit from being charged within the hive, perhaps leading to different evolutionary adaptations for different mechanisms of charge generation.

However, a separate study by Erickson (1975) found that bees were more positively charged when entering the hive (i.e. after flight) and that this charge varied over the course of the day (Figure 4.14; Erickson 1975), which is similar to the results shown in this chapter. Erickson also found that honeybees flying back to the hive were more positively charged on fair weather days than on overcast days, but there was no difference in the charge of bees exiting the hive (Erickson 1975). My results with bumblebees corroborate these findings (Figure 4.10), suggesting honeybees and bumblebees may charge using similar mechanisms and their charge may be affected by atmospheric conditions in similar ways. Both honeybees and bumblebees would benefit from charge generation whilst foraging resulting in increased transportation of pollen back to the hive, and the proposed mechanisms for charge generation such as triboelectrification and in-flight charging would therefore hold adaptive value for both species. The differences in electric charge of different bees and insects and the potential mechanisms are further explored in Chapter 5.

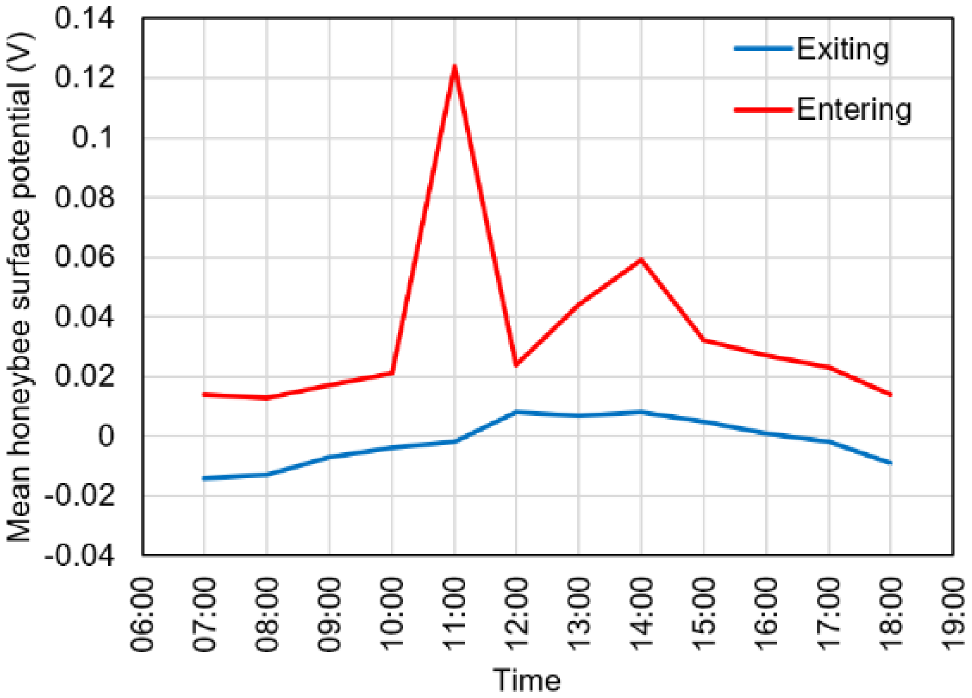

Figure 4.14. The surface potentials on honeybees entering and exiting a hive measured by Erickson (1975) over the course of a day. Data taken from Erickson (1975).

Bees carrying pollen back to the colony have significantly higher charges than those not carrying pollen (Figure 4.8). The charge on the bee is likely to affect the initial attraction and adhesion of pollen to the bee and is therefore may affect the amount groomed into pollen baskets, but once in the pollen baskets the bee charge is unlikely to highly impact the adhesion of pollen. Quantification of the pollen load of the bees may have provided further information as to whether the charge on bees affected the amount of pollen they brought back to the colony. Electrostatics is likely to promote the adhesion of individual grains to the bee, but the resolution on the cameras at the colony entrances was insufficient to resolve these grains. Interestingly, Colin et al. (1991) noted that the charge on honeybees returning to the colony was not affected by the presence of pollen in corbiculae, suggesting that whilst electrostatics is likely to affect the initial pollen acquisition and adhesion during foraging, at the point where the bee is at the colony the presence of pollen in corbiculae is not a good indicator of bee charge. Bee charge is more likely to affect pollen retention in bees without corbiculae, such as mason bees, who rely on specialised hairs for the transportation of pollen back to the colony. This relationship is further explored in Chapter 5.

Bumblebee charge was significantly affected by weather with humidity being the greatest predictor of charge. The exponential decay modelled relationship between bee charge and relative humidity was consistent between the summer, autumn and combined datasets, suggesting it is a robust relationship and that bees are likely to be the most positively charged in dry weather. This is supported by the literature on the triboelectric charging of powders and beads, where charge generation is negatively affected by humidity with a similar negative exponential relationship. Pressure and temperature also affect bee charge, suggesting that overall bee charge is highest in dry, warm, sunny conditions. Under fair weather conditions, when bee activity is highest and pollination is most likely to occur, the charges measured on bumblebees exceed those measured on bees in the laboratory. These results suggest that under these conditions greater Coulomb force could act on pollen due to the stronger charge difference between bee and flower.

5 CHARGING WITHIN THE HYMENOPTERA

5.1 INTRODUCTION

Throughout this thesis I have shown that bumblebees have a predominantly positive charge both in field and laboratory studies. This charge has profound influences on their pollination efficiency as well as potentially their sensory capabilities. Triboelectrically generated positive charges have been measured on other insects (McGonigle, Jackson, and Davidson 2002) but the adaptive value of this charge and its abundance in arthropods is currently unknown. Electric charge may benefit insects in a variety of ways. An electric charge on a pollinator causes increased acquisition and adhesion of pollen, benefitting pollinivorous insects who transport pollen to feed to larvae, and benefitting the plant by increasing the proportion of pollen reaching other flowers. Other potential benefits of charge include increasing the sensitivity of electroreception via charged mechanosensory structures (Sutton et al. 2016) allowing bees to perceive electrical information about their environment (Clarke et al. 2013) and potentially facilitating communication within the hive (Greggers et al. 2013). However, there may be a fitness cost associated with charging. A charged insect will attract and accumulate airborne pollen but also dust, soot, fungal spores and airborne pathogens (Lighthart, Prier and Bromenshenk, 2005). An electric charge on an insect will make it conspicuous to any electroreceptive predators, whilst charged predators will themselves be more likely to be detected by electroreceptive prey. The extent of electroreception in insect ecology has not been thoroughly investigated and as such it is hard to determine the extent of selection pressure on insect charge. For larger pollinators that are unlikely to be predated upon by electroreceptive insects, the benefits of being electrically charged (for electroreception and pollen adhesion) are likely to outweigh the costs. For insects that predate (or are predated upon) other potentially electroreceptive insects, the costs and benefits of electric charge are likely to be more complicated and will depend on the likelihood of being detected and the benefits of electric sensing.

In pollinivorous species morphologies have evolved to increase pollen adhesion and prevent pollen being lost during flight. Honeybees and many bumblebees have corbiculae (pollen baskets), on the tibia of the hind legs. They groom pollen attached to their hairs into these baskets which are smoothed down with nectar and oils for transportation (Thorp 1979). Whilst some of the pollen deposited on a bee is groomed into baskets, safe sites have been identified on the back of the head, top of the thorax and middle of the abdomen that bees are less able to reach (Koch, Lunau, and Wester 2017). These sites are thought to be integral for pollen transfer, as they are more likely to be transported between flowers and less likely to be consumed.

Other bee species rely on scopa for pollen transportation, e.g. some Fideliidae and Metachilidae (Thorp 1979). These are specialised hairs on the underside of the abdomen, with hooks or branches that collect pollen when brushed over anthers and retain it during flight. Because of this brushing, much more pollen is often dislodged and transferred between flowers, making mason bees highly efficient pollinators of many crops including almond (Bosch and Blas 1994), pear (Monzón, Bosch, and Retana 2004) and apple (Vicens and Bosch 2000). For this mechanism of pollen collection, electrostatics can be vitally important, as a charged bee will attract airborne pollen and transfer pollen efficiently between flowers and back to its nest. A female will lay her eggs in a suitable nest and provision them with nectar and pollen. The quality and quantity of the pollen provided to the developing larvae is highly correlated with offspring fitness (Radmacher and Strohm 2010; Bukovinszky et al. 2017), and hence provides adaptive value for mechanisms enhancing the pollen acquisition of the maternal bee.

Like bees, wasps comprise a very diverse group with very different diets, behaviours and social structures. Some wasps form complex eusocial colonies whilst some are entirely solitary (Nowak, Tarnita, and Wilson 2010). The yellowjacket wasp *Vespula vulgaris* provides a good comparison for mason bees and bumblebees. It is a similar size and shape but lacks the abundant hairs present on bumblebees and has no specialised pollen-collecting structures. *V. vulgaris* predominantly predates other insects, but also forage for sugar from nectar and fruit (Harris 1991; Kovac and Stabentheiner

1999), however it is not an effective pollinator. It does not forage for pollen and has no morphological adaptations for pollen collection or transportation. If electric charge is purely beneficial for pollen acquisition, the charges measured on wasps are expected to be lower than those measured on bees due to the reduced adaptive value of electric charge.

V. vulgaris has a similar body shape but lacks the abundant hair present in *O. bicornis* and *B. terrestris*. This difference in morphology may influence the generation and retention of electric charge, with structures such as hairs playing a role in charge generation. A long history exists regarding the triboelectrification of hairy surfaces, where parallel hairs are thought to accumulate charge well due to summation of charge in a particular direction (Jachowicz, Wis-Surel, and Wolfram 1984). The direction of triboelectrification appears important, as Jachowicz (1984) found that hairs rubbed with polycarbonate from root to tip became positively charged, and became negatively charged when rubbed from tip to root (Jachowicz, Wis-Surel, and Wolfram 1984; Kovac and Stabentheiner 1999). Hair and fur are often at the top of the triboelectric series (Freeman and March 1999) and bumblebees and mason bees are notably hairy, whilst wasps are mostly hairless. These hairs have multiple purposes, being used for insulation as well as mechanoreception and electroreception (Sutton et al. 2016). As electric charge is likely to be more beneficial for pollinators, especially pollinivorous insects, it would be expected that pollinivores would have a greater electric charge than non-pollinivores. The generation of charge may also relate to their behaviour, with specific behaviours (such as buzzing) perhaps causing the generation of charge

In an ideal study, a comparison between pollinating and non-pollinating flying insects with varying comparable morphologies would be established. This would allow a critical comparison of whether the enhancement of pollen capture has driven the evolution of insect charging, but a study of this size is beyond scope of this PhD. To analyse whether the hairy structures of pollinating insects, contribute to charging, the charges on a highly pollinivorous bee – the red mason bee (*Osmia bicornis*) and a non-pollinivorous, non-pollinating wasp (*Vespula vulgaris*) are measured and compared to that of the bumblebee (*Bombus terrestris*). *O. bicornis* and *V. vulgaris* differ in many respects. Though of a similar size, shape and evolutionary background they have different life cycles and have sufficiently different morphologies (from each other and from *B. terrestris*) that could cause them to gain charge differently.

In this chapter the charges on *O. bicornis, V. vulgaris* and *B. terrestris* are compared according to different methodologies previously established in this thesis. Their relative triboelectric position is characterised as well as their charge during flight in the laboratory (*O. bicornis* and *B. terrestris*) and in the field (*O. bicornis, V. vulgaris* and *B. terrestris*). These electric charge measurements alongside comparison of their physiology, diet and ecology allow insights into the evolution and mechanism of generation of electric charge in bees and wider insects. The experiments conducted and their research questions are shown in Table 5.1.

Table 5.1. Summary of the experiments within Chapter 5

Section	Location	Experiment	Research question	Method	Study organism	Page
5.2	Laboratory	Placing different insects on the triboelectric series	Do mason bees and wasps hold a similar triboelectric position to bumblebees?	Bumblebees, mason bees and wasps are rubbed against dielectric materials to establish triboelectric positions.	Bumblebees, mason bees and wasps	102
5.3	Laboratory	The charge retention of different insects	Do wasps lose their charge more readily than bees?	Insects are held in a Faraday pail to measure charge dissipation	Bumblebees, honeybees and wasps	105
5.4.4.1	Laboratory	The electric charge of mason bees in the laboratory	Are flying mason bees more positively charged than flying bumblebees? Is mason bee charge affected by sex?	The charges on mason bees and bumblebees are measured in the laboratory using the RCS	Mason bees and bumblebees	109
5.4.4.2	Laboratory	Bee charge before and after visiting flowers	Do bees gain charge by triboelectrification when visiting flowers?	The charge on mason bees and bumblebees are measured before and after foraging on a dandelion.	Mason bees and bumblebees	111
5.5.1	Field	Field measurements of mason bee charge	What is the charge of mason bees flying outdoors in natural conditions?	The charges on mason bees are measured before and after flight outdoors	Mason bees	114
5.5.2	Field	Field measurements of wasp charge	Do wasps have a lower charge than bees in natural conditions?	The charges on wasps are measured and compared to the charges measured on mason bees and bumblebees under similar conditions.	Bumblebees, mason bees and wasps	119

5.2 TRIBOELECTRIC CHARGING IN BEES AND WASPS

5.2.1 Methods

To compare the triboelectric properties of different insects, a triboelectric series was established where mason bees, wasps and bumblebees were compared to each other and to a variety of biological and artificial materials. Previous studies have placed bumblebees at the top of the triboelectric series (Clarke, Morley, and Robert 2017). Bumblebees are also shown to be more positive than flowers, gaining charge both when rubbed against flowers, and when vising flowers under their own volition (Chapter 3). These previous experiments cement the bumblebees position at the top of the triboelectric series, and suggest that the material properties of the hairs and the cuticle are sufficient to cause the bee to gain charge upon friction. An experiment was conducted to measure whether these triboelectric properties were representative of other insects specifically the mason bee *O. bicornis* and the wasp *V. vulgaris.*

Female mason bees (*O. bicornis*) were hatched from pupae and euthanised by freezing. Bumblebees (*B. terrestris audax*, Koppert UK) were reared in the laboratory and euthanised by freezing for use in the experiment. A worker wasp (*V. vulgaris*) was caught whilst foraging and euthanised by freezing. A triboelectric series was established using identical methods to the floral triboelectric series established in Chapter 3. The charges were compared on a female mason bee, a worker bumblebee, a wasp, a freshly cut dandelion flower, a cube of polyurethane foam (1cm x 1cm x 1cm), a nylon ball (approx. 1cm diameter), and a HDPE square (2cm x 2cm x 0.5cm). During a test, 2 samples of different materials were electrically grounded using a static eliminator gun (Zerostat, Milty, UK). The two materials were held with metal forceps and lightly rubbed against each other 5 times. The materials were then dropped one after the other into a Faraday pail. The increase in charge measured by the pail was registered for each material. This was repeated 5 times for each pair of materials and the mean charge of each material used for comparison. These tests were repeated for every combination of materials until all materials have been rubbed against all other materials. The triboelectric position of materials was established using the same method in (Clarke, Morley, and Robert 2017) where the material gains a score of 1 if it gains a positive charge and a score of 0 if it becomes negatively charged. The materials were ranked in this way based on the points to establish a triboelectric series (Figure 5.1).

5.2.2 Results

Both bee species were placed at the top of the triboelectric series, gaining a positive charge upon contact with wasps, dandelions, nylon and HDPE (Figure 5.1). Polyurethane foam is very triboelectrically positive, often placed at the top of the triboelectric series (Clarke, Morley, and Robert 2017). In this study bees are equally likely to gain a positive charge, with mason bees and bumblebees gaining very similar charges after being rubbed against identical materials. Wasps are positioned much further down the series, gaining a negative charge when rubbed against mason bees, dandelions or PU foam (Table 5.2).

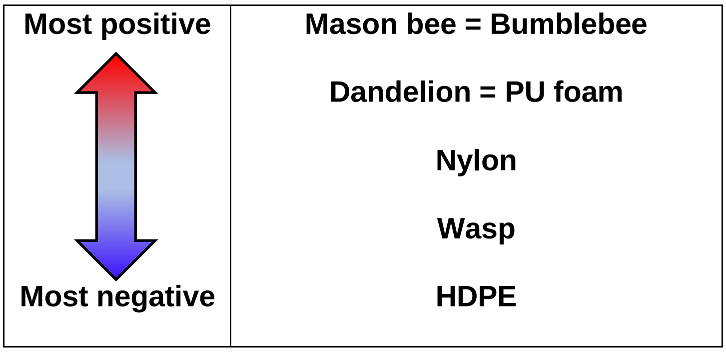

Figure 5.1. The triboelectric position of bumblebees, mason bees and wasps compared to some common polymer materials and a dandelion.

	Mean charge gain by material (pC)						
	Bumblebee	Mason bee	Wasp	Dandelion	PU	Nylon	HDPE
Bumblebee	x	1.72	0.62	2.48	-61.88	-0.42	-23.92
Mason bee	0.54	x	-0.18	1.28	6.42	0.62	-20.24
Wasp	1.56	11.06	x	-2.86	29.98	-2.74	-32.6
Dandelion	2.26	2.24	-1.92	x	-11.08	-0.62	1.3
PU	2.76	0.56	-2.86	2.26	x	6.2	-7.6
Nylon	1.9	1.68	2.26	-1.42	12.76	x	-0.08
HDPE	3.66	1.58	0.62	4.94	11.84	1.98	x

Table 5.2. The mean charge of each pair of materials after rubbing. Dark green boxes show materials with a positive charge gain of >10pC. Dark red boxes show materials with a negative charge gain of >10pC.

Mason bees were also positively charged after being rubbed against a variety of materials so it is likely that the triboelectric positivity is not specific to bumblebees but extends to other bees. Triboelectric studies with insects previously have found that many insects gain a positive charge due to triboelectrification including diptera (Edwards 1962; McGonigle, Jackson, and Davidson 2002; McGonigle and Jackson 2002; Jackson and McGonigle 2005), coleoptera (Edwards 1962) and lepidoptera (Edwards 1962). We can now add mason bees and wasps to this analysis and start to theorise reasons why these species charge up to different extents upon friction with different materials.

Rubbing two materials with different triboelectric properties together should cause one material to gain a more positive charge and the other to gain a more negative charge. This is not always evident in the above table, where two materials often have the same charge polarity after contact (e.g. bumblebee and mason bee, dandelion and wasp). This identical polarity is likely due to the material charge before triboelectrification being non-zero. Measurements of the charge pre-and post-triboelectrification would be more robust but were unavailable with the present equipment.

5.3 CHARGE RETENTION IN INSECTS

Insects are electrically charged and this thesis, alongside past studies of insect tribology, has shown that insects can generate a measurable charge via triboelectrification. For a pollinator to retain pollen during flight, they must not only gain a charge initially, but be able to retain it. Charged objects lose charge via conduction along the object surface, conduction through the volume of an object and neutralisation due to ions present in the surrounding air (Kindersberger and Lederle 2008a). Increasing relative humidity increases the rate of charge decay as charge is more rapidly conducted away from the object surface. Additionally, humidity increases surface conductivity, neutralising local charges.

The charge retention of bumblebees (*B. terrestris*), honeybees (*A. mellifera*) and wasps (*V. vulgaris*) was compared, to investigate whether the increased charge retention found on bumblebees in Chapter 3 was specific to this species or whether it extended to other insects. 10 bumblebees, honeybees and wasps were caught whilst foraging and euthanised by freezing. The bumblebees came from a laboratory-based colony, whilst the honeybees and wasps were caught near the honeybee hives at Langford Vet School. Mason bees were seasonally unavailable for this comparison. All insects were killed by freezing and affixed to the end of a wooden rod (20.4 cm long, 2.5 mm diameter) using a small amount of ethyl cyanoacrylate glue (Loctite, Henkel, Ohio) on the underside of their thorax. The insects were triboelectrically charged by friction with a length of polystyrene until they achieved a positive charge of 500pC. At this point the insect was held in a Faraday pail and the charge on the insect measured over a 30s period. The methods in this chapter are comparable to the measurements of charge decline in Chapter 3. An ANOVA was used to compare the end charges after 30 seconds of being held in the air of each insect species. Tukey's post-hoc showed individual differences between species.

Bumblebees and honeybees kept their charge significantly better than wasps (ANOVA, P < 0.0001, $F_{3,30}$ = 13.52, Figure 5.2), with wasps having a significantly lower charge after the 30s period than honeybees (P = 0.005) and bumblebees (P < 0.0001).

