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Pollination of invasive *Rhododendron ponticum* (Ericaceae) in

Ireland

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1. Abstract

Several insect species visited exotic *Rhododendron ponticum* flowers, but bumblebees were most common. Only bumblebees (and one *Vespula*) contacted the flower stigma whilst foraging and are probably the main pollinators. One-third of insect visits resulted in stigma contact and visitation rates were high. This explains the high seed set reported elsewhere. There were significant differences according to the body size of visitors, with large queen bees contacting stigmas more often than smaller workers. There were no significant differences in behaviour of three species (*Bombus jonellus*, *B. lucorum* and *B. monticola*) and all bees tended to move short distances between flowers on a single plant. Longer flights and movements among plants were rare. Pollen carryover was estimated to be high: there was no decline in the amount of pollen deposited on stigmas from the first to fifth flower visited. The impact of bee behaviour on reproduction and invasion by exotic *R. ponticum* is discussed.

2. Keywords

body size/exotic plant/invasion/pollen transfer/weeds

3. Introduction

Pollination of exotic plants by insects has recently become recognised as an important issue in invasive species biology (Chittka and Schurkens, 2001; Barthell et al., 2001; Ghazoul, 2002; Parker and Haubensak, 2002; Stout et al., 2002; Hanley and Goulson, 2003; Goulson and Derwent, 2004; Morales and

1   Aizen, 2006). Most angiosperms rely on mutualistic interactions with animal

2   pollinators (usually insects) for seed production and reproduction (Buchmann and

3   Nabhan, 1996). Unless pollinators are also introduced (as, for example, in the case

4   of European honeybees, *Apis mellifera* L., in Asia, America and Australasia; and

5   bumblebees, *Bombus* spp., in Australasia, and Japan), exotic plants often have to

6   rely on forming interactions with native pollinator species (Valentine, 1978;

7   Parker, 1997; Richardson et al., 2000). Failure to form these interactions with

8   native species can limit reproductive success and establishment. However,

9   successful pollination by generalist native species can facilitate seed set,

10   naturalisation and, in some cases, severe invasion (Parker, 1997; Richardson et al.,

11   2000).

12

13   Some flowering plants are visited by a range of generalist taxa which may have

14   varying efficiencies as pollinators (Macior, 1970; Herrera, 1987). Legitimate

15   pollinators are those that not only pick-up pollen from anthers, but also deposit it

16   on a receptive stigma. Several factors influence whether visitors actually pollinate

17   flowers, including body size (e.g. Stout, 2000) and the method by which food

18   (nectar and/or pollen) is collected (Inouye, 1983). Even if a taxon does act as a

19   legitimate pollinator, two further components of pollinator behaviour can affect

20   pollination success: the quantity of visits (visitation rate, abundance of flower

21   visitors) and the quality of visits (in terms of the proportion of visits resulting in

22   out-crossing events).

23

1 The size of the floral display can affect pollinator behaviour, with large plants

2 often attracting more frequent pollinator visitation (Augspurger, 1980; Geber,

3 1985; Klinkhamer et al., 1989; Klinkhamer and de Jong, 1990; Stout, 2000).

4 However, plants with large floral displays often suffer from increased

5 geitonogamy (within-plant pollen transfer), and hence inbreeding, as pollinators

6 often minimise inter-flower travel and move between adjacent flowers on large

7 plants (Geber, 1985; de Jong et al., 1992, 1993; Klinkhamer and de Jong, 1993).

8 Hence, pollinator behaviour can have profound implications for the genetic

9 structure of invasive plant populations (Levin, 1978).

10

11 In this paper, I examine how insect behaviour may affect the pollination success

12 of the ecologically damaging, exotic invasive shrub *Rhododendron ponticum*

13 (Ericaceae) in Ireland. Since its introduction to the British Isles from Spain in the

14 eighteenth century (Elton, 1958), *R. ponticum* has introgressed with other

15 *Rhododendron* species (Milne and Abbott, 2000) and become naturalised and

16 abundant in many Irish habitats. Successful spread in Ireland is caused by

17 effective seedling recruitment (Cross, 1981; Erfmeier and Bruelheide, 2004).

