

Flower Constancy and Memory Dynamics in Bumblebees (Hymenoptera: Apidae: Bombus)

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Flower visitors of several taxa (including bees, butterflies and hoverflies) are known to move preferentially between flowers of the same species, while neglecting other equally rewarding flower species. Darwin proposed memory limitations as a potential mechanistic explanation for this flower constancy behaviour. This reanalysis of a previously published dataset, relating a real-time sequential analysis of bumblebee flower choices to the distance distribution between flowers in a natural meadow, shows that Darwin was right in an unexpected way. Bees (*Bombus* spp) can learn how to extract rewards from several flower species, and switching between flower species does not compromise their handling efficiency (i.e. flower handling times do not increase following a species switch). However, bees do lose time flying between flowers. If a flower is encountered within a short time (0–2 secs) after the last flower visit, it is likely to be visited only if it is the same species. After longer intervals (3–6 secs), bees are more likely to switch to visiting different flower species. Thus, flower constancy is in part an effect based on comparing incoming stimuli (the flowers a bee detects in flight) with a transient form of short-term (working) memory, which holds the signal of the last visited flower. The relative weighting of working and reference memory changes as time passes following a flower visit. This results in a low probability of retrieving the memory for a different flower species (from that just visited) within a short time interval after the most recent flower visit (0–2 secs). This probability rises as the signal of the last visited flower fades from working memory (>2 secs).

Keywords: *Bombus pascuorum* (Scopoli 1763) – *Bombus veteranus* (Fabricius 1793) – *Bombus terrestris* (Linnaeus 1758) – *Bombus lapidarius* (Linnaeus 1758) – flower colour – floral signals – foraging behaviour – handling time – long-term memory – pollination ecology – short-term memory – working memory

RAINE N E & CHITTKA L [Biol & Chem Sci, Queen Mary, London, E1 4NS]: **Blütenstetigkeit und Gedächtnisdynamik bei Hummeln (Hymenoptera: Apidae: Bombus)**. – Entomol Gener 29(2–4): 179–199; Stuttgart 2007-01. – – – [Abhandlung]

Blütenbesucher mehrerer Taxa (inklusive Käfer, Schmetterlinge und Schwebfliegen) konzentrieren ihre Sammeltätigkeit auf oft nur wenige Pflanzenarten und ignorieren dabei andere, wertvolle Futterquellen. Darwin vermutete, daß diese Blütenstetigkeit durch eine begrenzte Gedächtniskapazität erklärt werden könnte. Die hier präsentierte Neuauswertung einer bereits publizierten Echtzeit-Analyse der Sequenzen des Blütenbesuchs von Hummeln zeigt, daß Darwins Vermutung auf ungeahnte Weise richtig war. Hummeln (*Bombus* spp) können mehrere Aufgaben im Gedächtnis speichern und das Wechseln zwischen mehreren Arten von Blütenpflanzen beeinträchtigt nicht die Effizienz der motorischen Muster, die die Hummeln ausführen, um an den Nektar zu gelangen. Allerdings treten deutliche Zeitverluste beim Fliegen zwischen verschiedenen Arten von Blütenpflanzen auf.

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Die Flugzeiten formen zwei deutlich voneinander getrennte Zeitgruppen, die in ihren Spitzenwerten klar getrennt sind nach konstanten Flügen (0–2 sec) und inkonstanten Flügen (3–6 sec). Eine mögliche Erklärung ist die, daß der zuletzt besuchte Stimulus in einer transienten Form des Gedächtnisses, einem Arbeitsgedächtnis, für etwa 2 Sekunden abgelegt wird. Während das Tier sich im Flug befindet, werden neue detektierte Stimuli mit dem Inhalt der Arbeitsspeicher verglichen. Wenn diese zur Passung gebracht werden können, so steuert das Tier mit hoher Wahrscheinlichkeit diesen Stimulus an. Nachdem dieses transiente Gedächtnis zerfällt (>2 sec), steigt die Wahrscheinlichkeit des Blütenartwechsels, weil nun das Referenzgedächtnis abgerufen wird.