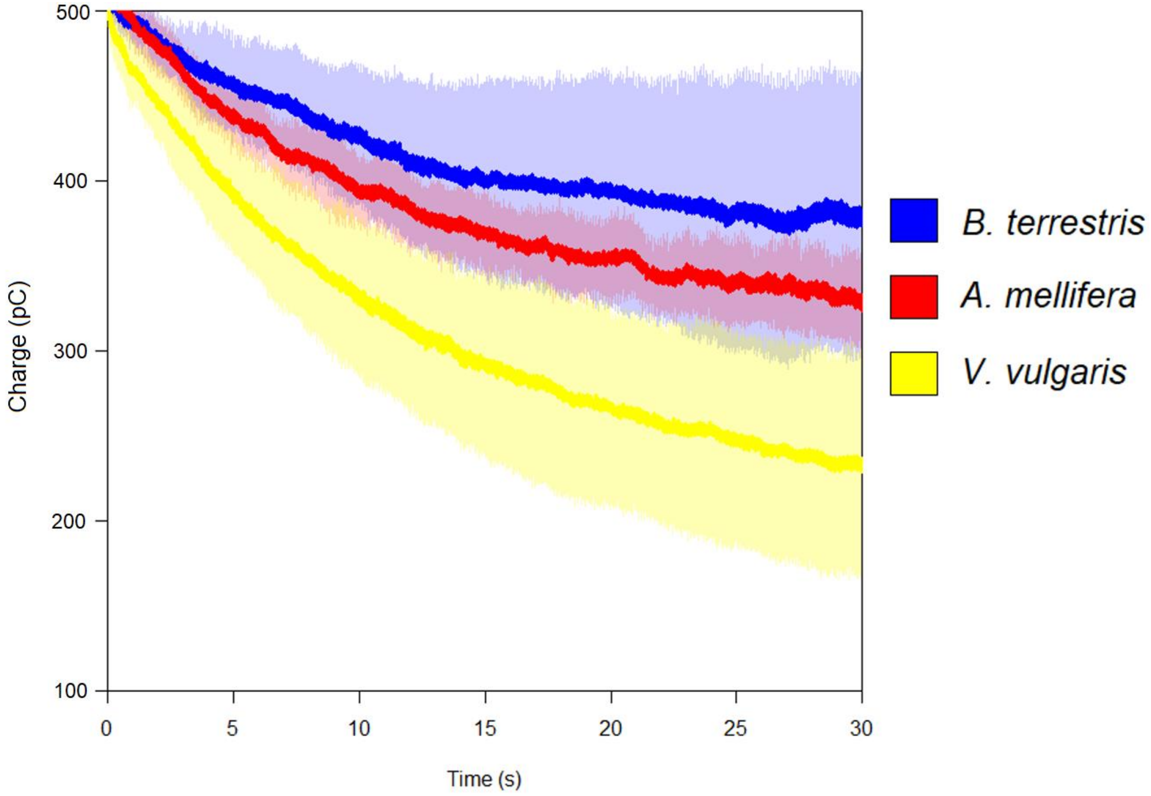

Figure 5.2. The charge decline of bumblebees, honeybees and wasps held in a Faraday pail for 30s. The end charge on the wasps was significantly lower than the end charge of honeybees (P = 0.005) and bumblebees (P < 0.0001). N=10 insects for each species.

5.4 LABORATORY MEASUREMENTS OF MASON BEE CHARGE

5.4.1 Husbandry

Whilst the majority of this thesis has detailed experiments conducted with bumblebees which are model animals for laboratory experiments, here I show the first laboratory measurements conducted with mason bees. The best studied solitary bee is probably the blue orchard mason bee (*Osmia lignaria*) (Amaya-Márquez et al. 2008). However lab-rearing of mason bees and their use and husbandry in captivity is still largely unexplored and all previous experiments using *Osmia* to date have been conducted in greenhouses or outdoors (Howell and Alarcón 2007; Amaya-Márquez et al. 2008). As such the rearing and maintenance of mason bees in the laboratory was subject to experimentation and method optimisation to ensure the best environment for the bees and to encourage reproduction and foraging.

To obtain measurements of the electric charges on mason bees under controlled conditions, red mason bees (*Osmia bicornis*) were obtained as pupae from Mason Bees UK (Shrewsbury, UK). Pupae were stored at 3°C until they were needed for experiments. Mason bee pupae were left to eclose into a foraging arena (112cm x 77cm x 30cm) and were provided with *ad libitum* sugar water (30%), pollen from oil seed rape flowers grown in the greenhouse at the Life Sciences GroDome, and fresh dandelions brought into the laboratory from Royal Fort Gardens. Hatching of the mason bees was staggered so that there were between 10-40 bees in the arena at any time. There was a 50:50 male female split, but the extended longevity of females ensured that there were often more females than males in the arena at any time. The bees were provided with a shelter of nesting tubes (8mm diameter; Figure 5.3) and 3 petri dishes containing fresh samples of dirt, sand and mud with varying moisture content were provided in the arena for the bees to cap the tubes. Nesting tubes were refreshed weekly and any sand/mud dishes not visited by the bees were removed from use.

Figure 5.3. The nesting tubes within the flight arena. A female mason bee is in the foreground.

5.4.2 Methods for the measurement of charge on mason bees

An experiment was conducted to measure the electric charge of flying mason bees using the Faraday pail as in (Clarke et al. 2013) and as in Chapter 3 of this thesis. Attempts to train the bees to fly into the Faraday pail were unsuccessful. Mason bees appeared more suspicious of the hole in the floor and whilst they could be trained to fly through it to access a sugar reward, they would not do so once the reward had been replaced with the pail. Instead bees were trained to fly through an RCS established and set up within the arena (Figure 5.4). The RCS was placed around the rim of a plastic cup. The cup rested on a block of floral foam and had a slit cut along its bottom, allowing the placement of flowers (*Brassica napus* and *Taraxacum officinale*) within the foam, with the heads of the flowers extending within the cup and being visible and accessible to the bees (Figure 5.4). The floral foam was locally saturated with water and electrically grounded as in the experiments in Chapter 3.

Figure 5.4. A female mason bee approaching the dandelions by flying through the RCS.

Mason bees readily flew through the RCS to access the flowers, with the first bees flying through within minutes of the flowers being introduced into the arena. This set up allowed measurement of the charges on mason bees flying in a controlled laboratory environment. This equipment and environment allowed comparisons between the electric charges of mason bees and bumblebees, the electric charges on male and female mason bees and the charges on mason bees before and after visiting flowers.

As mason bees are primarily pollen foragers and carry pollen with scopa, an electric charge is likely to hold more adaptive value for mason bees than bumblebees who use corbiculae for pollen transportation. As such it was predicted that mason bees would be more positively charged than bumblebees. As predominantly female bees forage for pollen and male bees lack pollen-collecting morphology such as scopa, it was expected that female bees would be more positively charged than male bees. Bumblebees have been shown to gain charge when visiting flowers (Chapter 3). As mason bees are also placed near the top of the triboelectric series, they were expected to also gain charge when visiting dandelion flowers.

5.4.3 Calibration

The RCS was calibrated in an identical way to previous calibrations (Chapters 3 and 4) by dropping cubes of PU foam through the RCS into the Faraday pail. The calibration showed a linear correlation between the charge measured by the ring and the Faraday pail with R^2 of 0.97.

5.4.4 Results

5.4.4.1 The electric charge on mason bees

Mason bees were typically positively charged with a mean charge of 20 ± 18 pC, N = 27 (Figure 5.5). These charges are similar in polarity and magnitude to those measured on bumblebees in the same flight arena under similar conditions (Clarke et al. 2013) (Figure 5.6A).

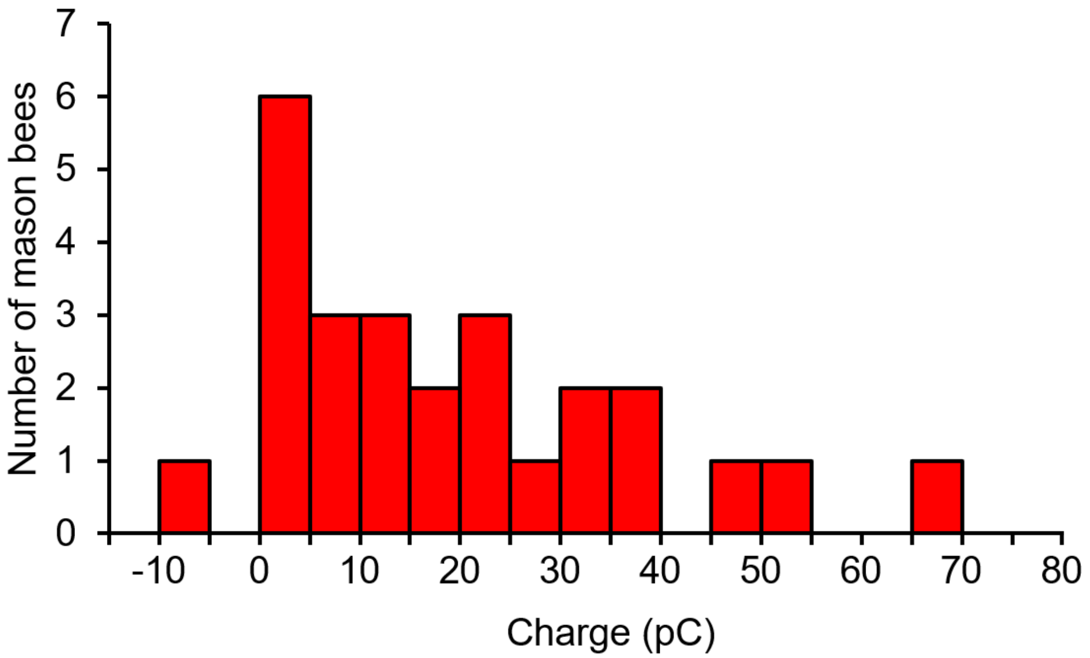

Figure 5.5. The distribution of electric charge measured on free-flying mason bees approaching dandelion flowers in laboratory conditions. N=26 individuals.

Despite their different physiology and body size, there was no significant difference in the charges on male and female mason bees (Figure 5.6B). Female mason bees are larger and hairier, whilst males are smaller and have hairy faces but lack abdominal scopa. Given that female mason bees are the primary pollen foragers whilst males do not provision for larvae, charge should hold more adaptive value for females than males and it was expected that female mason bees would be more highly charged than the males. In the conditions provided by this experiment, this does not appear to be the case. The morphological differences between males and females such as the presence of abdominal scopa does therefore not appear to significantly contribute towards charging. However, this experiment is conducted within a flight arena in a laboratory environment, precluding prolonged flight. Over longer flight distances (>1m), it is possible that morphological specialisations account for differences in charge acquisition, upper limit and retention.

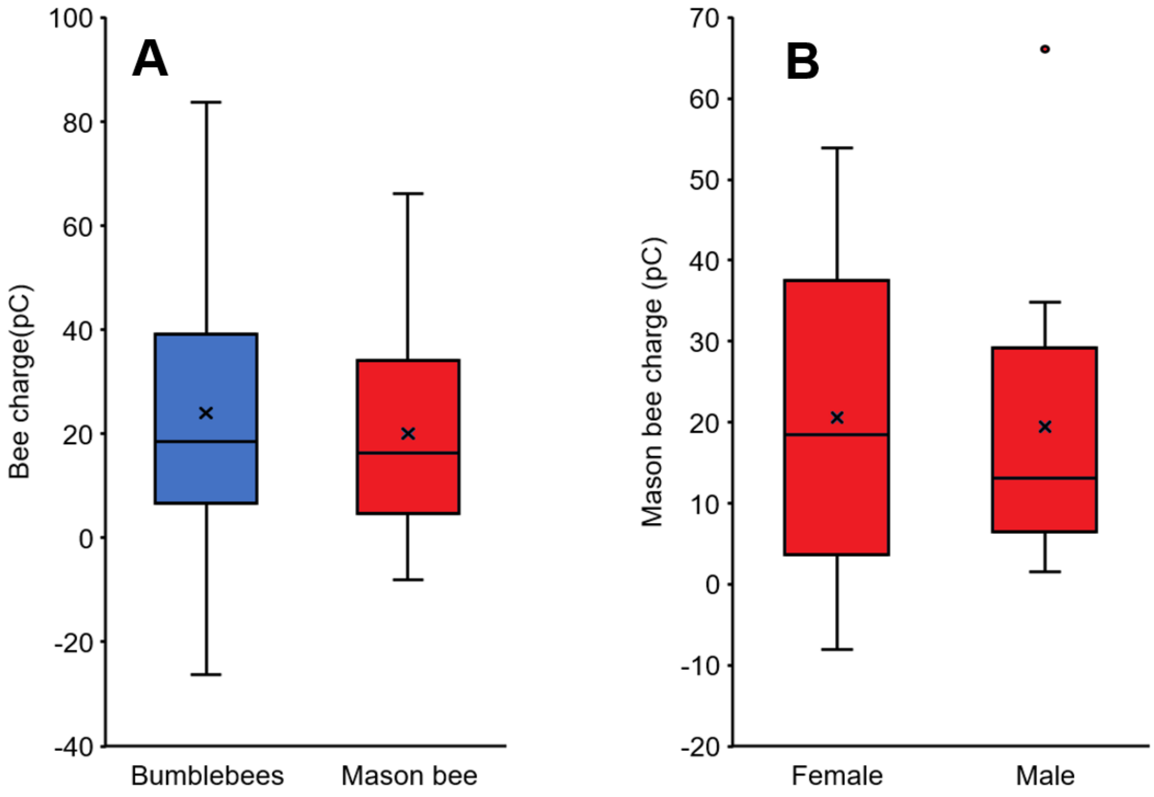

Figure 5.6. A) The charges measured on flying mason bees (red, N = 26) and bumblebees (blue, N = 27) under identical conditions. Charges are measured on both species of flying bees using the RCS in the same flight arena. B) The electric charges measured on male (N = 12) and female (N = 13) mason bees in the laboratory. Female mason bees did not have more positive charges than males but both bees were weakly positive with a similar charge magnitude to bumblebees.

5.4.4.2 The charge on mason bees before and after visiting flowers

As shown in Chapter 3, bumblebees gain a more positive charge after landing on flowers. Whilst bees approaching flowers are positively charged, they often leave the flower with a more positive charge than when they arrived. Female mason bees also gain positive charge upon visiting flowers (Figure 5.7A) of a similar magnitude to the charge gained by bumblebees upon visiting dandelions (Figure 5.7B).

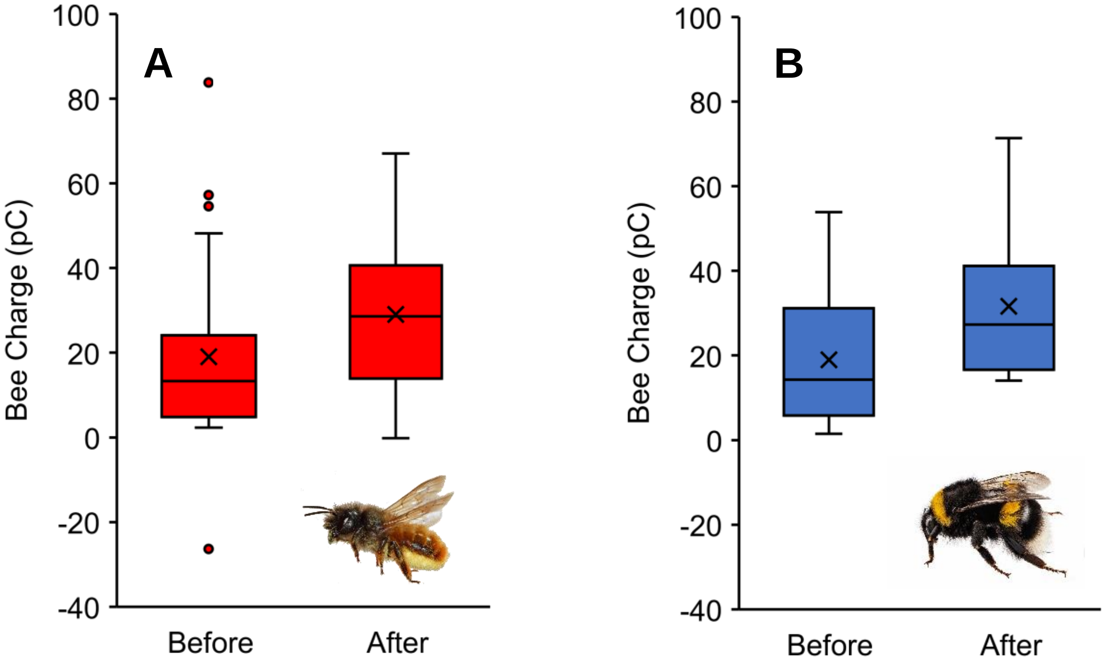

Figure 5.7. Flower visit results in an increase in bee charge. A) Charge carried by mason bees before and after visiting a dandelion flower (N = 8) and B) the charge of bumblebees (N = 29) before and after foraging on dandelion flowers in the laboratory. Measurements on bumblebees are taken from the floral triboelectric experiment in Chapter 3.

The observed similarities in charge gain of mason bees and bumblebees when visiting flowers makes sense given their similar triboelectric characteristics (established in Figure 5.1) and the relative triboelectric positioning of bumblebees and flowers (established in Chapter 3). Both bee species always had a positive charge after being rubbed against a dandelion flower (Table 5.1, Chapter 3). Indeed, the relative tribology indicates that if the charge gain is a triboelectric property, both bees should gain charge upon visiting dandelions and that this charge gain should be similar for both bee species, which appears to be the case. The electrical interaction between mason bees and flowers is therefore similar to that between bumblebees and flowers, indicating that the tribological studies conducted in Chapter 3 are representative of other bees and potentially other flying insects.

5.4.5 Summary

Laboratory measurements of the electric charge carried by bumblebees and mason bees show similar results. Mason bees are typically positively charged with comparable charges to those measured on lab-foraging bumblebees. This positive charge is not exclusive to pollen-foraging females, as male mason bees have identical charges. This result suggests that morphological features specific to pollen collection and retention such as scopa do not cause significant charge gain, at least in laboratory conditions.

Both mason bees and bumblebees gain charge when visiting flowers in the laboratory. In Chapter 3 it was shown that bumblebees gain and maintain their positive electric charge upon visiting flowers. This is also the case of mason bees although experiments with other flowers and in natural conditions further enhance our understanding about the extent of plant-bee triboelectrification in nature. However, as mason bees are triboelectrically similar to bumblebees, and bumblebees have been shown to both gain charge when visiting flowers and be more triboelectrically positive than all flowers tested in Chapter 3, it is logical to assume that mason bees will also gain charge when visiting other species of flowers. On the basis of the evidence gathered in this chapter and in Chapter 3, it can be proposed that the generation of electric charge is not specific to bumblebees, and that the mechanisms involved apply to mason bees, as well as potentially to other insects.

5.5 FIELD MEASUREMENTS OF INSECT CHARGE

As established previously in this thesis, laboratory experiments using calibrated instrumentation have been essential to elucidate the mechanisms behind charge gain and the effect of bee charge upon the plant-insect relationship. In this way, I have shown that bees can gain charge triboelectrically, that they appear to gain charge during flight and that their charge is influenced by meteorological conditions such as relative humidity. In the field, the conditions bees are exposed to during flight may differ to those encountered in the laboratory. Flight in laboratory conditions is constrained by several identifiable abiotic factors, such as the dimensions of the flight arena and the materials bees encounter in the arena, which are unlikely to be found in nature. Additionally, meteorological conditions can affect bee charge, causing greater variability. Measurements of the charges on free-flying bees in field conditions are therefore instrumental in determining how representative laboratory studies are, and how bee charge may be affected by the changing conditions found in nature.

As shown in Chapter 4, the charges measured from bumblebees flying outdoors are similar to those found for bumblebees in the laboratory but may be higher and more variable due to the variation in environmental conditions. Here, experiments are conducted to measure the electric charges on bumblebees, mason bees and wasps in natural conditions. At the Langford field site (see Chapter 4 for details), the bumblebee colonies began to be attacked and exploited by wasps (*V. vulgaris*) towards the end of the summer data collection period (24/08/2018). As bumblebees and wasps were both entering and leaving the same colony, this allowed an identical comparison between the charges measured on bees and wasps under identical conditions. The charges on mason bees were measured at a different site but under similar conditions using an identical RCS which was calibrated *in situ*. These measurements of charge on different pollinating and non-pollinating insects allow a direct comparison between them, and initial speculation on the wider role of insect charge on pollinator ecology.

5.5.1 Mason bees

5.5.1.1 Methods

A field site was identified in North Cadbury, Somerset UK (51° 2' 34.3932'' N, 2° 31' 24.1428'' W) containing a thriving population of mason bees. These had burrowed into the mortar between bricks in the south-facing side of an old farmhouse. Whilst mason bees have individual nests, they are gregarious and can often share a communal entrance (Fortel et al. 2016). Many bees nested in the wall but entered and exited through a single hole. This nesting arrangement made it very practical to fit an RCS over the hole to measure the charge of bees flying into and out of the wall (Figure 5.8). The RCS was calibrated on site (in an identical manner to the field calibrations detailed in Chapter 4). To record the flights of bees flying into and out of the wall, a camera was erected facing the RCS, (iPhone 5S, Apple). During flight recordings signals were also recorded manually on a laptop computer then validated to be caused by the flight of a bee using the video footage. During the flight recordings the temperature was 19°C with 51-65 % relative humidity. As only female mason bees lay eggs and provision for the young, only females used the hole and electric charge was therefore only measured on female bees.

Figure 5.8. A pollen-laden female mason bee approaching the nesting hole enclosed by the RCS.

5.5.1.2 Calibration

The RCS was calibrated at the end of the measurement period by dropping cubes of polyurethane foam through the RCS into the Faraday pail (JCI 141, Chilworth Global, Southampton, UK) *in situ*. For this field experiment, the linear correlation between the charge measurements by the RCS and the charge measurements from the Faraday pail had an $R^2 = 0.975$.

5.5.1.3 Results

5.5.1.4 Comparison with laboratory bees

Free-flying wild mason bees in a natural environment had much higher charges than those measured in the laboratory by an order of magnitude. Whilst the majority of bees had weakly positive charges of a similar magnitude to the laboratory, some charges were measured that vastly exceeded this range (Figure 5.9). As with the bumblebees, the charges on bees measured during flight outdoors were much more variable than those measured in the laboratory. This could potentially be attributed to the different flight durations of each bee, the different flowers they visited and/or the different conditions encountered during flight.