18 Seeds are sexually produced and despite being self-compatible, exclusion of

19 insects from flowers results in very low fruit and seed set (Mejías et al., 2002;

20 Stout, In press). Contrary to findings by Mejías et al. (2002) who studied native *R.*

21 *ponticum* in Spain, fruit and seed production in naturalised *R. ponticum* in Ireland

22 is significantly improved by out-crossing (pollen transfer between plants) (Stout,

23 In press). *R. ponticum* produces a massive floral display, with large (up to 6cm

1   corolla), brightly coloured (pink-mauve to the human eye), zygomorphic flowers

2   held in inflorescences of 9-21 flowers (Mejías et al., 2002; personal observations).

3   Flowers produce large volumes of sugar-rich nectar which accumulates in a

4   vertical groove formed by the upper petal (Mejías et al., 2002; Stout et al., 2006).

5   Although pollen is dispensed from anthers through an apical pore, insects do not

6   normally sonicate (buzz) flowers to release pollen because natural vibrations tend

7   to release it (King and Buchmann, 1995). Pollen is "sticky" and is released in

8   strings, which coat insect visitors (King and Buchmann, 1995; personal

9   observations). Flowers in native populations in Spain and invasive ones across

10 Ireland, are visited by a range of taxa, many of which become coated with strings

11 of pollen whilst foraging (Mejías et al., 2002; Stout et al., 2006).

12

13 Since the spread of invasive *R. ponticum* in the British Isles relies largely on seed

14 production (Cross, 1981; Erfmeier and Bruelheide, 2004), the behaviour of

15 pollinating insects has direct implications for the proliferation of this invasive

16 species. However, we do not know which species act as legitimate pollinators in

17 the invasive range, nor how pollinator behaviour may affect population structure.

18 This paper presents the results of the first investigations of pollinator behaviour on

19 invasive *R. ponticum* in the British Isles. The aims are to determine visitation rates

20 of different insect taxa, confirm which species act as legitimate pollinators of *R.*

21 *ponticum,* and examine how pollinators affect levels of inbreeding and

22 outcrossing.

23

1 **4. Methods**

2 *4.1 Study sites*

3 Observations were made in naturalised populations of *Rhododendron ponticum*

4 plants at Glencullen and Howth Head (Co. Dublin, Ireland) in May and June

5 (peak *R. ponticum* flowering period) in 2002 and at Howth Head in 2003 and

6 2005 (it was not possible to repeat observations at Glencullen as the population

7 was destroyed in 2003). At Glencullen, plants grew on a steep bracken and

8 heather dominated hillside on the edge of the Wicklow Mountains (N 53°13'28",

9 W 06°16'20", elevation 335m); and at Howth Head in heathland on the Ben of

10 Howth (N 53°22'36", W 06°04'12", elevation 130m).

11

12 *4.2 Visitation rates*

13 To determine visitation rates of different flower-visiting taxa, 72 replicate ten-

14 minute observations were made of small patches of flowers (average of 11.3

15 inflorescences per patch) between 09.30 and 16.30 on three days in Glencullen

16 and over five days in Howth in 2002. All insects entering the patch were recorded,

17 along with the number of flowers visited by each individual. Visitation rates for

18 each taxon (*Bombus*, other Hymenoptera, Diptera, Lepidoptera and Coleoptera)

19 were calculated as visits per flower per hour (number of flowers visited in 10 mins

20 / number of flowers in the patch × 6).

21

22 *4.3 Stigma contact*

1   In order to determine which insects might facilitate pollination, 136 insects (117

2   *Bombus* spp., 5 *Andrena* spp., 10 *Vespula vulgaris*, 1 *Seriocomyia silentis*, 1

3   *Eristalis tenax*, 1 *Palomena* spp., 1 unidentified Ichneumonidae) visiting *R.*

4   *ponticum* flowers were observed closely in 2003 and 2005. Insects were observed

5   opportunistically, and so the number of individuals observed of each species

6   reflects the relative abundance of the species at the site. Each individual insect

7   was followed for as many consecutive flower visits as possible (between 1 and 29

8   flowers, mean = 3.88, SE = 0.29). During each flower visit, I noted whether

9   insects contacted the stigma of flowers whilst foraging and whether insects were

10   collecting nectar only, pollen only or both nectar and pollen from the same flower.

11   The body size of a subset (82 individuals) of the insects observed was noted by

12   categorizing them according to their body length (<15 mm, 15-25 mm, >25 mm).