Schlüsselbegriffe: *Bombus pascuorum* (Scopoli 1763) – *Bombus veteranus* (Fabricius 1793) – *Bombus terrestris* (Linneaus 1758) – *Bombus lapidarius* (Linneaus 1758) – Arbeitsgedächtnis – Bestäubungsbiologie – Blütenfarbe – Blütensignal – Kurzzeitgedächtnis – Langzeitgedächtnis

1 Introduction

Aristotle observed that “during each flight the bee does not settle upon flowers of different kinds, but flies, as it were, from violet to violet, and touches no other till it returns to the hive” (quoted in [CHRISTY 1884]). This behaviour, known as flower constancy [WASER 1986, CHITTKA, THOMSON & WASER 1999, RAINE, INGS, DORNHAUS et al 2006a], is observed when bees make sequences of visits to flowers of one species, before switching to another species to which they will then stay temporarily faithful (when foraging in habitats containing several flower species): i.e constant flights occur between flowers of the same species, whereas transition flights occur between flowers of different species. One potential explanation for flower constancy is that it reflects the bees’ ability to learn and is thus adaptive in maximising foraging efficiency [FAEGRI & VAN DER PIJL 1980, MENZEL 2001]. It seems hard to see how such behaviour could be adaptive *per se*, since there is rarely only a single best food source, and specializing on one flower type while skipping other valuable ones encountered *en route*, would seem an unwise strategy to maximize energy intake per unit time [WASER 1986, CHITTKA, GUMBERT & KUNZE 1997, CHITTKA et al 1999, WEISS 2001, RAINE & CHITTKA 2005]. However, “no one ... will deny that there is ... some powerful influence at work which induces insects ... to continue visiting for a considerable time continuously the flowers of the same species of plant...” [CHRISTY 1884]. What then is the nature of this ‘powerful influence’? DARWIN [1876, p. 419] offered a mechanistic explanation: “the cause probably lies in the insects being thus enabled to work quicker; they have just learnt how to stand in the best position on the flower, and how far and in what direction to insert their proboscides. They act on the same principle as does an artificer who has to make half a dozen engines, and who saves time by making consecutively each wheel and part of them”.

In fact, this account contains two fundamentally different explanations. The first can be conservatively interpreted to mean that the reason for constancy is that an insect has learnt to handle one species of flower, but not any others. Although DARWIN’s [1876] statement has been taken by some researchers [e.g LEWIS 1986] to mean that insects *can* only remember one floral handling technique at any one time, DARWIN [1876] here only suggests that insects prefer to stay with flowers whose handling techniques they have already learnt, rather than visiting unfamiliar flower types.

However, DARWIN’s [1876] artificer has clearly memorised more than one manufacturing task, and is not suffering from a shortage of memory capacity; he is skilled in making all the parts of the engine, but saves time by making each sequence of identical parts before manufacturing the next set of identical items. Similarly, bees that have learned how to handle more than one flower species efficiently, might not forget how to handle one species as they switch to visiting another.

To put this in modern terms, DARWIN [1876] thus conjectures that there is short-term interference when different motor patterns are executed in quick succession, whether by artificer or bee. One prediction of this hypothesis is that bees should exhibit longer flower handling times immediately after switching from one species to another. However, tests of this prediction found no, or very small, differences between handling times depending on whether the last visit was to the same, or a different, flower species [WOODWARD & LAVERTY 1992, LAVERTY 1994b, GEGEAR & LAVERTY 1995].

In fact, flower constancy occurs in bees even when flowers differ only in appearance, but not in their handling procedure, e.g. two colour morphs of the same species [WASER 1986, KEASAR, BILU, MOTRO et al 1997, CHITTKA et al 1999, RAINE, INGS, RAMOS-RODRÍGUEZ et al 2006b]. Whilst increases in handling costs associated with switching can explain constancy under some circumstances, for example when flower morphologies are highly complex or species differ markedly in floral complexity [LAVERTY 1980, 1994a], they cannot be invoked when flowers differ only in appearance. Likewise, flower constancy cannot be comprehensively explained by a limited capacity of a bee's long-term memory for different motor patterns. However, limitations of short-term memory in retaining stimuli associated with rewards are likely to be highly relevant [GREGGERS & MENZEL 1993, MENZEL, GREGGERS & HAMMER 1993, CHITTKA et al 1999, MENZEL 1999]. If honeybees are given two consecutive learning trials in which different colours are associated with reward, the memory trace for the first colour can be effectively erased if the second trial follows in one of two sharply defined time windows: either (1) immediately (within 30 seconds), or (2) about 3 minutes after the first trial [MENZEL 1985]. In contrast, honeybees have no difficulty storing several different colours as predictors of reward in long-term memory. If the second colour is presented outside these specific time windows, both colours will be memorised [MENZEL 1990]. Moreover, retrieval of the memory for the flower (signal) just visited takes less time than retrieving another familiar signal: e.g. a different flower species [GREGGERS & MENZEL 1993]. This seems likely to have fundamental implications over whether to remain flower constant depending on how long after the last flower visit a bee perceives stimuli that indicate the encounter of another flower, and whether it is of the same or a different species.