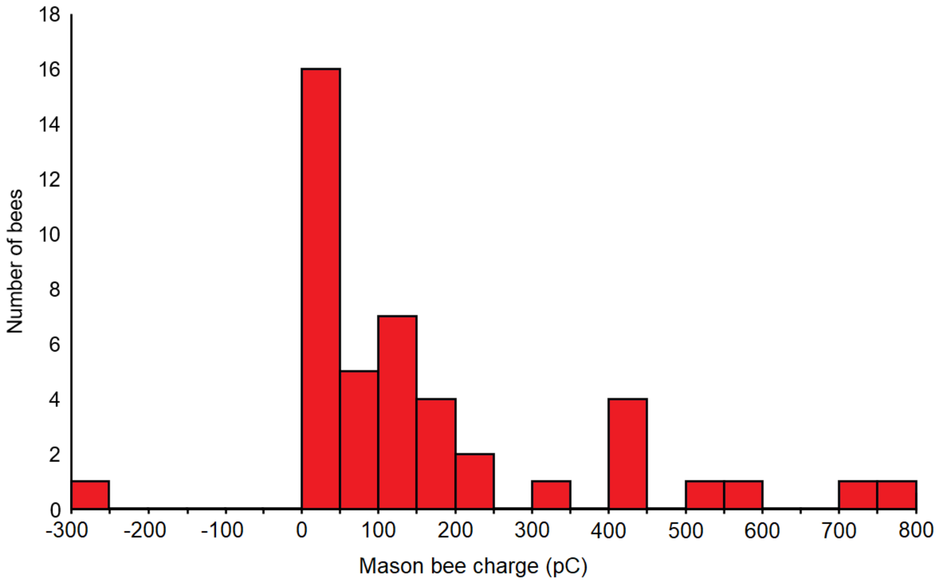

Figure 5.9. Distribution of the charges measured on flying mason bees flying outdoors (total number of charge measurements = 85).

The range of charges found on mason bees flying outdoors (Figure 5.9) vastly exceeded those measured in the laboratory (Figure 5.5). This is primarily due to the charges measured from inbound post-flight mason bees, while the charges from outbound mason bees are much lower and are similar to those measured in the laboratory (Figure 5.10). This pattern of results is very similar to those measured on bumblebees in Chapter 4, suggesting laboratory studies underestimate the charges that can be generated by flying bees. Whilst laboratory studies are integral for the understanding of certain mechanisms, it must be noted that space constraints prevent prolonged flight. Because of this, the measurements of charge gain during flight in a laboratory are limited, as bee flight can only be sustained in a flight arena for short periods of time without collision with or landing on the arena boundaries (walls, floor and ceiling).

In Chapter 3 I show that bumblebees gain charge during flight at a decreasing rate until they reach a saturation charge (of around +17pC). Above this charge, bees slowly lose charge to their surroundings. Bumblebees gain charge triboelectrically when visiting flowers, often vastly exceeding their saturation charge and leaving a flower with a more positive charge than they had when they arrived. Due to the similar triboelectric characteristics of bumblebees and mason bees shown at the start of this chapter, it is likely that mason bees also gain charge triboelectrically when visiting flowers and may also gain charge in a similar way to bumblebees during flight by triboelectrification with airborne particulates. Both mechanisms may explain the elevated charges found on mason bees in nature compared to the laboratory environment. Field measurements were taken on a dry, sunny day, which have been shown to be optimal conditions for bumblebee charging in Chapter 4. The low relative humidity and warm temperature is associated with greater charge retention and more highly charged bumblebees both in laboratory and field experiments. The optimal foraging conditions alongside triboelectric charging on flowers absent from laboratory experiments may explain the elevated charges found on free-flying outdoor mason bees.

5.5.1.5 Comparison of inbound and outbound bees

Charges measured on mason bees flying back to the nest site were significantly higher than those on bees exiting the nest site (inbound mean charge 39 ± 46 pC, N = 41; outbound mean charge 161 ± 208 pC, N = 44, Mann-Whitney U-test, P = 0.001, W = 1275.5, Figure 5.10). These results match the charges measured on bumblebees in Chapter 4, where bees flying back to the colony had significantly higher charges than bees flying out. This suggests some charging mechanism during flight over wide open spaces, and/or triboelectric charging when foraging on flowers.

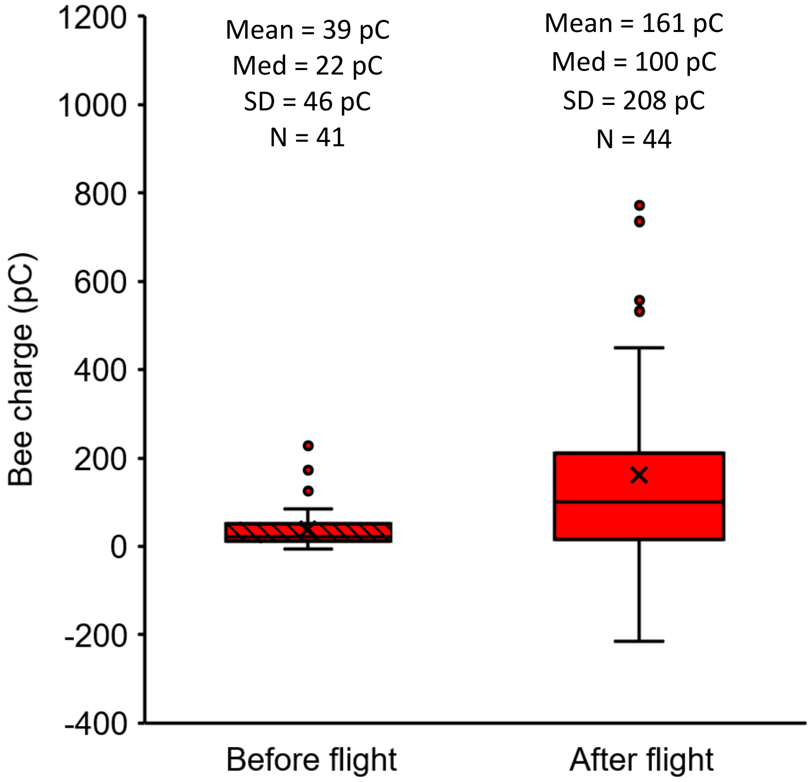

Figure 5.10. Electric charges on inbound and outbound mason showing mean (X), median, IQR and range excluding outliers. The charges measured after flight (ie on bees returning to the nest site) were significantly more positive than those measured on bees leaving the nest site before flight (Mann-Whitney U-test, P = 0.001, W = 1275.5).

The similar results shown from flying bumblebees and mason bees suggest again that these pollinators have similar charging mechanisms that are influenced by similar conditions. Both bumblebees and mason bees have significantly higher charges when returning to the colony after foraging outdoors, than when they are leaving the colony. Inbound charges are also much more variable (inbound SD: 208 pC, outbound 46 pC), perhaps due to variation in conditions encountered on foraging bouts. Conditions during charge measurements of mason bees were dry (51% RH) with negligible cloud cover and warm temperatures (19°C). Bumblebees flying in similar conditions show similar variability with bees exiting the colony having consistently lower charges then bees returning after flight.

5.5.2 Wasps

5.5.2.1 Methods

The charges on free-flying wild wasps (*V. vulgaris*) were measured in an identical manner to the methods used to measure the charges on bumblebees in Chapter 4. As the bumblebee colony neared the end of its life and the population declined, it became a target for opportunistic scavengers. The wasps eagerly flew in and out of the bumblebee colony alongside the bumblebees (Figure 5.11), allowing a direct comparison with bees still entering and exiting the colony in a well calibrated system using the same charge sensors (for calibration details see Chapter 4). The main activity of the wasps was largely confined to 1 day (24/08/2018). The weather was fine, dry and warm, with a similar temperature (16.2-19°C) and humidity (50-71% RH) to that measured when recording the charges on outdoor mason bees. As both experiments were calibrated *in situ* using identical methods, this allowed a reasonable and meaningful comparison between bees and wasps flying under the same conditions. For this comparison, only the charges measured on bumblebees on the same day as the wasps were included to allow comparisons between insects flying in identical atmospheric conditions.

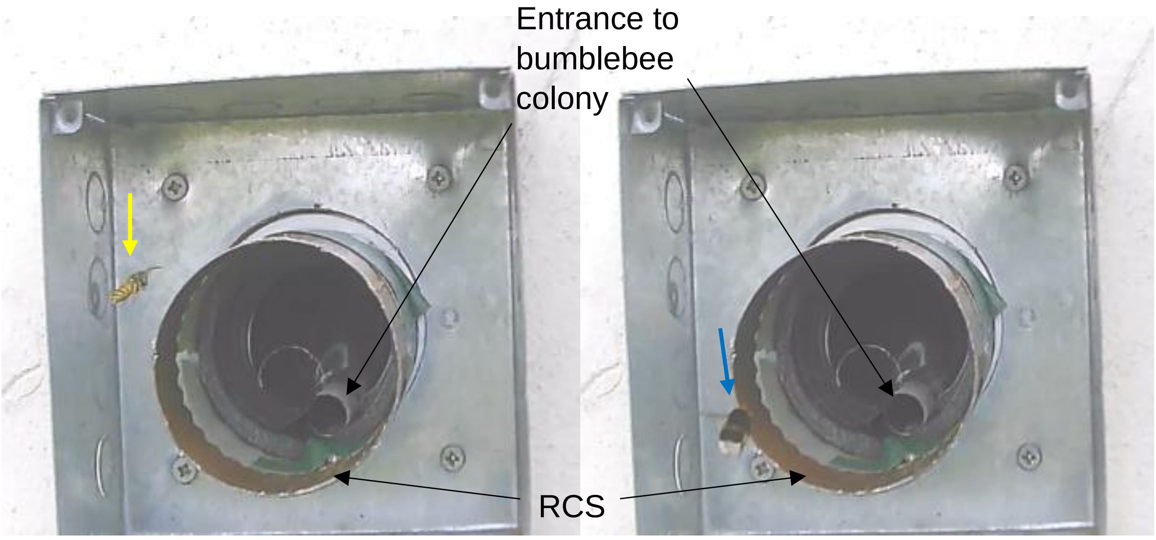

Figure 5.11. A yellowjacket wasp (*V. vulgaris*, left) and a bumblebee (*B. terrestris*, right) entering the same bee colony within minutes of each other. The entrance to the colony is a small tube in the centre of each RCS, so that the bee/wasp must fly through the RCS to access the colony. Wasp highlighted with yellow arrow. Bumblebee highlighted with blue arrow.

5.5.2.2 Results

5.5.2.3 The charges on wasps

The charges measured on wasps were much lower than the charges measured on either bee species, being predominantly below 20pC (Figure 5.12). Wasps entering the colony after flight did not have a significantly higher charge than those leaving the colony (Mann-Whitney, P = 1, W = 33, Figure 5.12). The number of wasps recorded was lower than that measured for either bee species (N = 13 wasps after flight, N = 5 wasps before flight). These results show that under natural conditions wasps have weak positive electric charges.

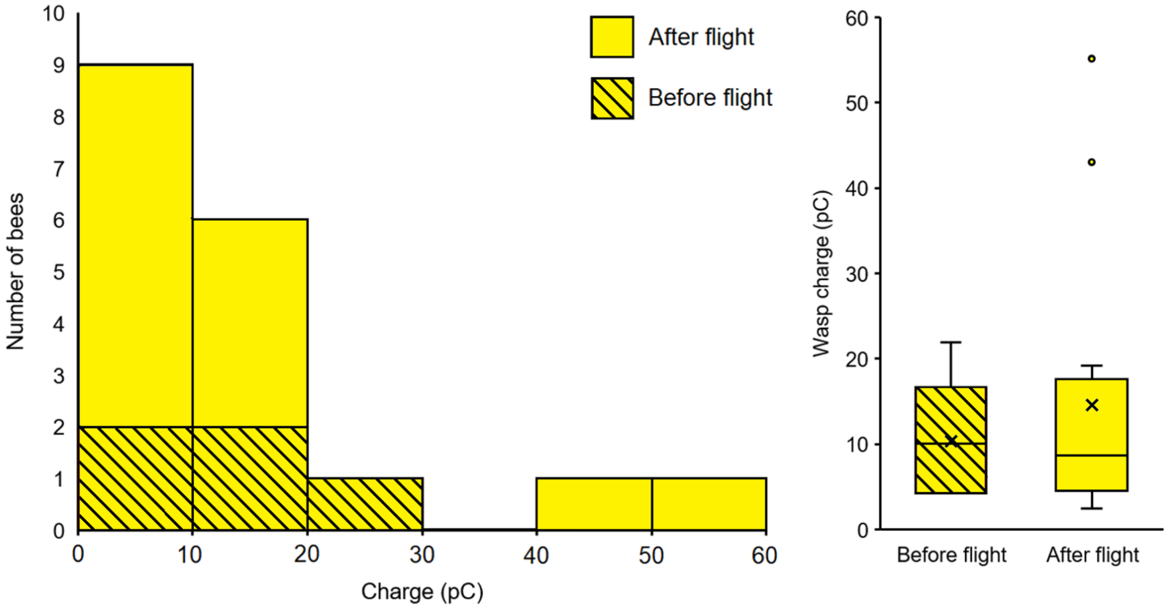

Figure 5.12. The electric charges measured on *V. vulgaris* before and after flight at the field site in Langford as a stacked histogram (left) and a boxplot (right) showing mean (X), median, IQR and range excluding outliers. N=13 after flight, 5 before flight.

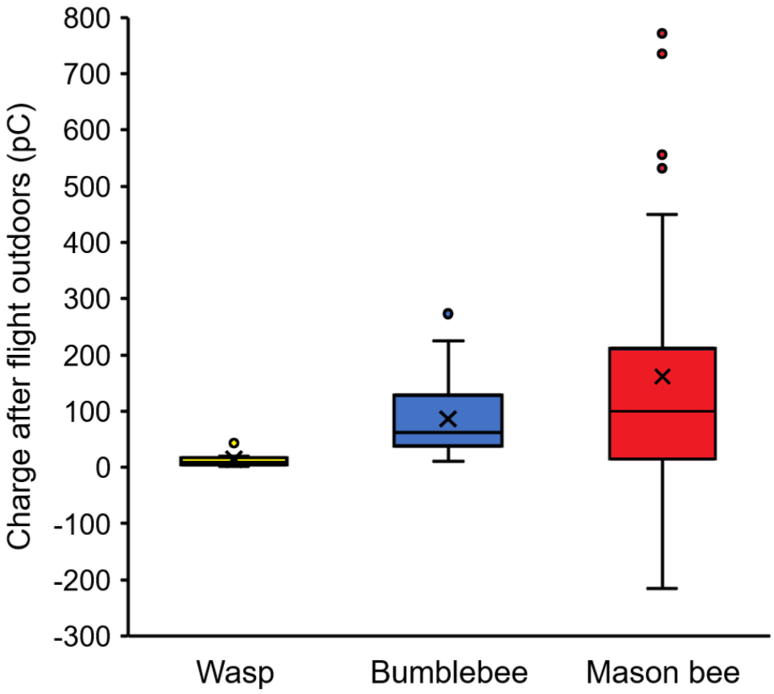

Figure 5.13. The electric charge measured on returning (inbound) wasps, bumblebees and mason bees after flight outdoors in dry, warm weather (fair weather conditions). The charges on mason bees and bumblebees were significantly higher than the charges measured on wasps.

The charges on the wasps after flight was compared with that of the bumblebees and mason bees after flying outdoors in similar environmental conditions. There was a significant difference in charge between the different insects (Kruskall-Wallis, $P < 0.0001$, $F = 13.6$). The charges on free-flying *V. vespula* wasps are significantly lower than those on bumblebees flying in and out of the colony on the same day under the same conditions (Figure 5.13, Dunn's post-hoc, $P < 0.0001$, $Z = 3.60$) and significantly lower than the charges on mason bees under similar conditions ($P < 0.0001$, $Z = 4.22$). The charges on both bee species are not significantly different from each other ($P = 0.715$, $Z = -0.36$). These results suggest that pollinivorous bees have higher charges than omnivorous wasps flying in the same conditions.

5.6 SUMMARY OF RESULTS

The charges measured on bees and wasps flying outdoors correspond to the relative triboelectric position of each insect, with mason bees and bumblebees having the most positive charges both when rubbed against various materials, and when measured during flight outdoors. This suggests that the triboelectric properties of the bees contribute to their greater charge measured during flight.. Mason bees returning to the colony after flight had a much higher charge then those leaving the colony (Figure 5.10). This was also observed with bumblebees (Chapter 4) but was not found to be the case with wasps, where wasps entering and leaving the bumblebee colony had approximately equal charges (Figure 5.12). It seems therefore that bees gain charge during flight or foraging, but that wasps do not, perhaps resulting from different mechanisms of charge generation, or differences in their morphology and associated tribology. These mechanisms can be investigated using laboratory studies with *V. vulgaris*, but this was beyond the scope of this thesis.

5.7 DISCUSSION

Throughout this chapter it is shown that mason bees and bumblebees are more positively charged than wasps, but that wasps are still very weakly positive. In all cases bees are more positively charged than wasps, with mason bees and bumblebees gaining a more positive charge through triboelectrification and during flight outdoors. This increased charge in bees may have adaptive value by facilitating pollen adhesion and potentially electroreception, whilst in wasps there is reduced value to being electrically charged. These differences can be explained by comparing differences in the foraging ecology and morphology of these insects, and investigating the potential mechanisms of charge generation and retention and whether these differ between species.

5.7.1 Mechanisms

Bees, beetles, flies and moths have very different morphologies, life histories and charges. The only study comparing the charge on different insects is Edwards (1962), where the author compared the triboelectric charging of these orders by rubbing dead insects against different materials with similar methods to this current study. However, there is no quantification of charge, so it is hard to compare the charging of the different insects. Edwards showed that generally all insects became positively charged when rubbed against glass, mica, wood and even metals (although it is not mentioned which metals), but negatively charged when rubbed against asbestos. Glass, mica and asbestos are commonly placed towards the positive end of the triboelectric series (Diaz and Felix-Navarro 2004) suggesting therefore that insects gain a positive charge upon contact with most materials. This implies that charging is a material property, where insects who may not benefit from charging up gain a positive charge upon friction with their surroundings. The insects used in the Edward’s study (Edwards 1962) and in this thesis are freshly killed, so we can show that the charge generation in this instance can be based on material properties alone. However, the charges on the insects in our study are very low after triboelectrification (<5pC apart from one case with the mason bee, Table

5.1) suggesting that while passive triboelectrification may contribute towards charging in insects, it is unlikely to be the sole mechanism.

Bees are shown to gain charge through triboelectrification with flowers and with dielectric materials with known electrical properties. Wasps do not gain charge so readily, resulting in them being placed further down the triboelectric series and having a more variable charge upon triboelectrification. The difference in charge observed on these insects during flight could therefore be due to differences in their tribology. Both mason bees and bumblebees gain charge upon visiting flowers. Although the charge on wasps was not measured directly before and after visiting a flower, the relative tribology suggests that wasps would not gain charge from this interaction. As charge transfer by triboelectrification results from differences in the work function between both materials, it is possible that the cuticle of wasps has a higher work function and will therefore lose electrons less readily than bumblebee or mason bee cuticle. Whilst both cuticles are made of chitin (Majtán et al. 2007; Baranek et al. 2018) they differ in the chemical composition of the waxy layer and have vastly different surfaces due to the presence of hairs over most of the bee body. Bees also spend much more time visiting flowers which could lead to an increased triboelectric accumulation of charge. As bees retain their charge well, this charge can accumulate over time, leading to greater charges after prolonged foraging bouts. Our results reveal that wasps are less likely to gain a positive charge when landing on flowers or other surfaces and will lose this charge to their surroundings more readily than bees.

Mason bees had much lower charges when exiting the colony than after flight. Mason bees returning to their egg chambers will squeeze through small holes in the wall made of stone and brick. In doing so, they may lose charge to the wall, effectively grounding themselves. Similar low charges were seen on bumblebees exiting their colony in Chapter 4, and it is therefore possible that bees lose charge by sharing charge with each other. In most bee colonies, bees are in often constant physical contact with each other. Assuming bees of the same species have the same or similar electron affinity, they will share charge where a more highly charged bee will impart charge onto a bee with a lower charge upon physical contact. Thus, a bee entering a colony with a higher charge will lose charge to its sisters and exit the colony with a reduced charge which may be then replenished during flight and foraging. The charge on a bee has even been suggested to be important in honeybee communication, with Greggers and colleagues suggesting it plays a role in communication via the waggle dance (Greggers et al. 2013). The charge on a returning honeybee may even provide information about foraging conditions (as favourable conditions such as warm, dry, sunny weather correspond to a higher charge). Charge would also correspond to local food availability, as if bees gain charge triboelectrically on flowers, this charge is boosted upon each flower visit but lost slowly over prolonged flight. Thus in constant conditions bee charge may demonstrate the availability of local foraging resources and may be a useful factor in honeybee communication.