13   Body lengths were estimated by eye whilst insects were foraging and samples

14   were captured to confirm estimations. The proportion of stigma contacts were

15   compared according to food collected (nectar, pollen or both) and body size, plus

16   the interaction, using a non-parametric equivalent of two-way ANOVA (the

17   Scheirer-Ray-Hare extension of the Kruskal-Wallis test, Sokal and Rohlf, 1995, p

18   446). The proportion of stigma contacts were compared among bumblebee species

19   using a Kruskal-Wallis test.

20

21   *4.4 Time, distance and switching*

22   To determine whether the main flower visitors behaved as a single functional

23   pollinating group and to quantify potential levels of outcrossing, 66 individual

7

1   bumblebees (which were by far the most abundant and easily observed visitors) of

2   three common species at the Glencullen site in 2002 (19 *Bombus jonellus*, 23 *B.*

3   *lucorum / magnus* and 24 *B. monticola*) were followed for between 3 and 48

4   consecutive flower visits (mean = 13.63, SE = 1.08). It was not possible to

5   distinguish between *B. lucorum* and *B. magnus* workers in the field and so these

6   species were grouped. All *B. jonellus* and *B. monticola* individuals were workers,

7   but the *B. lucorum / magnus* group comprised queens, workers and males. Bees

8   were followed until either they finished their foraging bout and flew out of the

9   site, they were lost when flying to the other side of a large plant, or were lost

10   flying between distant plants within the site. If a bee departed from the plant it

11   was foraging on, it was followed to a subsequent plant. If it was not possible to

12   follow it or to determine the subsequent plant, recording ceased and data collected

13   for that particular individual was discarded. Handling times, search times and the

14   distance moved between individual flowers and whether bees switched

15   inflorescence or plant were recorded. The average handling time per flower,

16   search time between flowers and distance between flowers were calculated for

17   each individual bee and compared among castes within *B. lucorum / magnus* and

18   then among bee species using one-way ANOVA. In addition, the proportion of

19   moves between flowers on an inflorescence, between inflorescences on a plant

20   and between plants were calculated for each individual bee and compared among

21   castes within *B. lucorum / magnus* and then among bee species using non-

22   parametric Kruskal-Wallis tests.

23

1 *4.5 Pollen carryover*

2 In order to assess levels of stigmatic pollen carryover by bumblebees to

3 successively visited flowers, 100 flowers were emasculated prior to anthesis (to

4 avoid contamination of stigmas with self pollen) and bagged with bridal veil

5 material (to prevent insect visits). This method of emasculation and bagging has

6 previously been shown to successfully exclude pollen from stigmas (Stout, In

7 press). On large, many-flowered plants such as *R. ponticum*, it is very difficult to

8 ensure free-flying insects visit a sequence of emasculated flowers. In order to

9 overcome this problem, once emasculated flowers were fully open, dead *B.*

10 *terrestris* bumblebees (of all three castes, from a colony which had been kept in a

11 glasshouse, hence not contaminated with *R. ponticum* pollen) were used to

12 simulate the behaviour of live ones to examine levels of pollen carryover (this

13 technique has been successfully used by Waddington, 1981, and Escaravage and

14 Wagner, 2004). The relaxed bumblebees were loaded with pollen by simulating

15 their natural positions during live visits to five unmanipulated *R. ponticum* flowers

16 with dehiscing anthers for 5 seconds (which is slightly longer than the 3.7 seconds

17 average time spent per flower by live foraging bumblebees). The test bee was then

18 inserted into five successive emasculated flowers for 5 seconds in the same way.

19 Since dead bees were of all three castes, they represented a range of body sizes

20 and so were assumed to pick up pollen and contact stigmas the same proportion of

21 times as live bees. After each "visit" to an emasculated flower, the stigma from

22 that flower was removed and later mounted on a microscope slide, stained with

23 0.5% safranin solution and the number of *R. ponticum* pollen tetrads counted. The

number of tetrads on flowers 1-5 were compared using Friedman's method for randomized blocks (a non-parametric test using ranks, with each block representing a sequence of five flower "visits", Sokal and Rohlf, 1995, p 440).