Further circumstantial evidence that working memory dynamics govern floral constancy was provided by observing bumblebee flower choices under natural conditions. These bees showed highly stereotypical flight times between flowers of the same species [CHITTKA et al 1997]: constant flights were most commonly between 2–4 seconds, almost never more than 8 seconds, and were surprisingly independent of the spatial distribution of flowers. Thus, CHITTKA et al [1997] conjectured that flight times were not determined by external factors, such as distances between flowers, but by working memory dynamics. It was thought that the signal of the most recently visited flower was held in working memory for a few seconds, and if newly incoming stimuli matched this (flower) signal, the bee would visit another flower of the same species. A few seconds later, after working memory of the last visited flower has decayed, bees might retrieve older memories for different flower species [CHITTKA et al 1997, MENZEL 2001].

A potential weakness of these approaches is that the time intervals at which bees encountered flowers (stimuli) were not controlled [GREGGERS & MENZEL 1993, CHITTKA et al 1997], therefore conclusions about memory dynamics can only be drawn indirectly. However, recent laboratory experiments in which the distance, and hence flight time, between two sequentially encountered stimuli was rigorously controlled [ZHANG, BOCK, SI et al 2005], provide strong support for the inferences drawn from earlier field research [CHITTKA et al 1997, CHITTKA & RAINE 2006]. Honeybees encountered a visual pattern whilst flying through a tunnel which they had to remember in order to make a correct binary choice decision at the end of the apparatus. ZHANG et al [2005] found almost exactly the same working memory dynamics as those recorded by CHITTKA et al [1997] under natural conditions. Recall was best in the first few seconds after encountering the first visual pattern, and working memory had largely decayed by 8 seconds [ZHANG et al 2005] – strikingly similar dynamics as in the field trials with bumblebees [CHITTKA et al 1997].

These new results have triggered a reanalysis of the field data collected by CHITTKA et al [1997] to address the following questions:

1. Over what timescale do free foraging bumblebees exhibit flower constancy under natural conditions? Do individual bees switch frequently when foraging, and if so how many flower species do they visit?

2. Does the flower handling time increase after bumblebees switch species? This is the prediction if recalling the motor skills required when handling flower species A interferes with retrieving the skills associated with species B.

3. Does the flight duration between flowers influence the chance of a species switch? If in general, longer flights are associated with higher chances of switching this would be consistent with the notion that working memory governs flower choices made in rapid succession.

4. Does the probability of switching change with the number of constant flights made before a switch occurs? If handling skills are more rapidly accessed from working, rather than long-term, memory, it is predicted that longer periods of flower constancy would generally reduce the chances of switching.

2 Methods

2.1 The experimental meadow

The experiment was conducted in a nature reserve near Berlin (Naturschutzgebiet Lange Dammwiesen, Strausberg, Brandenburg, Germany) containing 5 plant species in bloom: Bird's foot trefoil *Lotus corniculatus* (Linnaeus 1753), Meadow vetchling *Lathyrus pratensis* (Linnaeus 1753), Tufted vetch *Vicia cracca* (Linnaeus 1753), Fabaceae; Cabbage thistle *Cirsium oleraceum* ((Linnaeus) Scopoli 1769), Asteraceae and Purple loosestrife *Lythrum salicaria* (Linnaeus 1753), Lythraceae. Human observers cannot reliably distinguish flowers of *Lotus* and *Lathyrus* in the field using either colour or morphology. Instead, leaf shape and the presence of tendrils on *Lathyrus* plants were used to tell them apart when recording bee flower choices. These flower species are also predicted to be almost indistinguishable to foraging bumblebees due to their similarity in morphology and colour – both are bee green (human yellow) and lie extremely close to each other in the bee colour space [CHITTKA et al 1997]. In contrast, the flowers of the other species in the meadow are easily distinguishable to both humans and bees based on colour and morphology. Floral colours: *Cirsium* = bee blue-green (human yellow), *Vicia* = bee blue (human purple), *Lythrum* = bee UV-blue (human pink). Floral morphology: *Vicia*, *Lotus* and *Lathyrus* (Fabaceae) all have zygomorphic flowers of relatively low complexity handled by bees using similar motor patterns; *Lythrum* has radially symmetric, open flowers presented vertically on an elongated inflorescence; while *Cirsium* flowers have a long-corolla tube, and are presented as an upwards facing inflorescence [CHITTKA et al 1997].

All bumblebee flower choices were observed in an 8m x 20m area selected to maximise the homogeneity of each flower species. The study area contained 777 *Vicia* inflorescences (henceforth referred to as 'flowers'), 642 *Lotus*, 253 *Lathyrus*, 153 *Cirsium* and 120 *Lythrum*. The flower distance distribution within the study area was mapped by measuring the distance from a randomly selected focal flower to its nearest conspecific, and the nearest flower of each of the other (four) species. This procedure was repeated several times for each focal flower species [CHITTKA et al 1997].