All mason bees returning to the nesting site were laden with pollen, suggesting that this pollen may contribute to the charge on the bee. To obtain pollen, the mason bee must rub her abdomen all over the stamens to trap pollen between the scopa. This mechanism is likely to contribute towards triboelectric charging in mason bees, so a full scopa may be indicative of a triboelectrically charged bee. Bumblebees typically use their legs to remove pollen from stamens (Thomson and Goodell 2001), before grooming it into the corbiculae, causing relatively less frictional contact between flower and bee. Measurements of the electric charge on laden and unladen mason bees flying outdoors would be useful to elucidate the mechanisms behind charge generation. Additionally, since charge is likely to promote adhesion of pollen grains to the bee, it would be very interesting to compare the pollen load of a foraging bee with its charge. Mason bees will also bring mud and sand back to the nest for construction of nesting cells, using their mouthparts to transport the material. The charges on bees after a pollen foraging trip or a trip scavenging for construction materials could be compared to see whether bee charging is specific to bees foraging on flowers and carrying pollen, and if so, whether it reflects a triboelectric interaction or whether the bees could actively modulate their charge for maximum pollen transportation.

That bees retain charge better than other arthropods may be due to an adapted morphology. The obvious distinguishing factor is the hairs present on bumblebees and mason bees, which may contribute to their ability to gain and/or retain charge. Preliminary experiments with bumblebees were instigated to investigate a correlation between hairiness and charge. However, whilst it is possible to remove hairs from bumblebees to a reasonable extent using a scalpel or a corrosive hair removal agent, doing so alters the surface structure of the cuticle and may also influence charging capacity by changing the chemical structure. Additionally, removing hairs from bees also removes their willingness to fly through the RCS or into the Faraday pail. As such, direct comparisons of bees with and without hairs were attempted, but ultimately abandoned.

Both mason bees and bumblebees have a significantly more positive charge after foraging outdoors than they do when leaving the nest. This suggests a charging mechanism present during foraging, whether that is triboelectrification with flowers or other surfaces they encounter, or charging during flight due to contact with ions or triboelectrification with air-borne particulates. This increase in charge after flight is not seen with wasps flying outdoors, but there are many differences that need to be considered. The wasps measured in this chapter were not foraging on flowers but were stealing nectar from the bumblebee colony to take back to their own colony. Accordingly, they do not encounter the same surfaces as the bees, and their differences in charge may be due to the different materials they are encountering. In summary, there are several potential explanations for why bees gain charge whilst foraging whilst wasps do not:

1. Bumblebees and mason bees are charging when visiting flowers. Wasps do not visit flowers and even if they did their triboelectric position indicates they would not gain charge this way.
2. The low work function of bumblebee cuticle makes them lose electrons readily by triboelectrification whilst a higher work function of the wasp cuticle does not.

3. Bees and wasps charge to the same extent during flight or whilst foraging, but the bees are better able to retain the charge whilst with wasps it is lost to the surroundings.
4. Hairs facilitate charge acquisition and/or retention. Bumblebees and mason bees are therefore better able to acquire and/or maintain their charge whilst foraging whilst wasps are not.

These explanations are not mutually exclusive, and the likely mechanisms for the generation of charge in insects and the difference in charge acquisition and retention between different insects and wider arthropods is likely to be due to a whole range of factors. These are hard to isolate without concrete knowledge of the electrical properties of insect cuticles alongside measurements of charge on many different insects. This chapter is not intended to be comprehensive, rather to present new empirical evidence and provoke discussion into how charging may affect insects, how charge may be generated and whether this may have influenced the evolution of pollinator morphology.

5.7.2 Ecological role

5.7.2.1 Pollen adhesion

Electric charge is likely to benefit pollinating and pollinivorous insects by facilitating the adhesion of pollen to the insect body. The charge measured on flying insects varies significantly between species (Kruskall-Wallis, $p < 0.0001$, $F = 13.6$) with pollinivorous mason bees and bumblebees having significantly more positive charges than omnivorous wasps. Increased pollen adhesion would select for increased charge generation in mason bees by increasing the fitness of offspring. In effect, more pollen collected on each foraging trip is poised to ensure greater larval provision at reduced energetic cost. The composition and amount of pollen deposited in brood cells has profound impacts on the development of offspring in mason bees (Bukovinszky et al. 2017) with maternal provision of pollen correlating strongly with offspring fitness (Radmacher and Strohm 2010). The facilitation of pollen transport back to brood cells may therefore allow greater offspring provision, increasing offspring fitness and providing a selective advantage to charged bees. This would select for morphological adaptations on the bees (such as hair) that may contribute to their charge and to the adhesion of pollen.

This is also true of bumblebees despite the differences in pollen transportation. In bumblebees the majority of pollen is transported in corbiculae. However, these corbiculae are filled with pollen groomed from over the bee’s body. An increased charge on the bee would result in greater pollen adhesion to various parts of the bee body. This pollen can then be groomed into corbiculae (or transported on the body of the bee if electrostatic adhesion is sufficient) and brought to the colony with minimum additional effort, leading to increased offspring fitness and colony productivity. Given that pollen provision is correlated with increased offspring fitness in many bee species (Génissel et al. 2002; T'ai and Cane 2002; Radmacher and Strohm 2010), the generation and retention of an electrostatic charge provides adaptive value for pollinivorous bee species, potentially selecting for morphological features enhancing bee charge such as hair.

A charged insect will attract and accumulate any charged airborne particulates, not just pollen. This includes dust, soot and charged ions, as well as potentially detrimental pathogens such as fungal spores and airborne bacteria. Charged bees have indeed been observed to collect more airborne bacterial spores during flight than uncharged bees (Lighthart et al. 2000). Thus, there is a trade-off where, in bees, the benefits of pollen adhesion and enhancement of electroreception may outweigh the costs of attracting pathogens. As wasps do not forage for pollen, these costs are likely to select against the generation of charge to prevent the build-up of dirt upon the insect.

5.7.2.2 Electroreception

Alongside pollen adhesion, bee charge also benefits bees by promoting electroreception via the deflection of charged hairs in an electric field. In bumblebees this is thought to allow bees to sense additional electric floral cues such as the shape and strength of the floral electric field, allowing bees to make foraging decisions and respond to electrical information potentially signalling nectar availability (Clarke et al., 2013). This has also been theorised in honeybees to allow communication within the hive via the electrostatic deflection of antennae (Greggers et al. 2013). Honeybees communicate the location and distance of rewarding food sources via a waggle dance. The waggle dance of a charged bee has also been proposed to provide electrical information, as well as visual, auditory and percussive information. This may help explain why honeybees in the hive have been found to have greater charges than those out foraging (Colin, Richard, and Chauzy 1991), and suggests electroreception and possibly bee charge may play different ecological and communicational roles in different bee species. Insect charge may further be enhanced by the electrical properties of materials within the hive or colony. Social bees such as bumblebees and honeybees build structures out of beeswax and propolis, which are often insulating and dielectric, with beeswax having a dielectric constant of 2.4 (Von Hippel, 1954). Bees walking on beeswax gained a more positive charge than when walking on any other material except wool (Es'kov and Sapozhnikov, 1976) suggesting that wax enhances the charge on bees within the hive. *V. vulgaris* on the other hand make nests of chewed wood fibres which are largely triboelectrically neutral (Diaz and Felix-Navarro, 2004), suggesting wasps will not charge within the nest.

Electroreception has not currently been investigated in either wasps or mason bees. Electroreception in insects is generally understudied and may be more widespread than currently thought. For wasps, electroreception could allow detection of charged prey species against an uncharged background. However, the charge generated by a wasp may alert electroreceptive prey species to its presence. Electroreception has been shown in insects (Newland et al. 2008; Clarke et al. 2013; Greggers et al. 2013) and has been suggested as a means of detecting and subsequently avoiding predators (Newland et al. 2008). The electric charge on an approaching wasp would allow increased detection of that wasp by any prey insects with even primitive electroreception, although this is likely to be only over low distances. Without knowledge of the extent of electroreception in predator-prey insect interactions, it is hard to determine whether increased detection by prey insects could have selected against the evolution of electric charge in predatory wasps.

Whilst the inclusion of only 3 species limits this study, it nevertheless provides a good early comparison between similarly sized species living in similar environments. Widespread measurement of the charges on different insect species with different morphologies, diets, social structures and threats would greatly increase our understanding on the role of electric charge and electroreception in insect ecology. Greater understanding of the extent of electroreception and electric charge within and beyond the Hymenoptera is therefore necessary to increase understanding of the role of charge in insect interactions and ecology. This information has potential benefits outside of science, including conservation, ecology and agriculture. Knowledge of the charge on insects can be exploited for the development of technology used to target insect crop pests (Tanaka et al. 2008; Kakutani et al. 2012; Nonomura et al. 2012) and disease vectors (Brown et al. 1997; Whitmore et al. 2001). Thus, increased knowledge of insect charge and its variability and role in insect ecology has applications for agriculture and human health, whilst also providing information on how to conserve beneficial insects such as pollinators.

6 BUMBLEBEE CHARGE AND PLANT VOLATILE EMISSIONS

6.1 INTRODUCTION

The mutualistic relationship between plant and pollinator relies on successful communication. An insect-pollinated plant must advertise effectively to attract insects for reproduction. Insects must be able to receive and process this information in order to make foraging decisions and avoid feeding on unrewarding flowers. Plants release a wide variety of information to try and lure in potential pollinators. These include combinations of alluring scents, colours, and shapes (Whitney and Glover 2007) as well as temperature (Dyer et al. 2006), iridescence (Whitney et al. 2009), symmetry (Rodríguez et al. 2004) and local humidity (Von Arx et al. 2012). Bumblebees have also been shown to sense the strength and shape of floral electrical fields (Clarke et al. 2013).

In the context of pollination, scent is a pivotal floral cue. Scent fulfils a large number of communicative roles (reviewed in Dudareva et al. 2006; Das et al. 2013), is highly species-specific (Pichersky and Gershenzon 2002) and can be indicative of pollination status (Theis and Raguso 2005). Plant scents can be used for intra- and inter-species communication (Karban et al. 2000); reviewed in (Dicke and Bruin 2001). The flower actively advertises its nectar availability by producing scented compounds which can attract pollinators across great distances (Haverkamp et al. 2016). The production of volatile organic compounds (VOCs) is energetically expensive for the flower, so many plants coincide their scent release with the peak foraging times of their pollinators (Dudareva et al. 2000; Hoballah et al. 2005; Theis, Lerdau, and Raguso 2007). This can be mediated by circadian rhythms as well as in response to sensory cues such as light intensity (Matile and Altenburger 1988; Kolosova et al. 2001; Simkin et al. 2004; Hoballah et al. 2005). However, rhythmically controlled chemical release could allow unnecessary volatile emission when there are no pollinators nearby (e.g. in poor weather), as rhythmically mediated emissions can persist in a free-running cycle in full light or dark (Kolosova et al. 2001). If flowers could directly sense the presence of their pollinators, they could more accurately coincide their volatile emissions with peak pollinator activity, preventing unnecessary and wasteful volatile release whilst maximising chances of successful pollination.

Accurate, timely knowledge of the activity and abundance of pollinators hold the benefit of enhancing the plants' advertising efficiency by optimising the release of VOCs on a much finer timescale.

As information about local floral rewards is time-sensitive, and only useful if it indicates the current quantity of nectar reserves, dynamic signalling of nectar availability is a crucial aid for foraging pollinators. Local humidity may be indicative of nectar quantity in moth-pollinated flowers (Von Arx et al. 2012) whilst changes in floral hues can indicate nectar availability (Weiss 1991; Weiss and Lamont 1997). Many flowers change or reduce their scent production after successful pollination, as the requirement for advertising becomes largely unnecessary (Schiestl et al. 1997; Rodriguez-Saona et al. 2011). Significantly lower volatile emissions indicating decreased nectar resources can be observed 24 to 96 hours after pollination (Schiestl et al. 1997; Theis and Raguso 2005; Rodriguez-Saona et al. 2011). The exchange of electrical information on the other hand is comparatively instantaneous. A bee visiting a flower causes the flower's electric stem potential to change by up to 25 mV within seconds (Clarke et al. 2013). This could allow subsequent foraging bees to avoid the flower based on its new electrical information.

Bumblebees can distinguish between rewarding and unrewarding flowers on the basis of their electric charge with the likely sensory mechanism being mechanosensory hairs (Clarke et al. 2013; Sutton et al. 2016). Whilst the ability to sense electric fields benefits the bee, it is currently unknown how much the exchange of electrical information benefits the flower. When a charged bee approaches a flower it raises the potential in the flower up to 25mV (Clarke et al. 2013). Though applied electric fields have been shown to affect plants in different ways (reviewed in Volkov 2017), the extent to which this depolarisation affects the flower is currently unknown. A brief change in floral electric potential would provide information to the plant on the abundance and activity of foraging pollinators. This information could allow the plant to effectively moderate its VOC emissions with regard to the activity of foraging pollinators, to maximise its chances of successful pollination.

This potential for electrosensitivity in bee pollinated flowers is explored in this chapter. The volatile compounds produced by flowers were measured from a bee pollinated petunia, *Petunia integrifolia* and a bumblebee-pollinated snapdragon *Antirrhinum majus* ‘Maryland True Pink’ (MTP). Volatile emissions in *P. integrifolia* are modified by environmental conditions (Hoballah et al. 2005) and as such may be more likely to be influenced by external stimuli such as an electric field. Volatile emissions from *A. majus* MTP are mediated by an innate circadian rhythm, robust to changes in the environmental conditions (Kolosova et al. 2001).

The dominant volatile compounds were identified from both flowering plant species. Behavioural and electrophysiological experiments were conducted to identify whether these volatile compounds could be sensed by bumblebees and determine the ecological relevance for a foraging bumblebee. The emission rate of the identified volatiles was measured from flowers that were electrically stimulated by a triboelectrically charged rod mimicking a flying bumblebee. The volatiles emissions were also measured from flowers visited by free-flying bumblebees with measured charges. The overall aim of these experiments was to test the hypothesis that bumblebee-pollinated flowers increase their emission of attractive volatile compounds in response to electrical stimulation caused by a foraging bumblebee.

6.2 THE FLORAL VOCS

Two well-studied mellitophilous flowering plants that varied in their scent, shape and floral syndromes were used in these experiments. The petunia *Petunia integrifolia* has a simple, well characterised scent profile and has been previously used for studies with bumblebees. As there was the potential for subtle effects to be missed by using a model flower with a simple scent profile, another model flower was also chosen for comparison. The snapdragon *Antirrhinum majus* "Maryland True Pink" was chosen as it has a more complex floral scent profile and produces a greater quantity of volatiles than *P. integrifolia*. It was chosen over other snapdragon cultivars as it had a high volatile emission (Wright et al. 2005) and it was possible to obtain seeds. Plants were grown from seed in the GroDome at the University of Bristol with a 16:8 day:night cycle at 20°C. Where experiments were conducted at Rothamsted, plants were transported from Bristol and housed in the Rothamsted greenhouses with a natural light cycle and kept at 22°C.

6.3 METHODS: FLORAL VOLATILE COLLECTION AND ANALYSIS

6.3.1 Floral headspace analysis

There are several methods for collecting the volatile organic compounds (VOCs) emitted by plants, but one of the most efficient and useful is dynamic headspace collection (air entrainment). For the collection of floral volatiles, this involves encapsulating the head of the flower in a bag or glass chamber. Charcoal-filtered air enters this headspace, and another pump is used to draw air out through a glass tube at a lower rate, thereby maintaining a constant positive air pressure (Figure 6.1). This tube (3.9 mm outer diameter, 2.4 mm inner diameter) contains 50mg of Porapak Q powder (Supelco, Poole, UK), a volatile entrapment adsorbent powder contained by glass wool plugs. To prevent contamination before use, these Porapak tubes are washed with distilled diethyl ether and baked in a specialised oven under nitrogen flow at 132°C for 2 hours before use to remove any contaminants present. After the experimental volatile collection period the pump is turned off and the glass tube containing the adsorbent powder is removed. After use, the powder is eluted with 750 µl of diethyl ether into a glass vial. This solution can then be concentrated down by evaporating the diethyl ether under a gentle stream of nitrogen in a fume hood.

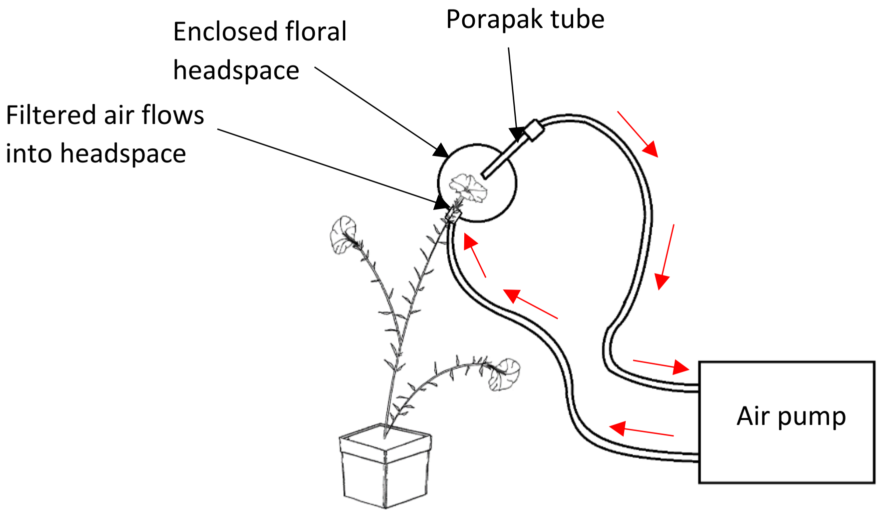

Figure 6.1. Diagram showing volatile collection via dynamic headspace collection. Direction of airflow is indicated by red arrows. Filtered air is pumped into the headspace to maintain positive pressure. Air is then drawn through the Porapak tube and back to the air pump and the floral volatiles are captured by the Porapak powder in the tube.

6.3.2 Gas Chromatography

The VOC composition of these samples can be measured by Gas Chromatography (GC). Gas chromatography consists of two phases; the mobile phase and the stationary phase. The mobile phase is a carrier gas, usually helium or hydrogen, pumped through a long, narrow glass tube. The walls of this tube are covered with the stationary phase, a film which absorbs the volatile compounds and elutes them at different times depending on their volatility or reaction with the stationary phase. Once the sample is injected the compounds adhere to the stationary phase film. The tube is heated at a constant rate and the chemicals are released, leading to a separation of compounds as a function of time (retention time). The compounds are subsequently moved down the tube by the carrier gas and are usually detected by a flame ionisation detector (FID). The FID pyrolyzes the VOCs leaving the column. The combustion of the compound creates charged ions, which are measured as a current by a biased voltage collector. The current is proportional to the rate of combustion, and hence proportional to the concentration of analyte in the sample. As the same volume and concentration of samples are injected each time, this method allows qualitative and quantitative analysis of the compounds within the sample.

Quantitative analysis of the sample can be discerned by integrating the peaks provided in the gas chromatogram. The integral of the peak is proportional to the amount of that compound present in the sample. Injection of known amounts of alkanes with different retention times allows an estimation of the amount of the analyte in the sample of interest. Other quantification methods include using internal standards or calibration curves. As the concentration and volume of the injected sample are known, this allows quantification of the ratios and amounts of the different compounds in the sample and hence the ratio and amount of VOCs produced by that flower over the volatile collection period.

For qualitative identification of the compounds, the VOCs present can be identified tentatively by the retention time of each compound. By comparing the retention time of the sample compounds with that of known alkanes analysed on the same polarity GC column and under the same temperature programme, the retention time can be standardised between GCs. This standardised retention time is known as the Kováts retention index (KI). The KI of the sample compounds can be compared against the KI of known compounds to identify likely candidates (Guan, Kiraly, and Rijks 1989), however for complete identification the samples must be analysed more comprehensively.

6.3.3 Gas Chromatograpy-Mass Spectrometry

To identify the compounds present in a sample, the GC is coupled with a mass spectrometer. This combination is referred to as gas chromatography-mass spectrometry (GC-MS). The compounds are separated out by GC by their retention time, and subsequently identified by the mass spectrometer by analysing the mass to charge ratio of the separated compounds. GC-MS allows tentative identification of compounds that may have similar retention times and therefore cannot be reliably identified solely by GC.

6.3.4 GC Peak Enhancement

For confirmation of identification, ideally the same amounts of pure samples (standards) of the compounds identified by GC-MS are co-injected with the original sample. Peak height in the chromatogram should have doubled for each injected sample but not widened, signalling correct identification of samples with identical retention times. Double peaks or significantly widened peaks indicate an incorrect identification and injection of different compounds with similar but differing retention times. For the analysis of the major compounds produced by *P. integrifolia* and *A. majus*, the VOCs were collected by a modified dynamic headspace analysis and eluted with diethyl ether. Samples were inspected via GC and their retention times compared to injected alkanes to calculate the KI for each peak. Compounds were subsequently identified by GC-MS and identification confirmed by GC coinjection with pure standards.

6.3.5 Volatile collection from petunias and snapdragons

The volatiles were collected from both petunias and snapdragons by modified dynamic headspace analysis. For initial identification of compounds, the flowers were enclosed in roasting bags and dynamic headspace collection was done as shown in Figure 6.1, with the Porapak tube placed at the floral opening 5mm from petals. The flow rate of air being drawn from the flower through the Porapak Q tube was 500 mLmin^{-1}, and the flow rate of purified air entering the roasting bag was 600 mLmin^{-1} to maintain positive pressure. A room control was done without flowers present to identify peaks corresponding to potential contaminants. Only peaks that were reliably present in the floral samples but not in the room control were analysed and identified.