5. Results

5.1 Visitation rates

A total of 555 insects were observed foraging on *R. ponticum* patches during 12 hours of observation. Bumblebees of six species (29 *B. jonellus*, 3 *B. lapidarius*, 163 *B. lucorum / magnus*, 110 *B. monticola*, 16 *B. pascuorum* and 121 *B. pratorum*) were recorded. In addition, 32 other Hymenoptera (from 8 taxa), 76 Diptera (11 taxa), 4 Lepidoptera (4 taxa) and 1 Coleoptera were observed. Mean visitation rates were different at the two sites: 1.29 (S.E. = 0.14, n= 40) insects per flower per hour during daylight at Howth, and 4.10 (S.E. = 0.32, n=32) at Glencullen. At both sites, most visits per flower per hour were made by bumblebees (Howth: mean = 1.10, S.E. = 0.10, n= 40; Glencullen: mean = 3.86, S.E. = 0.22, n= 32), with queens, workers and males observed visiting flowers (15% of individuals recorded were queens, 73% workers and 12% males).

5.2 Stigma contact

On average, 33.3% of visits by insects resulted in contact with the stigma of *R. ponticum*. Stigma contact was made by various parts of the insects' bodies, depending on the position in which they approached and landed on flowers. Some landed so that the ventral side of the insect contacted the stigma (in the way

1   described by Mejéías et al., 2002), but others flew down into the back of the

2   flowers so that the dorsal side of the insect contacted the stigma. Other contacts

3   were made as the insect flew into or out of the flower. Stigma contacts were only

4   made by bumblebees (38.6% of visits by bumblebees resulted in contact with

5   stigmas) and one *Vespula vulgaris* queen (1.3% of visits made by insects other

6   than bumblebees resulted in contact with stigmas). Of all visits to flowers, 62.5%

7   were to collect nectar only, 10.3% to collect pollen only and 27.2% to collect

8   both. The average proportion of stigma contacts did not vary according to whether

9   insects collected nectar, pollen or both from flowers (Figure 1, Table 1). However,

10 the proportion of stigma contacts did vary significantly according to body size

11 (Figure 2, Table 1), with larger insects (mostly queen bumblebees) contacting the

12 stigma of *R. ponticum* flowers more frequently than smaller ones (on average,

13 66.1% of queen bumblebee visits resulted in stigma contact). There were no

14 significant differences among bumblebee species in the proportion of stigma

15 contacts made (χ^2_3=7.45, p=0.06).

16

17 *5.3 Time, distance and switching*

18 There were no differences among castes within the *B. lucorum* / *magnus* group

19 (p>0.05 for all tests). There was no difference in the handling or search/flight

20 times of the three bumblebee species nor in the distance flown between successive

21 flowers (Table 2). There were no differences between species in the proportion of

22 moves made within inflorescences, between inflorescences and between plants

23 (Table 2). In total, 47.5% of all bumblebee moves observed were within

1   inflorescences, 50.1% of moves between inflorescences, and 2.4% of moves

2   between plants. The distribution of distances moved between successive flower

3   visits was highly leptokurtic, with short-distance movements far more common

4   than longer distance ones (Figure 3).

5

6   *5.4 Pollen carryover*

7   There was no decline in the number of pollen tetrads deposited on stigmas of

8   successively "visited" flower (from 1-5) (Table 3, $\chi^2_4 = 6.59$, p=0.16).

9

10   **6. Discussion**

11   Visitation by native insects to the invasive exotic, *Rhododendron ponticum*, was

12   very frequent in the populations studied. Even with only a third of visits resulting

13   in stigma contact and hence potential pollen transfer, individual flowers may be

14   pollinated at least once every three hours during the peak flowering season. This

15   explains the high seed set in these populations, reported elsewhere (Erfmeier and

16   Bruelheide, 2004; Stout et al., 2006; Stout, In press) and an absence of pollination

17   limitation (Stout, In press).

18

19   Visitation rates were three times higher at the Glencullen site compared with the

20   Howth site. This may be due to the fact that there were few other flowering plants

21   at the Glencullen site, and *R. ponticum* provided the primary resource to

22   anthophiles in the area. Indeed, *R. ponticum* flowers after *Vaccinium* and before

23   the heathers (*Erica* and *Calluna*) and so may provide an important resource for

1   bees at this time of year. By contrast, the Howth site is close to urban gardens

2   which may compete for pollinator attention.

3

4   Queens, workers and males of six bumblebee species, including relatively

5   uncommon heathland specialist species, *B. monticola* and *B. jonellus*, were the

6   most frequent diurnal visitors to flowers, with other taxa rarely seen visiting

7   flowers. Previous studies have also shown that bumblebees comprise the majority

8   of flower visitors and pollen was found on the bodies of most individuals

9   examined (Mejías et al., 2002; Stout et al., 2006). Bumblebees were the only

10   taxon (except the occasional *Vespula* queen) to contact the stigmas of flowers

11   whilst foraging. Since *R. ponticum* flowers are relatively large, small insects,

12   including worker bees, can visit flowers without touching the reproductive

13   structures of the flower by flying directly towards the back of the flower to extract

14   nectar from the groove in which it accumulates. The larger queens, however,

15   many of which are foraging during the peak flowering for *R. ponticum*, more

16   frequently contact flower stigmas.

17

18   No differences were found among three bumblebee species in terms of their

19   search or handling time per flower, distance flown between successive flowers, or

20   proportion of switches within and between plants. These three species have

21   similar, short-medium length probosci (Stout, unpublished data), and seem to

22   forage in a similar way. Although we only examined three species, it is possible

23   that all the bumblebee species visiting *R. ponticum* form a single functional

1   pollinating group (Fenster et al., 2004). If this is the case, even though a wide

2   range of insect species visit *R. ponticum*, tempting us to conclude that the plant-

3   pollinator interaction is a broad, generalised one (Mejías et al., 2002; Stout et al.,

4   2006), bumblebees are the most frequent flower visitors and if several species are

5   comprising a single functional pollinating group, the pollination ecology of *R.*

6   *ponticum* could be more specialised than previously thought, with specialisation

7   towards pollination by large bees.

8

9   Bumblebees are known to be efficient foragers, frequently moving between

10   flowers, often visiting adjacent flowers to reduce flight times, and avoiding re-

11   visitation of depleted flowers (Goulson, 2003). This efficiency on the part of the

12   pollinator can often affect levels of inbreeding and outbreeding, and ultimately,

13   the population structure of the plant. Most (97.6%) successive flower visits

14   recorded in this study were between flowers on the same plant. It is possible that

15   this is an underestimation, as bumblebees are often very difficult to follow if they

16   fly over the top of large *R. ponticum* plants. Nonetheless, with individual plants

17   producing hundreds of flowers (Cross, 1981), it is likely that within-plant

18   movement accounts for most successive flower visits. This might result in high

19   levels of inbreeding via geitonogamy. Even if bees move between plants, if they

20   move between adjacent individuals, it is possible that they are moving between

21   clones (because *R. ponticum* can spread locally by vegetative layering, Erfmeier

22   and Bruelheide, 2004), causing inbreeding. Indeed, since many exotic species

23   consist of small isolated populations with low genetic diversity, or descend from

1   small founder populations (Lee et al., 2004), inbreeding may be relatively

2   common.

3

4   However, relatively high levels of pollen carryover might mitigate the negative

5   impacts of bees visiting many flowers on a single plant and may increase pollen

6   dispersal and outbreeding (Broyles and Wyatt 1991). In this study, stigmatic

7   pollen carryover was not shown to decline at all over the first five flowers visited.

8   Other studies of pollen carryover have found a rapid decrease in pollen deposition

9   (e.g. Geber, 1985; Cresswell et al., 1995; Cresswell, 2000), with more than half of

10   all pollen deposited going to the first two flowers visited (e.g. Escaravage and

11   Wagner, 2004). However, Carré et al. (1994) also found that the quantity of pollen

12   deposited was very variable and depended on the individual forager, and in 50%

13   of cases the pollen deposition was independent of the visitation order. Pollen

14   carryover may have been over-estimated in this study by the use of dead bees to

15   simulate flower "visits". This may have caused two problems: firstly, dead bees

16   may not have been inserted into flowers in the same way that live bees visit

17   flowers (although every attempt was made to mimic natural behaviour) and,

18   secondly, dead bees are unable to groom pollen off their bodies, increasing the

19   chances of pollen deposition in the experiments (Escaravage and Wagner, 2004).