The volatiles were collected from potted petunia flowers with the flower intact on the stem. With the snapdragons, the stem was cut below the inflorescence, and the stem was placed in a conical flask containing water. Using a portable dynamic headspace sampling kit (Pye volatile collection kit, Kings Walden, Herts, UK), volatiles were collected from the flowers for 2 hours at a flow rate of 500 mLmin^{-1} by placing a glass tube containing 50mg Porapak Q polymer sandwiched by two glass wool plugs, at the opening of the flower 5mm from the petals (Figure 6.2). The soil at the base of the plant was lightly watered before volatile collection took place.

For subsequent experiments, the flower needed to be accessed by an electrical stimulus so encapsulation inside an inert container was impractical. As such the Porapak tube was placed very close to the flower of interest, but the flower or inflorescence was not enclosed (Figure 6.2). To control for environmental contamination, control samples from the room without the flowers present were taken and analysed. The floral compounds previously identified from enclosed flowers were not present in the room controls. Any compounds present in the room controls were not analysed in the floral samples.

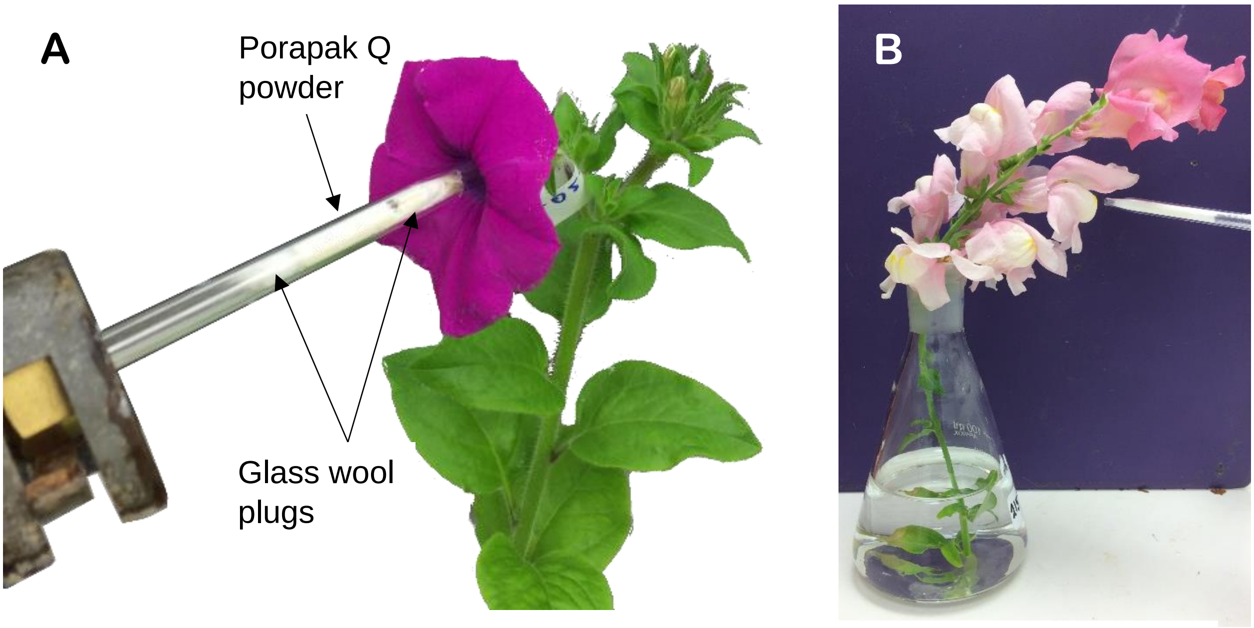

Figure 6.2. The position of the glass tube containing Porapak Q polymer relative to the petunia flower (A) and the snapdragon inflorescence (B).

The volatiles were removed from the polymer tubes by flushing the tubes with diethyl ether. The samples were then concentrated to 50µl and analysed by GC-MS. For the identification of the compounds present in *P. integrifolia* and *A. majus* MTP, a Hewlett-Packard 5890 series II GC fitted with a capillary HP-1 GC column (50 m × 320 µm i.d., 0.52 µm film thickness; J&W Scientific, Folsom, CA) and equipped with a cool on-column injector was directly coupled to a mass spectrometer (Hewlett-Packard 5972 mass-selective detector). Ionisation was by electron impact at 70 eV, 220 °C. The oven temperature was maintained at 40 °C for 1 min and then programmed at 5 °C/min to 250 °C (hold time 17.2 min). The carrier gas was helium. Tentative identification by GC-MS was confirmed by comparing retention index of the unknown peak with that of synthetic compounds and by GC peak enhancement by co-injection with an authentic sample (Pickett 1990), using an Agilent 6890N GC equipped with a cool on-column injector, flame ionisation detector and a 50 m × 320 µm i.d., 0.52 µm film thickness HP-1 column. The oven temperature was maintained at 30 °C for 1 min and then programmed at 5 °C/min to 150 °C for 0.1 min, then 10 °C/min to 250 °C for 20 min. The carrier gas was hydrogen.

6.4 RESULTS: IDENTIFIED FLORAL VOCS

6.4.1 Petunias

Petunia integrifolia has a very simple but well characterised scent profile. Unlike some other *Petunia* species such as *P. axillaris*, it does not invest in a complex scent production and is pollinated predominantly by bees, not moths (Hoballah et al. 2005). The relatively simple scent profile allowed comparison of scent production between different flowers. Benzaldehyde was the only major compound identified from *P. integrifolia* flowers (Figure 6.3A). This result is supported by the literature where the lack of other significant floral compounds has been previously remarked upon (Hoballah et al. 2005).

6.4.2 Snapdragons

The floral volatile emissions of *Antirrhinum majus* “Maryland True Pink” cultivar snapdragons were collected and analysed by GC. The main volatiles were tentatively identified by GC-MS and by their Kováts Index. The three main compounds present were myrcene, (*E*)-ocimene and methyl benzoate (Figure 6.3B), which is consistent with the compounds identified from this cultivar in the literature (Dudareva et al. 2000; Dudareva et al. 2003; Wright et al. 2005; Table 6.1). However, bumblebees were later found to respond to a fourth compound, 3, 5-dimethoxytoluene, which whilst not as abundant as the other three, caused consistent electrophysiological responses in bumblebees (as shown in subsequent experiments) and is also indicated on the GC profile (Figure 6.3B).

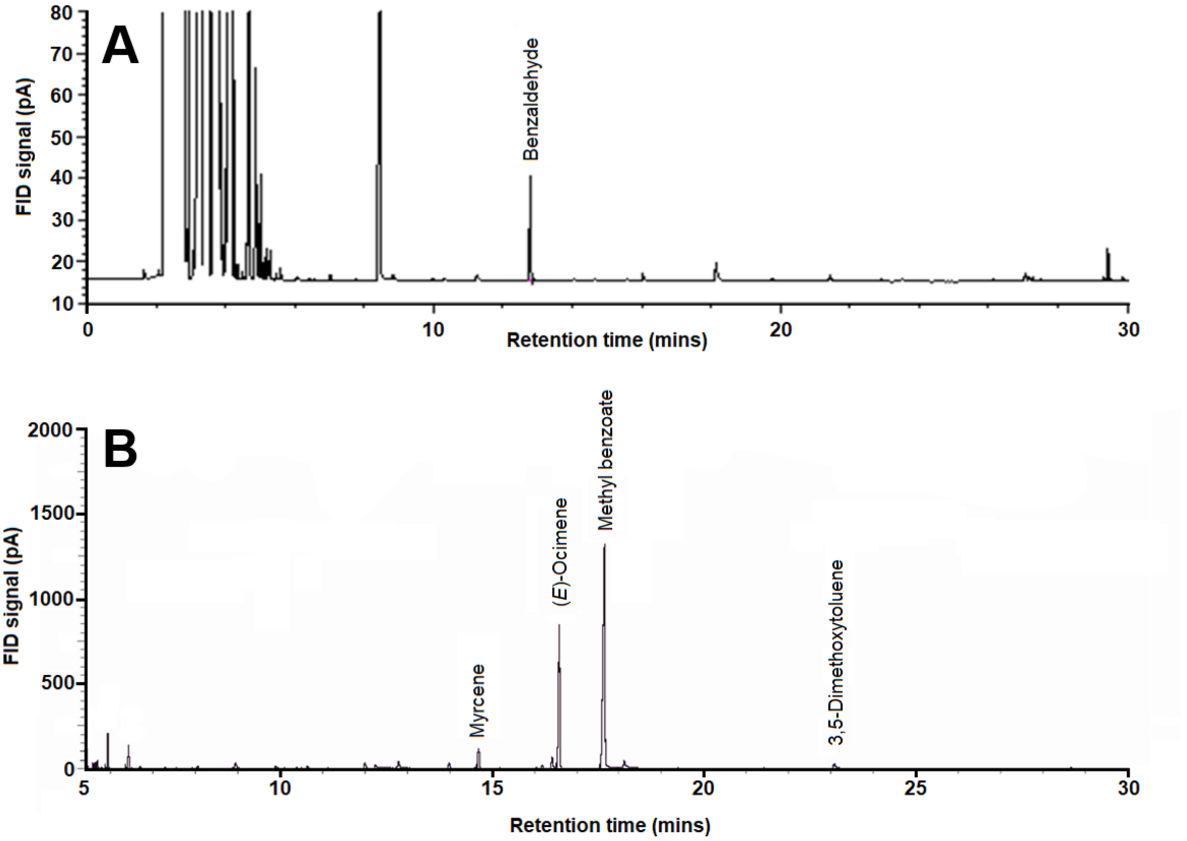

Figure 6.3. A typical gas chromatographic profile for A) *P. integrifolia* and B) *A. majus* flower headspace extract, showing the identification of the major compounds present in the floral samples but not in the controls. These compounds were identified by GC-MS and confirmed with GC co-injection with synthetic standards.

Table 6.1. Comparison of the volatile composition of *A. majus* "Maryland True Pink" from Dudareva et al., (2000) and identification of floral volatiles from the same species in this thesis.

	Dudareva *et al.,* (2000)		This thesis	
Compound	Volatile production (µg/flower/24hr)	Relative amount (%)	Volatile production (µg/flower/2hr)	Relative amount (%)
Myrcene	7.7 ± 2.1	8.1	0.21 ± 0.12	5.5
(*E*)-Ocimene	26.0 ± 5.9	27.4	1.56 ± 0.92	40.4
Methyl benzoate	56.5 ± 7.3	59.5	2.00 ± 1.14	51.7
3, 5-Dimethyoxytoluene	-	-	0.10 ± 0.07	2.4

6.5 THE COMPOUNDS SENSED BY BUMBLEBEES

To determine whether the identified floral VOCs could be used by bees to detect rewarding flowers, it is necessary to establish which compounds can be detected by bees. Bumblebees have been previously shown to sense and respond to some of the compounds present in these two flowers. Bumblebee electroantennography performed by Suchet and colleagues in 2011 showed that bee antennae responded to benzaldehyde, the main compound in *P. integrifolia*, as well as methyl benzoate, the main compound in *A. majus* MTP (Suchet et al. 2011; Figure 6.4). Myrcene and (*E*)-ocimene have also been shown to elicit electrophysiological responses from antennae of the bumblebee *Bombus vosnesenskii,* using GC-EAG (Byers, Bradshaw, and Riffell 2014).

The hypothesis that bees could detect the compounds present in petunia and snapdragons was tested using a proboscis extension reflex (PER) experiment and coupled Gas Chromatography Electroantennography (GC-EAG).

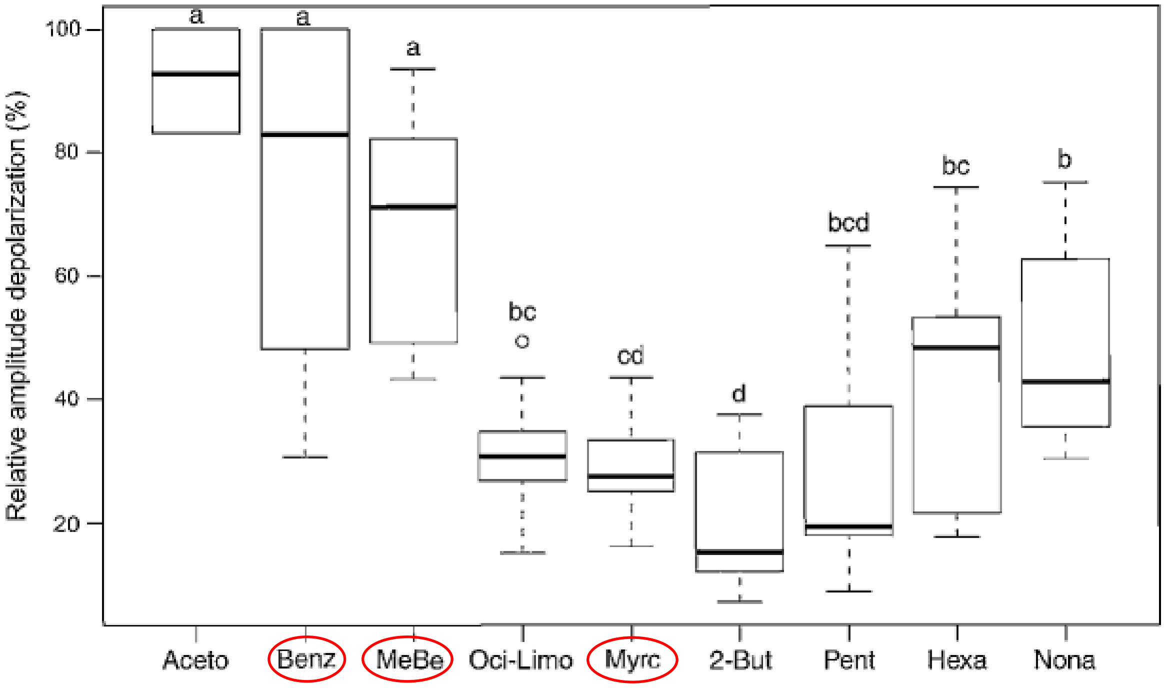

Figure 6.4. Relative amplitude depolarisation of ten bumblebee antennae for the ten synthetic VOCs analysed by electroantennography method (EAG). The ten tested VOCs were: Acetophenone, Benzaldehyde, Methyl benzoate, Z-ocimene and Limonene, Myrcene, 2-Butanone, Pentanal, Hexanal and Nonanal. The compounds present in *P. integrifolia* (Benzaldehyde) and in *A. majus* MTP (Methyl benzoate and Myrcene) are circled in red. A pairwise comparison of the signal was performed: box plots with the same letter at the top were not significantly different. Figure from Suchet *et al.,* 2011.

6.5.1 Proboscis extension reflex (PER)

The proboscis extension reflex (PER) experiment is a common behavioural experiment used to test memory and learning in insects. PER involves pairing a scent (conditioned stimulus) with a sugar reward (unconditioned stimulus). Over a series of trials, the bee is taught to associate the scent with the reward. During each trial the bee is presented with the scent and given the opportunity to extend its proboscis (unconditioned response). The antenna of the bee is then touched with a tissue containing sugar solution, causing the bee to extend its proboscis and the bee is allowed to consume some sugar. Once the association is learnt, the bee will extend its proboscis in anticipation of the reward upon detecting the scent (conditioned response). An overview of PER in bumblebees is found in Laloi et al. (1999).

The PER experiment exposed bumblebees to the scent of benzaldehyde administered as a puff of air from a pipette containing a filter paper onto which 2µl of pure benzaldehyde was applied. Bees were starved of sugar water 12 hours prior to the experiment. One bee was anaesthetised using CO_2 and placed in an enclosure formed from the head of a pipette, where the end had been removed to allow the head and tongue to protrude out the front of the enclosure (Figure 6.5). The bee enclosure and the end of the stimulus pipette were held down with plasticine. The stimulus pipette was placed so the tip was 1cm away from the head of the enclosure. The reward was administered as a drop of 30% sugar water on tissue paper rolled around a wooden rod (Figure 6.5).

16 bees were conditioned through 10 trials to associate the puff of air containing benzaldehyde with a reward. The trial consisted of slowly depressing the stimulus pipette for 12s ensuring flow of scented air past the head of the bee. During the first 6s of this period, the bee was observed for proboscis extension. During the second 6s, the bee was presented with a sugar solution and allowed to drink.

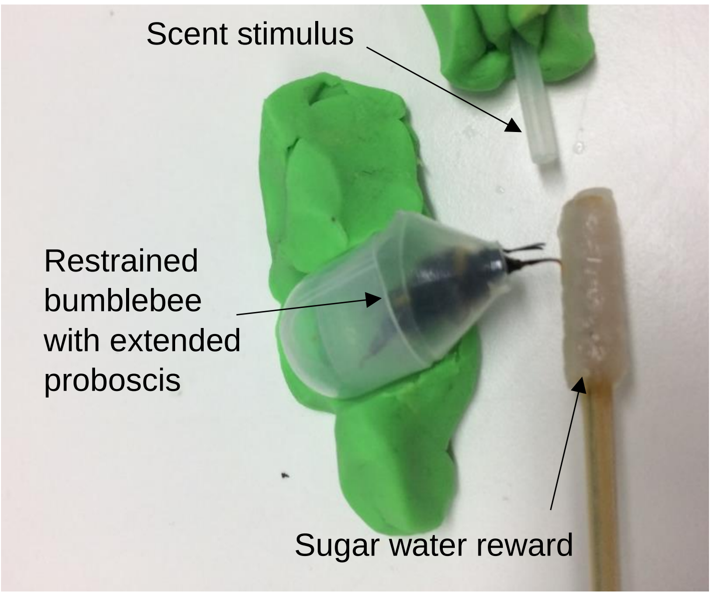

Figure 6.5. The experimental set-up for PER showing a bee in its restraining container being rewarded with a taste of sugar water after extending its proboscis.

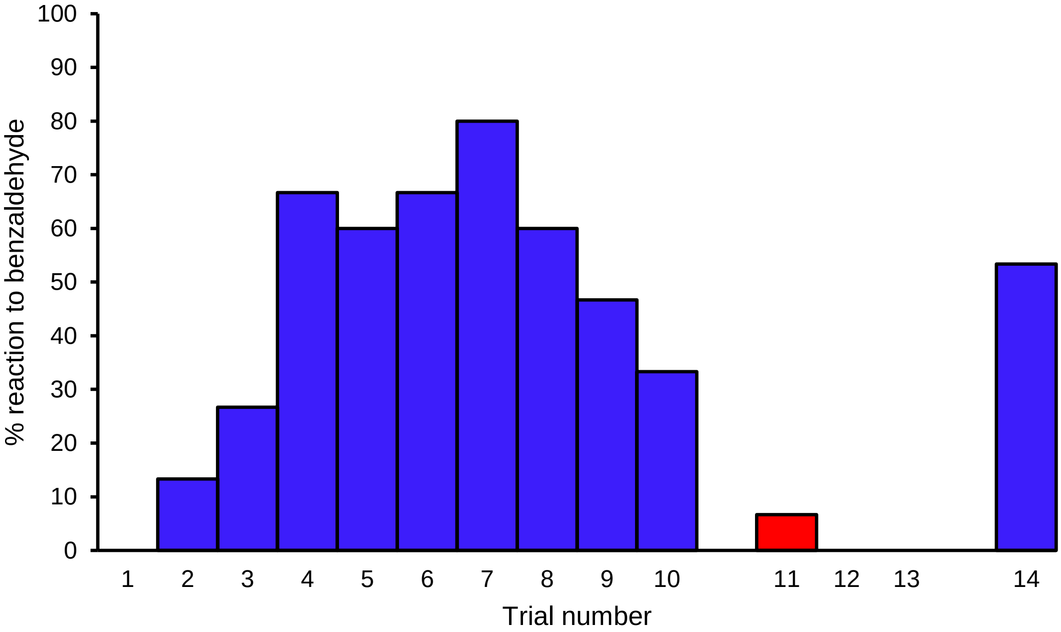

Figure 6.6 The percentage PER response rate of the 16 bees trialled. Trials 1-10 are conditioning trials. Trials 11-13 are control trials using an unscented puff of air. The lack of response during these trials shows the bee is conditioned to the scent, not the airflow. Trial 14 is the final confirmation trial using the scented stimulus.

The bee was left for 5 minutes between trials to allow the benzaldehyde scent to dissipate. After ten conditioning trials, three control trials (Trials 11, 12 and 13) were administered where the stimulus pipette was replaced by a control pipette not containing filter paper. In all but one case, these failed to elicit a PER response from the bee (Figure 6.6). After the three control trials, a final stimulus trial was conducted with the original benzaldehyde scent stimulus. The purpose of the control and final stimulus trials was to confirm the bee was responding to the scent of benzaldehyde and not just to the mechanical stimulus of the puff of air.

In the PER experiment, the percentage PER response increased up to 80% in trial 7 and remained above 30% (Figure 6.6). The unscented control trials failed to elicit a response in all but one case, whereas the final scented trial had a 53% response, showing that the bumblebees can reliably sense and respond to the scent of benzaldehyde.

6.5.2 Coupled Gas Chromatography-Electroantennography (GC-EAG)

6.5.2.1 Methods

Identification of the compounds that bumblebees responded to from snapdragons and petunias was possible using coupled Gas Chromatography-Electroantennography (GC-EAG). GC-EAG couples the outflow from the GC with a stream of humidified air past an insect antenna, allowing simultaneous measurement of the quantity of identified compounds via GC and the electrophysiological response produced by the insect antenna. GC-EAG can therefore be used to detect the response of insects to specific compounds within a sample (Wadhams 1990).

GC-EAG was used to confirm which compounds produced by petunias and snapdragons the bees can sense at the olfactory level. Volatiles were collected from a petunia flower and from a snapdragon inflorescence using enclosed headspace analysis for 2hr duration. The samples were eluted with diethyl ether and concentrated to 50 µl.

Bumblebees (*Bombus terrestris audax*) were taken from a colony (Koppert, UK) at Rothamsted Research. A worker bee was anaesthetised by cooling on ice, and an antenna was excised below the scape. Electrodes were constructed using borosilicate glass capillaries (2mm outer diameter, 1.6mm inner diameter) using an electrode puller. These were filled with ringer solution (7.55gl^{-1} sodium chloride, 0.64 gl^{-1} potassium chloride, 0.22gl^{-1} calcium chloride, 0.86gl^{-1} sodium bicarbonate, 1.73gl^{-1} magnesium chloride, 0.61gl^{-1} sodium orthophosphate). The electrodes were attached to a holder on a micromanipulator and threaded on so that a silver wire connected to the circuitry was inside the electrolyte.

The bumblebee antenna was cut just above the pedicel and a slit was made in the tip to ensure contact between the electrolyte and the antenna. Either end of the excised antenna was placed in the tip of the electrodes. Airflow was established by placing a glass tube with a hole in the side 5-10mm in front of the antenna. Air was passed through activated charcoal filter, humidified and pumped through the tube at 1l/min. The effluent from the GC was split 1:1 between the FID and a heated transfer line, the latter connected to the side of this tube so that it combined with the stream of purified humid air and passed over the antenna at a constant rate. The output from the GC and the EAG were compared using Syntech software (Ockenfels Syntech GmbH, Kirchzarten, Germany) to locate the peaks in both petunia and snapdragon samples which produced an electrophysiological response. Three repeats for each plant sample were done to confirm findings with separate bee antennae. The electrophysiological recordings were filtered (16x filter) to reduce noise and compared with the GC output to identify simultaneous depolarisations corresponding to the peaks of the different compounds.

6.5.2.2 Results

From GC-EAG I identified that bumblebees responded to benzaldehyde present in *P. integrifolia* flowers (Figure 6.7, N=3), and (*E*)-ocimene, methyl benzoate and 3, 5-dimethoxytoluene present in *A. majus* (Figure 6.7, N=3) but not to myrcene present in the same sample. This largely agrees with the results found by Suchet et al. (2011) who also found a depressed response to myrcene but a strong response to the other compounds. However it contradicts the findings of Byers, Bradshaw, and Riffell (2014) who found that bees responded to Myrcene. This may be due to different bee species being used in the studies, and *B. terrestris* may simply be able to sense different compounds than *B. vosnesenskii.* As neither study measured the response of bees to 3,5-dimethoxytoluene, this is the first study to show that *B. terrestris* can sense this compound.

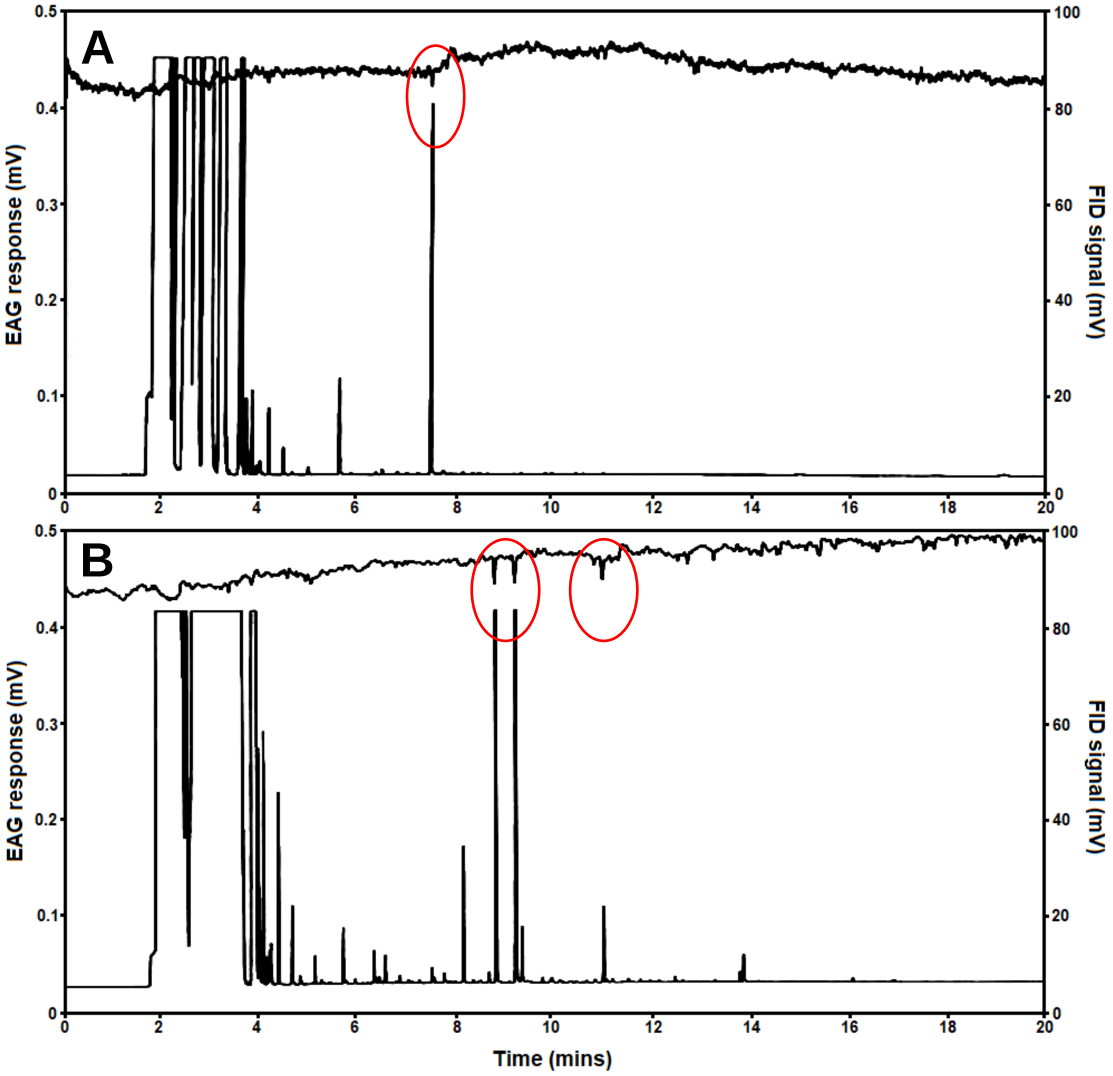

Figure 6.7. The EAG responses of a bumblebee antenna to compounds present in the floral volatiles of both flowers. FID signal shows the proportion of compound present in the sample shown as the GC trace along the bottom. Where a bee has a positive response to a compound the depolarisation in the antennae (top) align in time with the compounds detected by FID (bottom). Positive electrophysiological responses to compounds are shown by red circles. A) In *Petunia integrifolia* bees showed a response to benzaldehyde. B) The bees responding to volatiles collected from *A. majus* "Maryland True Pink" showed consistent electrophysiological responses to (*E*)-ocimene, methyl benzoate and 3, 5-dimethoxytoluene. No response was observed for myrcene.

6.6 THE CHARGED ROD STIMULUS

Various methods were used to expose the flowers to the electrical activity of foraging bumblebees. Previous measurements from Clarke et al. (2013) showed that bumblebees had a predominantly positive charge of 32pC ± SD during flight. Systematic measurements presented in this thesis confirm that bees are nearly always positively charged.

Plants were electrically stimulated using two different methods to simulate the electrical information provided by a foraging bumblebee. These provided stimulation which could be applied to the flower whilst volatile collection was taking place, allowing changes in the floral VOC emission to be analysed. The electrical stimuli were:

1. A triboelectrically charged nylon ball on a wooden rod
2. Exposure to free-flying foraging bumblebees

Using a bee as a source of charge provides the most biologically relevant test of charge upon plant volatile emission. However, a bee provides other physical and chemical stimuli such as its smell and other variations in its physical movement, such as vibration during buzz pollination and scraping with its tarsi. To this end, a stimulation instrument was constructed using a triboelectrically positive object (a nylon ball) on the end of a wooden rod. This ball could be charged triboelectrically using polystyrene to simulate the electrical presence of the bee without embodying any of its other physical or chemical properties that could potentially influence VOC emission. This technique of stimulus presentation also allowed the charge of the electrical stimulation to be controlled and changed with a degree of accuracy. Whilst the ball will not perfectly mimic the charge or movement of an approaching bumblebee, it can be reliably charged by triboelectrification and the charge can be quantified using the Faraday pail.

The charge on the ball changed over time, attenuating quickly, so the exact charge on the ball at the moment of touching the flower could not be determined with absolute accuracy. However, to estimate the charge on the ball at the point of contact the charge decay curve of the ball was analysed and characterised (Figure 6.8). The ball was charged triboelectrically to a charge of between 1000-1500 pC and held in a Faraday pail connected to a data acquisition board, so that the decline in charge on the nylon ball could be continuously monitored over a 30 second period. A least squares model was created in MATLAB to fit a curve to the charge decline on the ball (red line, Figure 6.8, equation 6.1). This curve was used to calculate the approximate charge on the rod at the point of touching the flower in subsequent experiments. The charge on the charged rod at the point of touching the flower was calculated from the modelled decay curve shown in Figure 6.9. The equation for this model is

$$Q_t = (Q_0 - 100)e^{-0.2859t} + 100 \tag{6.1}$$

Where Q_t is the charge on the rod at any point in time and Q_0 is the starting charge on the rod.

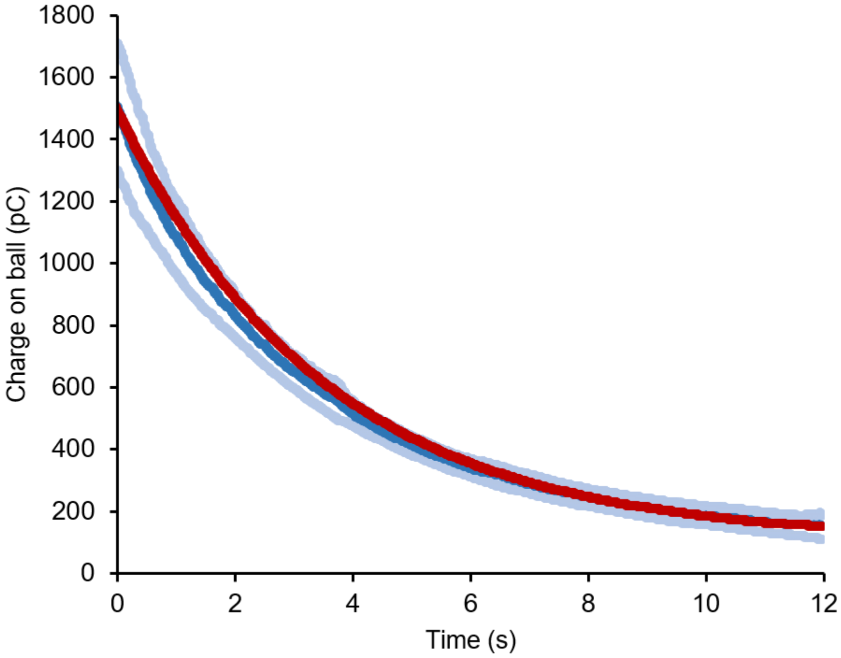

Figure 6.8. The mean decline in the ball charge (blue line, faint lines showing SD) and the modelled charge decline used to calculate the ball charge at the point of touching the flower (red line).

To test the effect of an electrically charged object touching a bee pollinated flower, experiments were conducted to touch a charged rod with a strong and weak positive charge and measure the volatiles released by *P. integrfolia* and *A. majus* MTP before and during stimulation. These experiments were designed to address whether the electric charge typical of a bee flying in different conditions affected the volatile emissions of two very different flower species.

6.6.1 Petunias and the charged rod

6.6.1.1 Strongly positive charge

Sixteen *Petunia integrifolia* plants were grown from seed in the GroDome at the University of Bristol and brought to Rothamsted Research at 8 weeks of age when they were starting to flower. Plants were housed in a greenhouse with a natural light cycle and kept at 22°C.

Plants were randomly allocated to the control group (touched with electrically grounded rod) or the experimental group (electrically stimulated by touching with a positively charged rod). Plants with flowers of the same age were randomly paired into control and experimental groups. As floral emissions can vary due to flower age (Dudareva et al. 2000), flowers were used at 2-4 days post anthesis corresponding with the likely peak emission period. Unlike nocturnally scent-emitting plants such as *P. hybrida* cv ‘Mitchell’ and *P. inflata,* benzaldehyde production by *P. integrifolia* has been shown not to be driven by circadian rhythm and shows little diurnal variation (Hoballah et al. 2005; Boatright et al. 2004). Because of this, time of day was not controlled for, although all experiments took place between 9:00 and 17:00. A control and an experimental plant were placed at opposite ends of a room. Using a portable dynamic headspace sampling kit, volatiles were collected from the control and experimental flowers for 2 hours at a flow rate of 500 mLmin^{-1} by placing a Porapak tube at the opening of the flower 5mm from the petals (Figure 6.2). The soil at the base of the plant was lightly watered before volatile collection took place and the plants were electrically grounded by piercing the soil at the base of the plant with an earthed metal wire. Volatiles were collected from both plants whilst undisturbed for 2 hours. The volatiles were then collected for a further 2 hours during which the experimental flower was electrically stimulated every 10 minutes by lightly touching the flower with a positively charged nylon ball. The stimulus consisted of a nylon ball fixed to a wooden rod which was given an electric charge of +500 to +1000pC by rubbing the ball with polystyrene. The charge on the ball was measured using a JCI 147 Faraday pail with a JCI 140 voltmeter (Chilworth Global, Southampton, UK) before and after touching the plant. The control flower was touched at the same 10 minute intervals with a metal rod that was electrically grounded.

The electric charges on the rod at the point of touching the flower were predominantly between 600-700pC. This is higher than the typical charge of a flying bumblebee but well within the range of the charges measured on flying bees in this thesis. After each plant had been used in the experiment, the groups were switched so the experimental plants underwent the control treatment and vice versa. This allowed each plant to be used twice, once as a control and once as part of the experiment and accounted for the substantial differences in VOC emission quantities observed between individual plants. Flowers were discarded after use to prevent pseudoreplication, and there was at least a 3 day gap between the first and second use of each plant. Volatiles were collected and the benzaldehyde emission was quantified using GC. The increase in volatiles produced by each plant as a result of touching with a grounded and a positively charged rod were calculated and compared using a repeated measures *t*-test in R.

6.6.1.2 Weakly positive charge

A second experiment was conducted to ascertain whether any change in volatile production could be due to charges of the magnitude of those carried by bumblebees. The methodology is identical, but trials were conducted in a Faraday cage, to prevent any fluctuations in the ambient electric field from influencing the experiment. The plants in this instance were touched with an electric charge of up to 150pC, mimicking the charge carried by a foraging bumblebee. The charge on the rod was again measured before and after touching the plant.

Petunia flowers significantly increased their volatile emissions when touched with a 600-700pC rod (paired t-test; P<0.0001, t=-5.701, df = 15) (Fig. 6.9A) whilst no increase was seen from flowers touched with the grounded control rod (paired t-test; P=0.240, t=-1.223, df = 15). When plants were touched with a rod with a much lower charge (<100pC) there was no significant increase in emissions from either the flowers touched with the charged rod (Paired Wilcoxon; P=0.0597, W=39, N=12; Fig. 6.9B) nor flowers touched with the grounded rod (Paired Wilcoxon; P=0.0519, W=38, N=12). Together, these results suggest that strong electric charges found on bumblebees foraging in fair weather cause an increase in plant volatile emissions from *P. integrifolia,* but that this may only occur when strongly charged pollinators are present.

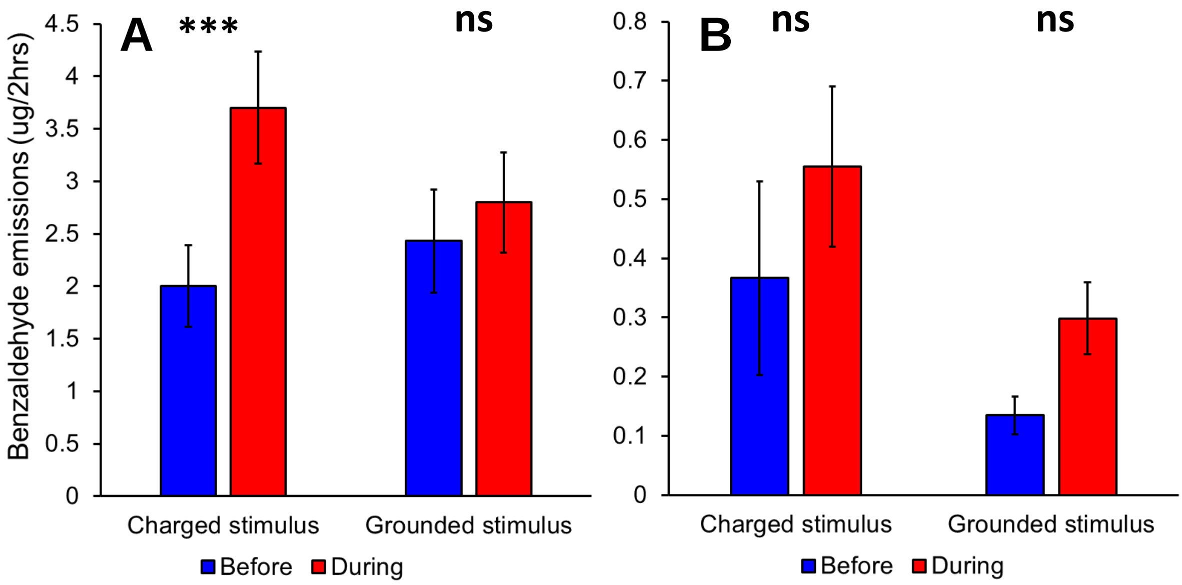

Figure. 6.9. A) The mean benzaldehyde emissions from a *P. integrifolia* flowers before and after electrical stimulation with A) a triboelectrically charged nylon ball of 600-700pC relative to a grounded control rod and B) A nylon ball charged to <100pC does not cause a significant increase in volatile emissions relative to a grounded control. Error bars show SE. The amount of benzaldehyde produced by the plants in the weaker charge experiment was much lower than in the higher charge experiment, shown by different scales on the y axis.

Together these results suggest that the presence of a strong positive electrical charge (600-700pC) causes an increase in benzaldehyde emission from petunias, but this effect is not seen with much lower charges. As bee charge has been shown to be highly weather dependent (Chapter 4) and vary significantly between insects (Chapter 5), bee charge may influence plant volatile emissions under certain conditions. In hot, dry weather insect charge could enhance the volatile emissions of flowers and may act as a signal of pollinator abundance. Outside of these conditions, it is unlikely that bees would generate sufficient charge to cause an increase in floral volatiles.

Petunia floral VOC emissions are independently regulated by environmental factors such as light intensity, with increased emission in bright conditions (Hoballah et al., 2005). As pollinator abundance is likely to correspond to the same environmental conditions that cause increased emissions, environmental conditions may act as a good indicator of pollinator abundance, and there is therefore limited adaptive value to responding directly to pollinators. However, increased scent production also increases the risk of being detected by florivores, herbivores and insects seeking oviposition sites (Theis, Lerdau, and Raguso 2007). Whilst increased temperature and light intensity would correspond to an increase in pollinator abundance, it is likely to also correspond to an increase abundance of insects detrimental to the plant. If pollinators are generally more highly charged than florivores or herbivores, insect charge may provide a good indication of the relative abundance of beneficial and detrimental insects. Increased emission in response to highly charged insects may therefore have adaptive value whilst an increased response to low positive charges could advertise floral presence to florivores.

6.6.2 Snapdragons and the charged rod

6.6.2.1 Methods

Volatiles were measured from snapdragons under similar conditions to the petunias. The methods for touching the snapdragons with the high and low positive charges are identical. 11 snapdragon plants were transferred to Rothamsted and kept under greenhouse conditions. Two inflorescences were cut from each plant and placed in a conical flask containing water. A strip of aluminium foil connected to a grounding point was also placed in the water to electrically ground the base of the stem. Flowers of a similar age on each inflorescence were randomly allocated to be touched with the earthed rod or the experimental charged rod. The volatiles were then collected from the control and experimental inflorescences over a 2 hour period, during which every 10 minutes the outer lobe of the flower was touched with the grounded or charged rod. This experiment was done with separate inflorescences at both 600-700pC and <150pC of charge. The rods were charged in an identical manner to the petunia experiments and the charge was measured the same way.

6.6.2.2 Results

The amount of each snapdragon volatile produced by the charged and the control flowers was compared. The amount of each volatile was highly correlated within each flower so volatiles were combined for each flower and the total volatile emissions were compared. In the high charge experiment the flowers touched with charged rod emitted a greater quantity of volatiles than the flowers touched with the grounded rod but not significantly (Figure 6.11A, paired t-test; P = 0.0935, N = 11, t = 1.854). In the low charge experiment the quantity of VOCs produced by the control plants was similar to that of the charged plants and there was no significant difference between the two (Figure 6.10B, Wilcoxon; P = 0.8311, N = 11, V = 30).