20   However it is quite plausible that the patterns shown in this experiment are an

21   accurate reflection of the natural situation. *R. ponticum* flowers are big, and not all

22   bee visits contact stigmas (depending on the position of the stigma when a bee

23   visits and, as shown above, the size of bee). Therefore, in natural situations, bees

15

1   may well visit five flowers without ever touching the stigma and depositing

2   pollen. In addition, as *R. ponticum* pollen adheres in "strings" (King and

3   Buchmann, 1995), we would expect a clumped distribution of deposition, not a

4   linear one.

5

6   If *R. ponticum* plants are suffering from inbreeding, we might expect a reduction

7   in individual plant fitness through decreased quantity and quality of seed

8   produced (Keller and Waller, 2002; Wallace, 2003). Recent experiments suggest

9   that inbreeding in naturalised *R. ponticum* in Ireland does reduce seed set, but that

10   it has little impact on seed germination (Stout, In press). In addition, at the

11   population level, inbreeding can affect genetic diversity and the ability of a

12   population to cope with environmental change (Lande, 1995). Although it is

13   possible that introgression with North American *Rhododendron* species has

14   occurred (Milne and Abbott, 2000), currently we know little about the genetic

15   diversity of invasive *R. ponticum* populations in Ireland. Work is currently

16   underway to address this issue (Stout et al., in prep).

17

18   In conclusion, invasive exotic *R. ponticum*, which benefits from animal-mediated

19   outcrossing, has succeeded in forming legitimate pollination interactions with

20   native generalist bumblebee species in the British Isles. This is not altogether

21   surprising considering the native range of *R. ponticum* is within the same

22   continent, where it is also pollinated by generalist large bees (Mejías et al., 2002;

23   Stout et al., 2006). It would be interesting to discover what pollinates introduced

1   *R. ponticum* in areas where bumblebees are not native and to examine rates of

2   invasion in these places. Many exotic plants with a large floral display, and

3   copious nectar production form associations with native insects, particularly

4   bumblebees. This may not only promote invasion by the exotic plant, but may

5   disrupt native plant-pollinator mutualisms and so have further impacts on native

6   biodiversity and ecosystem functioning (Traveset and Richardson, 2006). Clearly,

7   there is a need for more research into exotic-plant – native-pollinator interactions.

8

14

15   **8. General Summary (for translation into French and German)**

16   The behaviour of flower-visiting insects affects the quantity and quality of

17   pollination service a plant receives, and affects both individual plant fitness and

18   population dynamics. Animal-pollinated exotic plants must form pollination

19   mutualisms with native taxa in order to become established and spread in non-

20   native habitats. Thus the behaviour of the native pollinators can have direct

21   impacts on the invasion dynamics of exotic species. The invasive exotic

22   *Rhododendron ponticum* in Ireland is visited by a range of insect taxa, but in two

23   naturalised populations in Co. Dublin, Ireland, only bumblebees and one *Vespula*

1   queen were observed to contact stigmas. Bumblebees were the most frequent

2   visitors to flowers, with more than one insect visit per hour recorded during the

3   peak flowering season in 2002. This accounts for the vast seed production and

4   successful invasion by this species, reported elsewhere. Six species of bumblebees

5   (comprising 15% queens, 73% workers and 12% males) were observed visiting

6   flowers and collecting nectar and/or pollen. There was no significant difference

7   between insects foraging for nectar and/or pollen in terms of their ability to

8   pollinate flowers (measured as proportion of visits contacting the flower stigma,

9   Figure 1), but larger insects (mostly queen bees) were more likely to contact

10   stigmas than smaller insects, including worker and male bees (Figure 2). There

11   were few differences in foraging behaviour between three abundant species of

12   bumblebee (Table 2), suggesting these species form a single functional pollinating

13   group. *R. ponticum* plants produce a massive floral display and 47.5% of all

14   bumblebee moves observed were within inflorescences, 50.1% of moves among

15   inflorescences on the same plant, and 2.4% of moves between plants. The

16   distribution of distances moved between successive flower visits by bumblebees

17   was highly leptokurtic (Figure 3) which could lead to high levels of inbreeding. In

18   addition, since *R. ponticum* is capable of local vegetative spread, plants close to

19   one another may in fact be clones. Hence even apparent out-crossing events may

20   actually result in inbreeding. However, pollen carryover is probably extensive as

21   there was no decline in the pollen deposition over subsequent flowers visited

22   (Table 3). As the primary pollinators of *R. ponticum* in Ireland, bumblebee

behaviour has important consequences for outcrossing and population dynamics of this exotic species.

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1   Table 1: Analysis of proportion of stigma contacts according to body length of

2   insect (size), food collected (nectar, nectar+pollen, pollen) and the interaction.

3   Test statistic given is H (as calculated by the Scheirer-Ray-Hare extension of the

4   Kruskal-Wallis test). ** = p<0.01

5

6

	SS	MS_{total}	H	df	p
Size	5533.9	516.8	10.71	2	0.005**
Food	1662.4	516.8	3.22	2	0.200
Size × Food	637.8	516.8	1.23	4	0.872
Error	34546.0			73	

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1   Table 2: Mean (S.E.) time spent per flower (Handling), time spent searching

2   between flowers (Search), distance between successive flowers (Distance),

3   proportion of moves within inflorescences, between inflorescences and between

4   plants, for *B. jonellus*, *B. lucorum* and *B. monticola*, and results of statistical tests

5   for differences among species (ANOVA for Handling, Search and Distance,

6   Kruskall Wallis for proportion of moves).

	B. jonellus	*B. lucorum*	*B. monticola*	Test statistic	*p*
Handling (s)	3.51 (0.38)	3.42 (0.29)	4.14 (0.36)	$F_{2,63} = 1.370$	0.262
Search (s)	1.07 (0.06)	1.14 (0.07)	1.18 (0.11)	$F_{2,63} = 0.365$	0.695
Distance (cm)	15.79 (1.97)	34.92 (14.62)	13.11 (1.35)	$F_{2,63} = 1.839$	0.167
Within inflorescence	0.45 (0.04)	0.51 (0.05)	0.46 (0.04)	$X^2_2 = 1.58$	0.452
Between inflorescences	0.53 (0.04)	0.44 (0.05)	0.53 (0.04)	$X^2_2 = 3.46$	0.178
Between plant	0.01 (0.008)	0.05 (0.03)	0.01 (0.006)	$X^2_2 = 1.08$	0.584

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1   Table 3: Mean (plus standard error) number of pollen tetrads on stigmas of the

2   first to fifth "visited" flowers and mean rank of each flower within each visitation

3   sequence (where flowers receiving least pollen are ranked 1 and those receiving

4   most are ranked 5). Flowers "visits" were simulated using dead bumblebees.

5

6

Flower number	Mean number of pollen tetrads (S.E.)	Mean rank
1	53.7 (18.53)	3.3
2	20.75 (11.02)	2.6
3	9.85 (3.51)	2.425
4	26.5 (10.25)	3.25
5	38.55 (17.92)	3.425

7

8

1   **Figure Legends**

2

3   Figure 1: proportion of flower visits with stigma contact (mean ± S.E.) according

4   to food collected from flowers (nectar, both, pollen).
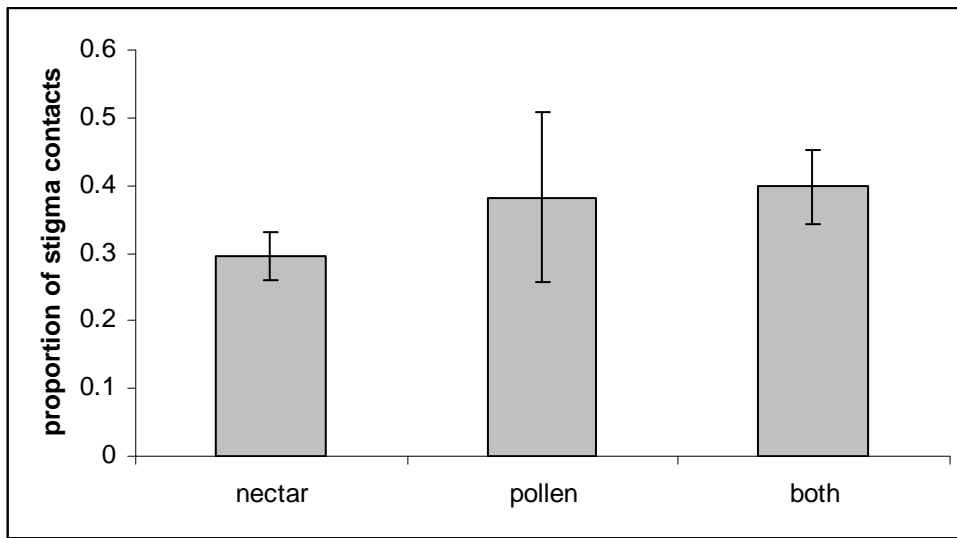
5

6   Figure 2: proportion of flower visits with stigma contact (mean ± S.E.) according

7   to body length of visitor (<15mm, 15 – 25mm, >25mm).