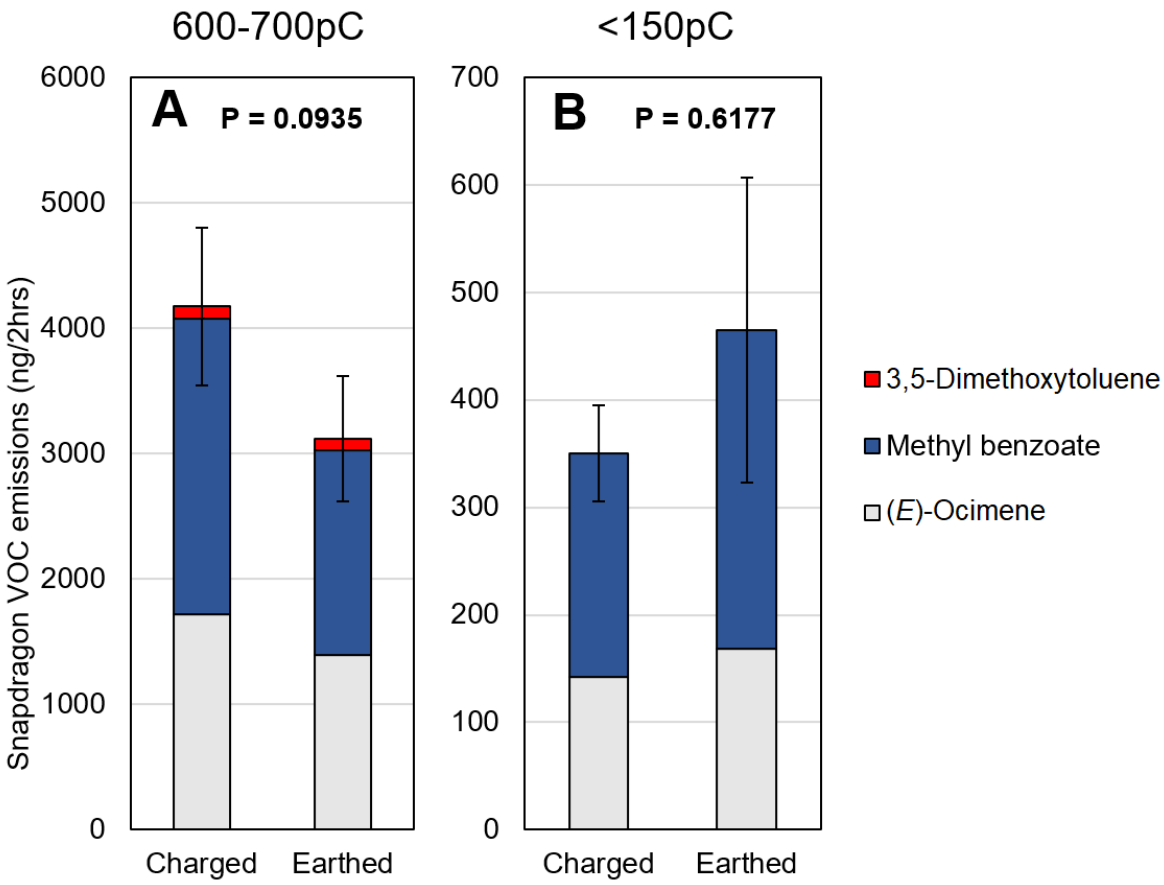

Figure 6.10. A) A comparison of the median volatiles produced by plants touched with a strongly charged nylon ball (600-700pC) and an electrically earthed control rod. The flowers touched with a charged ball released more volatiles than the flower touched with an earthed rod but not significantly (P = 0.0935, N = 11, t = 1.854). B) The volatiles produced by snapdragon plants touched with a nylon rod with a charge of 150pC and a grounded (control) rod. There was no significant difference in volatile emission between plants touched with a charged or earthed rod (P = 0.8311, N = 11, V = 30). The amount of 3,5-dimethoxytoluene emitted during the low-charge experiment was too low to be reliably and consistently quantified and contributes a negligible amount to the overall volatile emission. Error bars on both graphs show standard error of the mean sum of the compounds. The quantity of volatiles produced by the low charge experiment were much lower than in the high charge experiment, reflected by the different scales on the y axis.

6.6.3 Summary of the charged ball experiments

Using a charged ball as a proxy for the electrical activity of a bumblebee is convenient but flawed. It is very difficult to precisely charge a ball to less than 100pC, and the exact charge of the ball at the point where it is touched to the flower can be estimated but not precisely known.

Flowers were touched with a nylon ball charged to 600-700pC or >150pC. These range of charges were used to measure the effect of a strong or weak positive electric charge on the volatile emissions of petunia and snapdragon flowers to test whether electric charges of foraging bees may affect plant volatile emissions. *P. integrifolia* flowers significantly increased their volatile emissions when touched with a 600-700pC ball relative to a grounded control but did not produce significantly more volatiles when touched with a <150pC ball. Snapdragons touched with a charged ball did not have a higher emission of volatiles than those touched with a grounded control.

It is important to note that for both petunias and snapdragons, even the control plants in the high charge experiments released greater quantities of volatiles than either plant in the low charge experiment. As physical access to each flower is required for these experiments, it was not possible to enclose the flowers. As a result of this, it is very possible that the elevated concentration of volatiles measured in the high charge experiments was a result of cross contamination where volatiles from the experimental flower are picked up by the control Porapak tube. In each experiment the pair of plants were placed as far apart as possible (approximately 2m). However, each pair of control and experimental flowers were always measured simultaneously in the same room to control for time of day and environmental conditions. As the quantity of volatile emissions produced by the snapdragons a are approximately an order of magnitude higher in the high charge experiments than in the experiment using a lower charge (Figure 6.10), cross-contamination between plants could have allowed volatiles from the experimental plant to be collected in the Porapak tube form the control plant. As such, an even greater increase may be seen were there greater physical separation between the flowers, and the effect of induced charge upon floral volatile emissions is likely greater than that seen here.

6.7 VOLATILE COLLECTION FROM FLOWERS VISITED BY BEES

Isolating and mimicking the electrical information provided by a foraging bee is valuable, as it allows investigation into how a flower may respond to specific stimuli without confounding effects caused by bee flight (such as vibration, airflow, scent marking). Ultimately however, the best way to see whether floral volatile emission is affected by the presence and charge of bumblebees is to measure the emissions from flowers exposed to live, free-flying bumblebees. In this section I measure the volatile emissions from flowers exposed to a colony of foraging bumblebees whilst simultaneously measuring the electric charges of the bees approaching the flower.

In this experiment petunia flowers are used rather than snapdragons. This is due to two reasons: Firstly, because bumblebees visiting snapdragon flowers provide significant mechanical stimulation. When a bee lands on a snapdragon it must prise apart the upper and lower lobes of the flower to allow access to the nectary. This interaction is hard to mimic as a mechanical control, and the increased friction between bee and flower could cause other confounding problems such as increased wear on the flower, and scent being transferred between bee and flower. Secondly, it was impossible to isolate a single flower from a snapdragon inflorescence without significantly damaging the plant. A single, intact petunia flower could be threaded into the bee arena in both experiments whilst still being connected to the plant. The relatively simple floral shape and pollination syndrome of *P. integrifolia* made it a useful model plant for these experiments. To investigate the effects of bee charge on petunia volatile emissions, an experiment was conducted to measure the electric charge of foraging bumblebees as they approached petunia flowers, as well as any changes in volatile production that happened as a result.

6.7.1 Methods

A bumblebee flight arena was split into two distinct flight sections (Figure 6.11A). Both sides were connected to the same bumblebee colony via polycarbonate tubes which contained doors that could be closed and opened to control bee access to each side of the arena. Each side contains an identical metal ring connected to a picoammeter. Bees were trained to fly through the RCS to access a sugar reward.

During trials, the sugar reward below each RCS was replaced by a petunia flower so that the bees would have to fly through the sensor to reach the flower (Figure 6.11B). All bees were removed from the arena and volatiles were collected from both flowers for 2 hours. The Porapak tubes were then refreshed and bees were then allowed to forage in one side of the arena (and visit the experimental flower) but were excluded from the other side of the arena so that only the experimental flower could be visited by bees (Figure 6.11C). Volatiles were collected from both flowers for a further 2 hours. The charge on each bee visiting the experimental flower over the 2 hour period was measured. Whenever a bee visited the experimental flower, the control flower was touched with a grounded rod to control for the mechanical stimulus. The increase in benzaldehyde produced by each flower was compared over the 2hr period before and after adding bees using Wilcoxon signed rank tests for the experimental and control flowers.

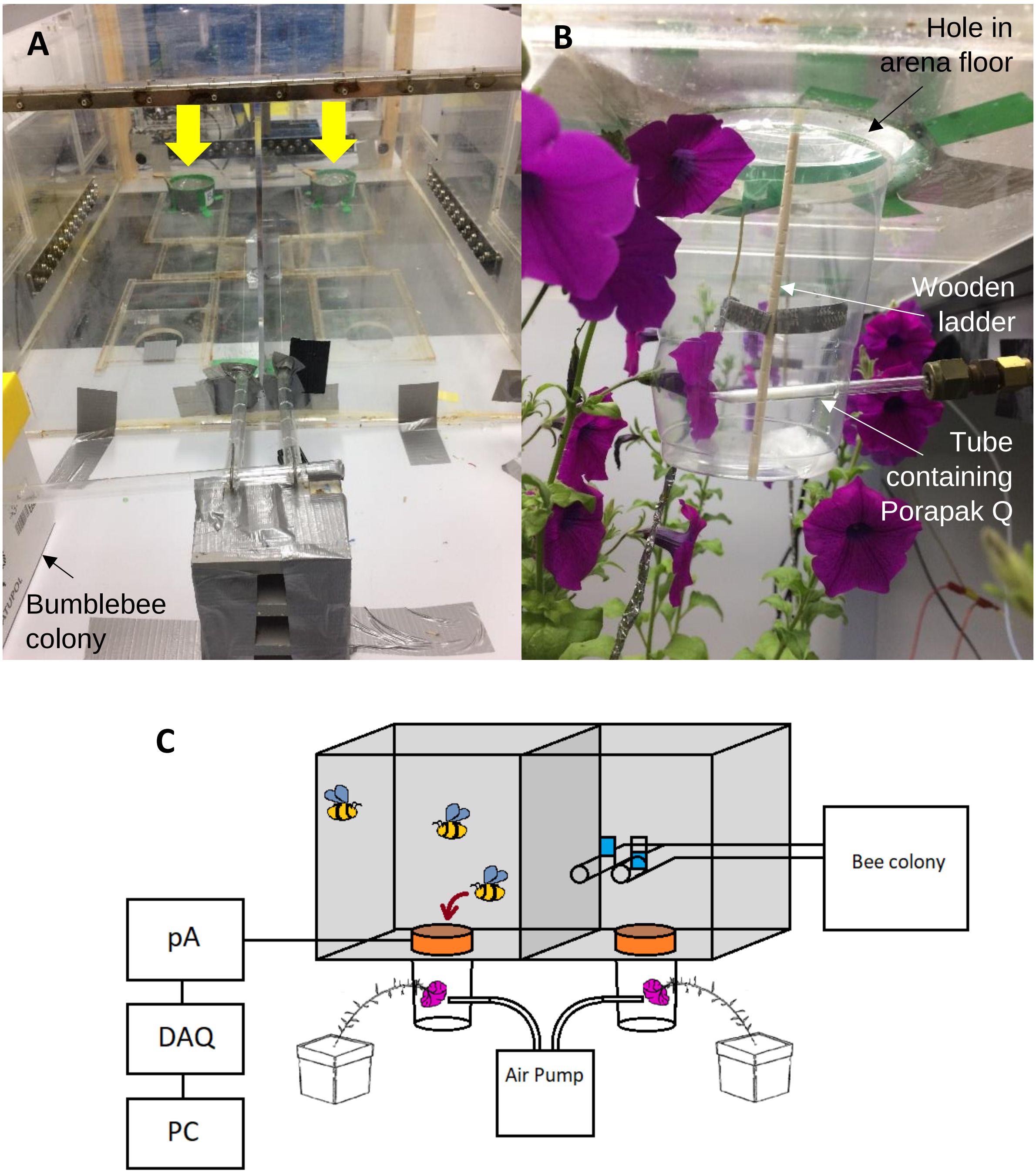

Figure 6.11. A) The flight arena was split into two identical sides. During training the bees were able to access each side and were trained to fly through an RCS over a hole in the arena floor to access a sugar reward (yellow arrows). B) During trials, a plastic cup was placed under each RCS. The cup contained the head of a petunia flower, a tube containing Porapak Q powder, and a small amount of sugar solution on cotton wool. There was also a wooden ladder to allow the bees to exit the cup. B) The view of the arena with the bumblebee colony in the bottom left. The holes in the arena floor covered by an RCS are shown as yellow arrows. C) Diagrammatic representation of experimental set up during a trial. The doors are opened to allow the bees to access the experimental flower on the left. The flower on the right is touched with a grounded rod.

6.7.2 Results

The bees visiting the flower were nearly all positively charged (Figure 6.12) with charges being predominantly in the range of 10s of picocoulombs, whilst some bees had much higher charges. These charges are very similar to those measured on outdoor, free-flying bees shown in Chapter 4, indicating that these foraging bees provide realistic electrostatic stimulation representative of bees foraging outdoors.

There was a significant increase in the amount of benzaldehyde produced by the flowers visited by free-flying bumblebees (Paired Wilcoxon test, P = 0.021, V = 68, N = 12). The flowers touched with the electrically grounded rod did not have a significant increase in benzaldehyde emission (Paired Wilcoxon test, P = 0.077, V = 62, N = 12, Figure 6.13). This suggests that mechanical stimulation alone is insufficient to cause an increase in volatile emissions, but that bee charge may cause increased emissions. However it should be noted that in both cases the statistical significance is relatively marginal and as such these results should be treated with a degree of caution.

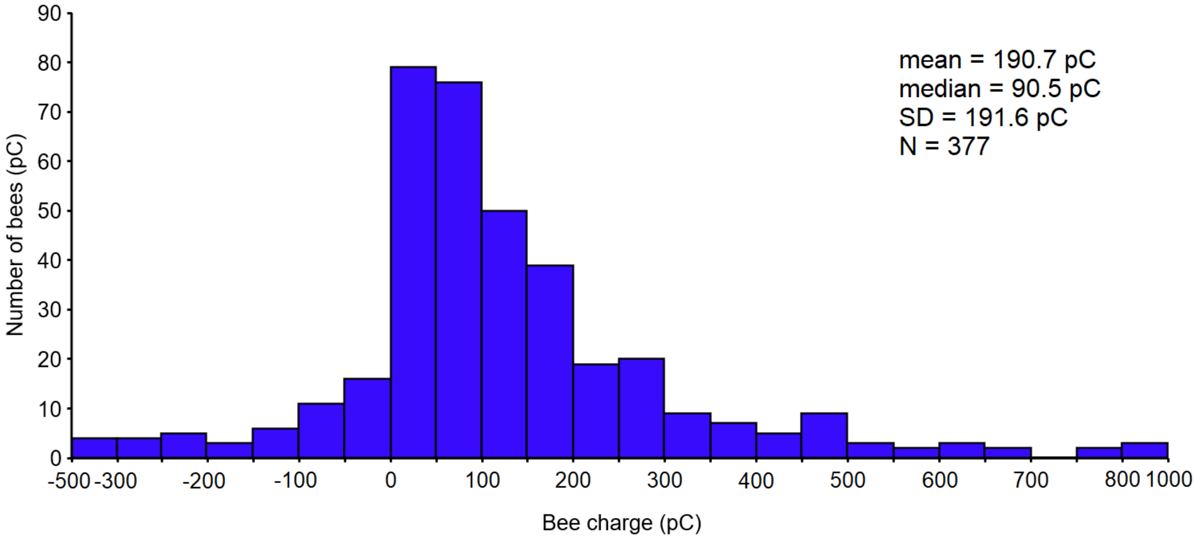

Figure 6.12. Histogram showing the distribution of charges measured on bumblebees approaching flowers.

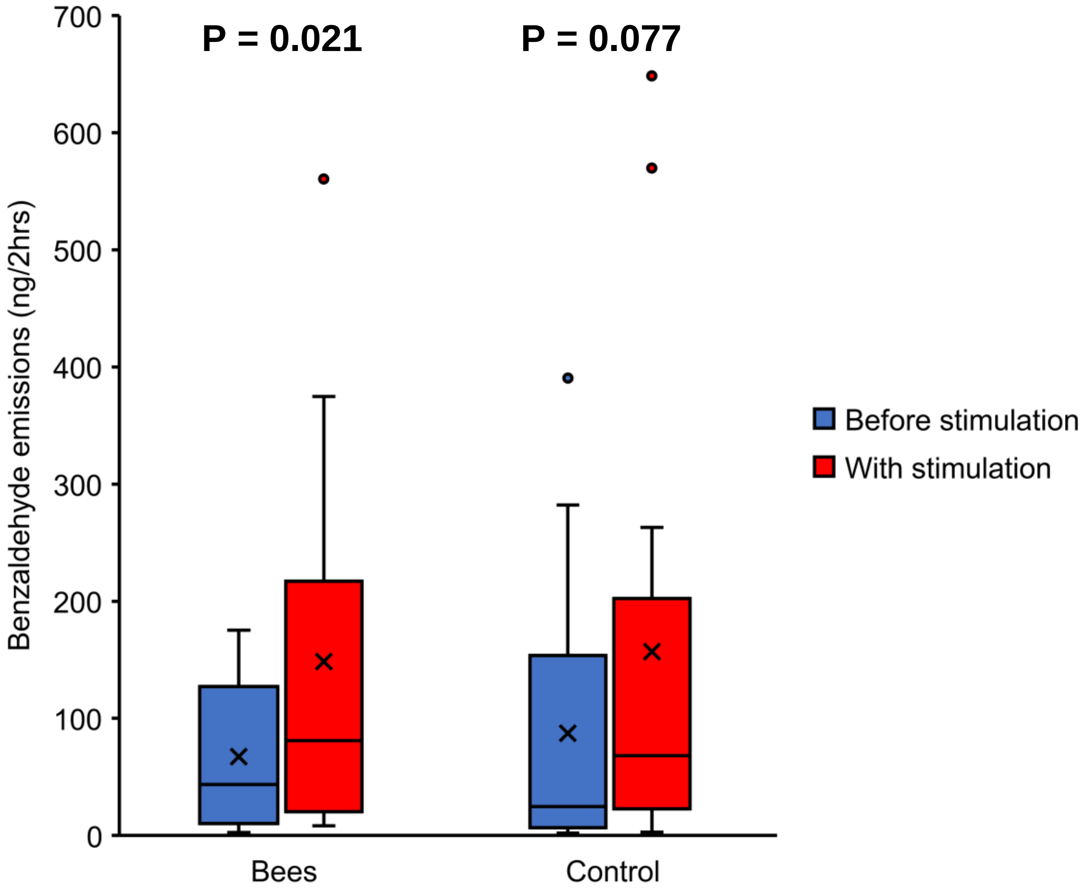

Figure 6.13. The amount of benzaldehyde produced by petunia flowers visited by bees or touched with a grounded rod. Blue boxes show the benzaldehyde emission before being exposed to bees or grounded rod. Red boxes show benzaldehyde emission after 2 hours of being exposed to foraging bumblebees (left) or touched with a grounded rod (right). Error bars show SE. N=24.

6.8 DISCUSSION

6.8.1 Summary of the results

Petunia integrifolia flowers increase their volatile emissions in response to electrical stimulation from foraging bumblebees. A positively charged nylon ball caused increased benzaldehyde emissions from petunia flowers at charges of +600 to 700pC but not at lower charges of <150pC. *Antirrhinum majus* MTP flowers touched with a charged nylon ball did not have higher emissions than a control flower touched with a grounded rod. Bumblebees visiting petunia flowers in the laboratory caused an increase in benzaldehyde emissions of the flowers they visited. These results suggest that the emission of benzaldehyde in *P. integrifolia* is influenced by electrostatic stimulation from foraging bumblebees.

6.8.2 Electrical stimulation vs mechanical stimulation

To separate the effects of mechanical stimulation from bees landing on flowers and electrical stimulation caused by the charge of the bee, a mechanical control was performed for each experiment where VOC emissions were simultaneously measured from a control flower which was repeatedly touched with an earthed metal rod rather than electrically stimulated. These control flowers sometimes also showed an increase in volatile production (Figure 6.10, 6.13) but this increase in production was not significant and was in all cases lower than the increase in VOC emission shown in electrically stimulated flowers.

The flowers visited by flying bees with measured charges had a significant increase in volatile emissions whilst flowers that were just mechanically stimulated did not. It is possible that mechanical stimulation from the bees is much greater than the mechanical stimulation with the grounded rod – bees would rub around the flower and occasionally scratch it with their tarsi. This scratching could cause greater damage than the simple touching with the earthed rod and result in a change in VOC emissions. This was controlled for as much as possible by making the control mechanical stimulation in the bee-visiting experiment as representative as possible to the foraging movements of the bees. In the experiments where plants were touched with earthed or charged rods, the mechanical stimulation from both rods was identical. As there was a significant increase in VOC emission from electrically but not mechanically stimulated petunias, it is unlikely that mechanical stimulation alone from a foraging bee is sufficient to cause an increase in volatile emissions. Subsequent experiments should attempt to locate the minimum charge required to cause an increase in volatile emissions from insect-pollinated flowers. These would isolate the role of electric charge in plant-pollinator communication.