8

9   Figure 3: the number of flights of different distances made by all bumblebees
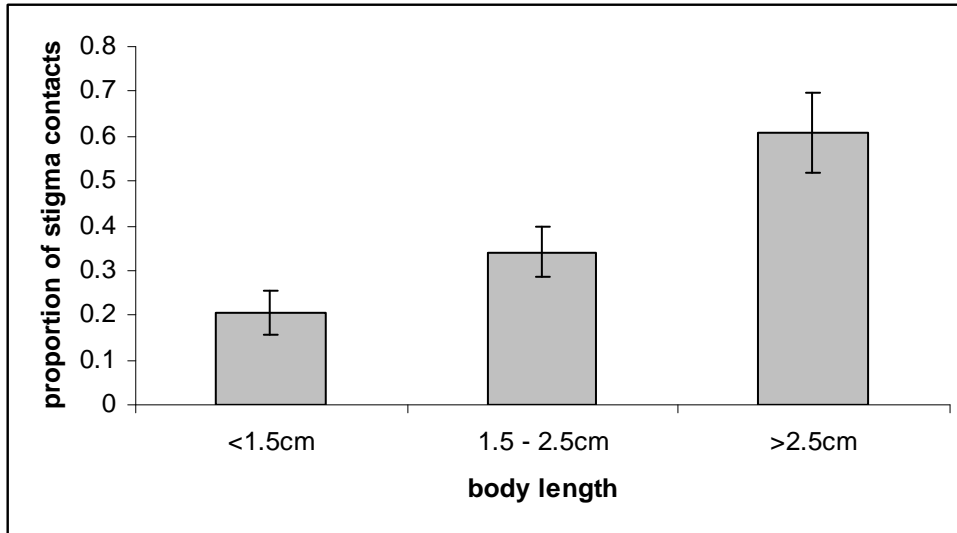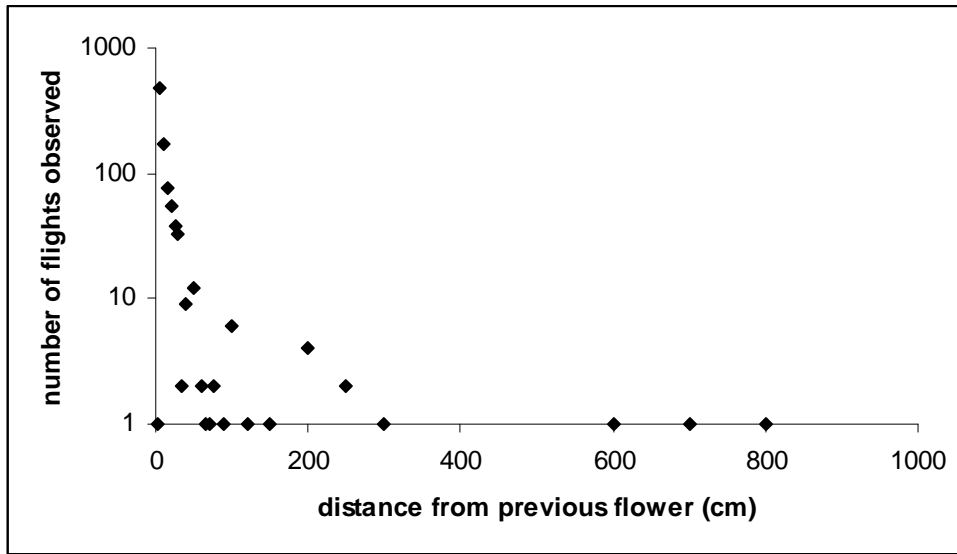
10   observed (logarithmic scale).

11

1   Figure 1

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1   Figure 2

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1 Figure 3

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