6.8.3 Plant-pollinator communication and ecology

The peak emissions of insect-pollinated plants coincide with the maximum foraging period of their pollinators in both petunia and snapdragons (Verdonk et al. 2006). Whilst both pollinator activity and plant volatile emissions correlate with environmental conditions such as temperature and humidity, there is little previous evidence to suggest that the plants directly sense the presence of their pollinators and target their VOC emissions directly. The experiments presented in this chapter provide evidence that plants may be able to respond directly to the activity of their pollinators by changing their VOC emissions. This suggests that floral volatile emissions may be responsive to pollinator activity, as well as predictive. Flowers use environmental cues to target their volatile emission towards periods of maximum pollinator activity (Hoballah et al. 2005; Dudareva et al. 2000), but I show here that they may also change their emissions in response to contact with electrically charged, foraging bees. This provides adaptive value to the plant by optimising volatile emissions, preventing wasteful production of volatiles at times where there are no pollinators present. For the plant, it is only worth advertising if you know you have an audience.

The ability of plants to sense their pollinators has been recently explored when it was shown that *Oenothera drummondii* flowers responded to the sound vibrations produced by flying bees by increasing the nectar sweetness (Veits et al. 2019). This provides further evidence that plants may respond directly to pollinator presence and not just rely on environmental cues. Using nectar sweetness as a response mechanism allows measurement of rapid responses from the plant without having to rely on long experiment times. These rapid responses are likely to be of more use to a pollinator, as nectar sweetness is a similarly expensive resource for the plant to produce and so the plant nectar production should be highly targeted. Nectar sweetness is therefore likely to be a more dynamic signal than scent, although both play a role in the attraction of pollinators. Investigations into the effect of electric charge on floral nectar sweetness would provide further information into the role of bee-charge on plant-pollinator communication.

The electric charge, or chargeability, of an insect may provide electrical information corresponding to its ecological role. Pollinators are likely to be more highly charged than non-pollinating insects (Chapter 6). Insect charge may therefore provide electrical information to the flower regarding the abundance and ratio of beneficial and potentially harmful insects and allow plants to target their volatile emission more efficiently to attract pollinators whilst not attracting florivores or herbivores. Studies with much greater scope are required to confirm whether pollinating insects do indeed have greater charges than non-pollinating insects, and whether the volatile emissions of different plants change in response to the electric charges of foraging insects at a greater scale. More efficient targeting of volatile emissions has adaptive value to the plant as volatile production is energetically expensive. A reactive, insect-induced volatile emission would therefore allow more efficient resource use.

The increased emissions of benzaldehyde from petunia flowers following electrical stimulation is also interesting when considering the electrical properties of benzaldehyde. Benzaldehyde is a polar molecule with a dielectric constant that varies depending on its solvent (Tekin et al. 2004). Aerolised benzaldehyde will therefore be attracted towards charged bodies via Coulomb force. Erickson (1982) suggested chemosensory placoid sensilla on honeybee antennae were electrically charged and would therefore attract charged or polarised odorants. Charged bees may therefore be highly sensitive to the increase in benzaldehyde produced by their presence.

6.8.4 Post-pollination changes in VOC emission

An increase in volatile emissions from bee-visited flowers is likely to occur at the whole-plant level rather than at the level of a singular flower. The reproductive benefits of repeated pollinator visits to a single flower decreases with each subsequent pollinator, as the likelihood of that flower being already pollinated increases. Post-pollination reduction in floral VOC emissions is well documented alongside visual effects. At a short range this provides an honest signal to foraging bees, discouraging them from visiting flowers with depleted reserves and instead diverting them to fresh, unpollinated flowers. This signalling strategy benefits both plant and pollinator, by preventing the pollinator from wasting energy visiting an unrewarding flower and increasing the reproductive success of the plant by increasing visits to unpollinated flowers. However, a whole-plant response of increased VOC emission following a visit from a pollinator would benefit the plant by attracting the pollinators already present to unpollinated flowers. It is likely that charge-mediated emission of volatiles is of most value to plants with composite flowers or inflorescences where multiple flowers require pollination. A plant with few or single flowers has less need to advertise its presence to successive pollinators as demand for insects is decreased following successful pollination.

As the volatiles measured in these experiments were measured from a single flower, we are unable to tell whether there is an increase in emission at a whole-plant level. Although an ideal experiment, it was impractical to engineer VOC measurements of whole plants due to access requirements for floral stimulation. As such it was much more practical to measure from a singular flower, although preliminary results indicate this may also be an effect at a whole plant level. There will always be a delay between a pollinator landing on a flower, and a decrease in emissions post-pollination due to the time taken for pollen to reach the ovary, the subsequent chemical cascade, and finally the change in chemical emissions. In the experiments in this chapter, the bees only ever had access to the one flower so there was no chance of pollination and no likelihood of post-pollination effects confounding the experiment. In nature, post-pollination effects may act against increasing volatile emissions, unless there was a short term increase before pollination (due to mechanical or electrical stimulation) followed a by a post pollination decrease.

6.8.5 Speculation on mechanisms

There was an increase in benzaldehyde emission from *P. integrifolia* upon electrical stimulation from both bees and a triboelectrically charged nylon ball. This increase could be due to an increase in production of benzaldehyde, where at some stage in the metabolic pathway more benzaldehyde was actively synthesised, or it could be due to an increase in emission of already present benzaldehyde potentially due to increased permeability of membranes. As plant VOCs must cross multiple cellular barriers before release into the environment (Widhalm et al. 2015) it is possible that an electric current induced by the charge on an incoming bumblebee could affect the permeability of any of these cellular barriers to different compounds in a manner similar to electroporation.

6.8.6 Limitations

There were several other limitations which should be taken into consideration. The flower would move when a bee visited it which made it very hard to position the rod inside the flower and keep it there reliably for the 2 hour period. Sometimes the flower had to be repositioned which may have affected the volatile release. Airflow from the bee’s wings was impossible to replicate with a metal rod so might have been a confounding factor. The Porapak tube in front of the flower may have changed the electric field surrounding the flower. Likewise, the external electric field in the flight arena and the room are not necessarily representative of natural environmental conditions. In addition, if the flowers were pollinated during bumblebee visitation it could cause a post-pollination reduction in volatile emission which could confound the results (Theis and Raguso 2005).

In most volatile collection experiments, when volatiles were collected from the same flower over the total 4 hour period, their production generally increased regardless of whether the flower was touched by a bee, a charged rod or an uncharged rod (Figures 6.10, 6.13). As the volatiles increased generally throughout most experiments as the experiment went on, this should be further investigated. This might be due to a mechanical effect of touching the plant, thus stimulating volatile emission, or could be due to the plants adjusting to the room in which the volatile collection took place. The volatile collection chamber was brightly lit both in Bristol and in Rothamsted. A delayed adjustment to this increased light intensity could have caused the plants volatile emission to increase during the course of the experiment as plant volatile emissions are affected by light intensity (Schuh et al. 1997). The increase in volatile emissions was not seen in the experiment that took place in Bristol suggesting that although the temperatures and light intensity were the same at both locations, other local conditions could be influential.

7 GENERAL DISCUSSION

Appreciation of the invisible forces underpinning biological interactions is vital to understanding how these interactions occur and how human activity may affect them. The results shown in this thesis regarding the triboelectric charge exchange between bees and flowers raises further questions about the electrical landscape in which insects forage, and the electric interactions occurring in nature. To what extent has electrostatics influenced the evolution of floral morphology? Can the pollinating efficiency of some insects be explained by an elevated electric charge? Can insects sense their own charge and, more intriguingly, can they modulate it? If charged insects accumulate airborne pathogens as well as pollen, does this drive the evolution of charge in airborne organisms? Is pollen adapted to take advantage of Coulomb force? The results presented in this thesis are a significant advancement to our limited understanding about the potential roles of charge in insect ecology, and will inform the further studies that are required to address these important questions.

7.1 HOW DO BEES GAIN CHARGE?

It has been previously stated that bees generate an electric charge during flight e.g. (Gan-Mor et al. 1995) but the evidence to support this is conflicting. In this thesis I show that bumblebees do gain an electric charge during flight both in the laboratory and when flying outdoors. This is not the sole charge gain mechanism. The previously reported positive triboelectric properties of bees and other insects e.g. (Edwards 1962; Es'kov and Sapozhnikov 1976; Clarke, Morley, and Robert 2017) suggest that bees will gain a positive charge upon contact with most materials. This is supported by the results shown in this thesis, and I extend this hypothesis to include the interaction between bumblebees and flowers. I show that bees gain charge when visiting flowers, which may contribute to the elevated charges found on bees foraging outdoors.

It has been previously shown that bumblebees visiting a grounded petunia flower cause a temporary increase in stem potential, which dissipates slowly after the bee has left (Clarke et al. 2013). This temporary increase in potential in the flower may act as a cue for subsequent electrosensitive foragers, who may associate an increased flower potential with temporarily decreased nectar and pollen resources. Whilst a potential measured in the stem of the flower will decay rapidly due to the relatively high conductivity of the phloem and xylem (Barlow 2008), charge deposited on the petals may remain for longer due to the relatively lower conductivity of petals (Corbet et al. 1982). The higher conductivity of the stigma will conduct charge away (Fromm, Hajirezaei, and Wilke 1995) but an elevated charge may remain in the petals, thus changing the electric profile visible to subsequent bees. Additionally, the deposition of pollen can cause a change of potential within the stigma, also altering the electric profile (Spanjers 1981). The residual charge on the petals after a triboelectric interaction would be an honest signal of nectar availability, further adding adaptive value to the charge exchange.

The triboelectric gain of charge in bumblebees is supported by the similar results seen with mason bees and their similar triboelectric position. As the charges measured on bees flying outdoors are more variable, it would be interesting to measure the charges on bees before and after visiting different flowers outdoors, to see whether this is true of flowers in their natural environment. This would be of significant value for studies with crop plants requiring insect pollination, where the application of fertilisers and pesticides may affect the electrical properties of the plant. The application of different chemicals to crops may affect the floral electric field, which would be of particular importance to plants that are pollinated by electrostatically charged pollen sprays.

The triboelectrification of bees on different substrates has the potential for use in agricultural techniques such as entomovectoring (Mommaerts and Smagghe 2011; Karise et al. 2016). This emerging technology uses bees to transfer beneficial fungal spores to flowers, acting as a biocontrol. These fungi prevent fruit spoilage by preventing the establishment of pathogenic fungi associated with fruit rot, allowing precisely targeted biocontrol. At present, bumblebees and honeybees pick up the spores by walking over a spore covered surface. However, by modifying the materials in the hive/colony to increase the charge on the bees prior to contact with the spores, the spore carrying capacity of the bees could be radically increased, leading to greater crop coverage and greater protection against spoilage. The triboelectric charging of bumblebees could therefore be used to enhance emerging biocontrol technologies, providing solutions to global agricultural problems. Knowledge of insect electric charge has additional extensive value outside agriculture. Knowledge of the electric charge on mosquitoes has led to the development of targeted insecticide sprays that utilise Coulomb force to enhance the electrostatic attraction and adhesion of pesticide to the mosquito, reducing the prevalence of mosquito borne human disease (Whitmore et al. 2001). Thus, studies of insect charge have wide-ranging applications for agriculture and food security, but also for human health.

7.2 ARE LABORATORY MEASUREMENTS REPRESENTATIVE OF THE CHARGES ON OUTDOOR FLYING BUMBLEBEES?

The electric charges measured in this thesis on bumblebees and mason bees flying outdoors exceed those measured in the laboratory. These measurements suggest that the models of pollen transfer by Clarke et al. (2017) are largely representative although may underestimate the Coulomb force acting on pollen grains in nature in low humidity. Bumblebees were predominantly positively charged except for during periods of rain. As bees are most active during warm, dry conditions and bee activity often negatively correlates with relative humidity (Abrol 2013), the conditions with the highest bumblebee charges are likely to be those in which most pollination occurs. Additionally, it has been shown that bumblebees target their resource acquisition depending on humidity, collecting pollen when it is dry and nectar when it is wet (Peat and Goulson 2005). Peat and Goulson suggest this is because it is harder to groom wet pollen into corbiculae, but this may also be due to the reduced Coulomb forces acting on pollen in more humid conditions making pollen collection more energetically costly for the bees. Anther dehiscence is also negatively correlated with relative humidity, whereby maximum pollen release occurs under the driest conditions (Yates and Sparks 1993; Gradziel and Weinbaum 1999). This may increase the reproductive success of the plant by correlating the availability of pollen with the conditions most favourable for electrostatic pollen transfer.

The charges measured on bees are highly influenced by the local relative humidity, with bees charging up less in more humid conditions and losing their charge more rapidly. It would be interesting to compare the charge and morphology of both pollinators and pollen from arid and tropical regions. As floral morphology, insect physiology and pollen exine ornament have each been suggested to have adapted to an extent for the exploitation of electrostatics for pollination (Chaloner 1986; Armbruster 2001) perhaps any morphological adaptations may become evident by comparing species in arid conditions with those in more humid areas. The pollination of swamp-dwelling flowers may be less influenced by electrostatics even if they share pollinators with arid species. Potential morphological features adapted to take advantage of the electrostatic relationship between pollinators and flowers (such as protruding stigma in flowers, hairiness in bees, spikes in pollen grains) may be lacking in species perpetually exposed to humid conditions. On the other hand, these features may be more present in desert-dwelling species, where pollinators are more likely to generate higher charges and the Coulomb force acting on pollen grains is likely to be greater. Morphological analysis of closely related species in dissimilar habitats may allow identification of these features and add evidence for the adaptive value of electrostatics in the evolution of floral morphology.

7.3 IS CHARGING AND CHARGE RETENTION WIDESPREAD AMONG INSECTS?

In chapter 5 I increase the scope of this study to include measurements of electric charge on mason bees and wasps and compare the triboelectric properties of different insects. These species have very different diets, social structures and behaviours, reflected by differences in their morphology, with pollinivorous bees having specialised pollen-collecting apparatus and having a greater density of hairs. These adaptations for pollen collection could extend to their generation of electrostatic charge, as electric charge is likely to facilitate the attraction and adhesion of pollen to the bee.

Comparison of charge on different insects allows speculation into how morphology and behaviour may affect charge generation. Previous studies of the tribology of different insects suggest that insects can be considered triboelectrically positive and as such will gain charge upon walking over most materials (Edwards 1962; Es'kov and Sapozhnikov 1976; McGonigle, Jackson, and Davidson 2002). In this thesis, mason bees and bumblebees were found to be more triboelectrically positive than wasps or the variety of materials they were tested against, suggesting that their morphology and perhaps cuticular composition may increase their ability to gain and retain charge. Keratin-rich fur and hair are often placed at the top of the triboelectric series and hence readily gain a positive charge (Pan and Zhang 2019). Interestingly, hairs become more positively charged when rubbed in the direction from root to tip (Martin 1941). As on bumblebees, the abdominal and thoracic hairs are typically oriented anterior-posterior, a bumblebee moving forward brushing up against any material will cause the material to rub against the hairs in an anterior-posterior direction, stroking the hair from root to tip and likely causing it to acquire a positive charge. The placement, density and orientation of hairs on bumblebees and potentially other bees may therefore affect the bees' ability to gain a positive charge.

There may also be behavioural reasons for the differences in charge seen on the different insects. Mason bees, bumblebees and honeybees all forage on flowers for nectar and pollen. As shown in Chapter 3, bumblebees gain charge when visiting flowers through a triboelectric exchange of charge. Mason bees also appear to gain charge in this way, and as mason bee and honeybee tribology was found to be similar to that of bumblebees, it is likely these other bees also gain charge when visiting flowers. Whilst *V. vulgaris* does visit flowers occasionally for nectar, it is much less frequent than pollinivorous and nectivorous insects and as such its charge is unlikely to be boosted by triboelectric interactions with flowers to such an extent. Thus, both the relative morphology and foraging ecology of different insects is likely to affect their ability to gain and maintain an electric charge.

A better understanding of charge generation in insects could be gained by performing a large-scale comparison of the electric charge on different insects. Comparisons of the electric charge of different insects with different diets and different trophic interactions would highlight the roles that charge generation, or the lack thereof, could play in insect ecology. Comparison between hairy and hairless flying insects would test the hypothesis that charge generation is facilitated by hairs on insect bodies, whilst study of whether charge affects behaviour in predator-prey interactions would suggest a role for insect electroreception in the detection of predators or prey. Electric charge has been largely overlooked in studies of insect sensory and behavioural ecology and a better understanding of its roles would enhance our understanding of insect and plant interactions. As anthropogenic influences spread to our surroundings, studies of electric charge and electroreception in ecology could also inform conservation strategies regarding how human activity affects electrosensitive species.

7.4 DOES BEE CHARGE AFFECT FLORAL VOLATILE EMISSIONS?

Plants have been previously proposed to react to electrical stimuli, with the application of external electric fields being shown to affect growth rate (Mizuguchi et al. 1994), compound synthesis (Inaba et al. 1995) and movement (Volkov et al. 2007). In this thesis, I show for the first time that electric interactions may play a role in plant-pollinator communication. Flowering plants respond to the electric charge on foraging bumblebees and charge stimulation alone is sufficient to elicit an increase in volatile emissions in petunia flowers. Dynamic signalling between flower and pollinator has been recently shown in *Oenothera drummondii*, where flowers were shown to increase their nectar sweetness in response to the vibrations produced by a flying honeybee (Veits et al. 2019). This is the first instance of plants responding to an insect-specific cue, where an increase in nectar sweetness is likely to increase floral constancy in subsequent recipient pollinators, benefitting the plant by increasing the likelihood the pollinator will carry pollen to other flowers of the same species.

The increase in nectar sweetness makes the plant a more attractive target for pollinators, increasing the likelihood of future visits to other flowers on the same plant. Increasing the output of pollinator-attractive volatiles has the same effect, where the plant can more accurately target the advertising of its presence to periods when pollinators are active. Of the two model plant species tested in this thesis (*P. integrifolia* and *A. majus*), this increase in costly volatile emissions was only seen in petunias. The volatile outputs of both species are mediated by different methods, with *P. integrifolia* changing its emissions in response to environmental factors such as light intensity and temperature rather than being regulated by an innate circadian clock (Hoballah et al. 2005), whilst *A. majus* releases its volatiles in a circadian-mediated rhythm, unaffected by environmental conditions (Kolosova et al. 2001). For *P. integrifolia*, the electric charge stimulation may simply act as another environmental cue, indicating that pollinators are present and hence it is beneficial to advertise floral presence. Electrical stimulation could also cause an increase in nectar sweetness. Both charge and vibration are indicative of pollinator presence, and thus the results presented in (Veits et al. 2019) and within this thesis suggest that plants can dynamically respond directly to the presence of their pollinators without having to solely rely on environmental cues that may correlate with pollinator activity. The enticing possibility now arises that some plants have the capacity to sense the presence of the vectors of their reproduction

The demonstration of electrosensitivity in flowering plants raises many new questions. What is the timescale of this volatile increase? Do plants respond in any other ways? Is it a local reaction or does touching one flower with a charge cause an increase in emissions from other flowers on the same plant? Is it dependent on other conditions? Can this electrically-mediated response be sensitised with repetitive presentation, offering the possibility of contextually modulating the amount and duration of volatile emissions? Does it require repeated stimulation for prolonged increased emission? If a plant was electrically stimulated without corresponding environmental cues (e.g. *P. integrifolia* in the dark) would you still see an increase in emissions, or does it require multimodal stimulation? Does an electric charge stimulate the synthesis of compounds or just the emission of compounds already present in petal cells? What is the mechanism responsible for the increase? Is it due to a temporary increase in membrane permeability? Is this electrically modulated increase in emissions specific to *P. integrifolia* and the emission of benzaldehyde or can it be found in other species with different volatiles and different metabolic pathways? For some of these questions, I have begun tentative investigations. Others are beyond the scope of this thesis.

7.5 FINAL REMARKS

The role of electrostatics in plant-insect communication and for the facilitation of pollination is a largely understudied area. Despite recurring interest over the last century, our understanding of electroreception and the generation of electric charge in insects is severely lacking. In this thesis I show that bumblebees gain electric charge during flight, and when foraging on flowers. I show that these charges are not limited to bumblebees in the laboratory but can be found on free-flying outdoor bees foraging in a natural environment. I show that strong positive electric charges are not limited to outdoor-flying bumblebees but are also present on mason bees, suggesting an adaptive advantage to electric charge for pollinating and pollinivorous species. Finally, I show that bumblebee charge causes an increase in the emissions of volatiles in a bumblebee-pollinated flower species, suggesting for the first time that electric charge affects plant-pollinator chemical communication.

As human activity changes the electric landscape experienced by foraging bees, the electric ecology of flying pollinators becomes increasingly relevant for global food security. The electrical interactions between the bee, the flower and the electrical environment have the potential to affect plant reproductive fitness and hence affect the yield of insect-pollinated crops. Electric charge can affect how insects respond to their environment, their ability to gather resources and communication between plants and pollinators. Thus, further research of electric charge in insects and other terrestrial animals has the potential to be of great benefit by increasing our understanding of complex trophic interactions in nature.

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APPENDIX: COMMON ABBREVIATIONS

APG	Atmospheric potential gradient
HDPE	High-density polyethylene
PTFE	Polytetrafluoroethylene
PU	Polyurethane
PVC	Polyvinyl chloride
RCS	Ring charge sensor
RH	Relative humidity
TENG	Triboelectric nanogenerator
VOC	Volatile organic compound