Distances between common conspecific flowers (e.g. *Vicia-Vicia*) were shorter than those between rare conspecifics (e.g. *Cirsium-Cirsium*). Also, whilst heterospecific distances from *Vicia* to all other species were significantly longer than those from *Vicia-Vicia*, distances from *Cirsium* to all other species were not significantly longer than those from *Cirsium-Cirsium* [CHITTKA et al 1997]. Thus, it might be more favourable to switch if a bee has just visited a rare, rather than a common, flower species. For *Lathyrus*, a species growing at an intermediate density, the distance to the less common species (*Cirsium*, *Lythrum*) were significantly longer than to conspecific flowers, whereas distances to the more common species (*Vicia*, *Lotus*) were not [CHITTKA et al 1997]. The distributions of inter-flower distances showed no indication of localised clumping of any flower species within the study area.

2.2 Recording bumblebee choices

Four bumblebee species were observed foraging in the meadow patch: *Bombus pascuorum* (Scopoli 1763), *Bombus veteranus* (Fabricius 1793), *Bombus terrestris* (Linnaeus 1758), and *Bombus lapidarius* (Linnaeus 1758). The sequence of flower choices made by each individual bee was recorded in real time using pocket tape recorders [further details in CHITTKA et al 1997]. In each choice sequence the flower just visited is the ‘reference’ flower, and the flower to which the bee subsequently flies is the ‘target’ flower. The foraging choices of an individual bee were recorded for as long as possible, until it either left the mapped area or it could no longer be tracked by the observer. Bee foraging choices were recorded by three observers working 3 hours per day (1100-1400h) during a 5 day period. During 205 foraging sequences (*B pascuorum* 86, *B veteranus* 57, *B lapidarius* 51 and *B terrestris* 11) 4464 bumblebee flower choices (*B pascuorum* 2368, *B veteranus* 1122, *B lapidarius* 867 and *B terrestris* 107) were observed, totalling 11 hours and 27 minutes of foraging behaviour (*B pascuorum* 345 mins, *B veteranus* 170 mins, *B lapidarius* 152 mins and *B terrestris* 20 mins).

3 Results

3.1 Foraging bumblebees switch frequently between flower species

Whilst observing the course of a single foraging sequence, most individual bumblebees visited more than one (134 of 205 = 65%), and many visited more than two flower species (75 of 205 = 37%: **Fig 1**). Some individuals, 7 *B pascuorum* (**Fig 1a**) and 1 *B lapidarius* forager (**Fig 1c**), visited all five flower species present in the meadow. In general, if bees were observed for longer periods they were more likely to be seen visiting more than one flower species. For example, *B pascuorum* foragers observed to visit all five flower species were observed for an average of 693 ± 180 (mean \pm 1SE: $n = 7$) seconds, whereas foragers seen visiting only one flower species were observed for an average of $85 (\pm 19; n = 17)$ seconds. At the species level, the general order of flower constancy observed was *B terrestris* > *B lapidarius* > *B veteranus* > *B pascuorum* (**Fig 1**). Observations of bee foraging over sustained periods revealed that individual bees can switch flower species very frequently. For example, one *B pascuorum* forager switched 64 times during a sequence of 182 flower visits, observed over 1610 seconds. However, it is also clear that bees regularly remain flower constant for sequences of several, or even tens of, flower choices over relatively short time periods (generally less than 10 minutes). For example, 4 of the 11 *B lapidarius* foragers, observed continuously for more than 300 seconds, visited only a single flower species (**Fig 1c**).

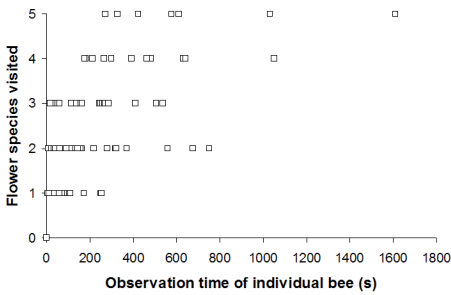
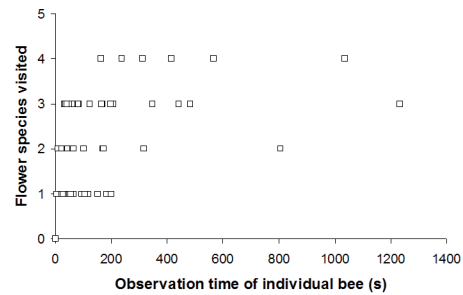
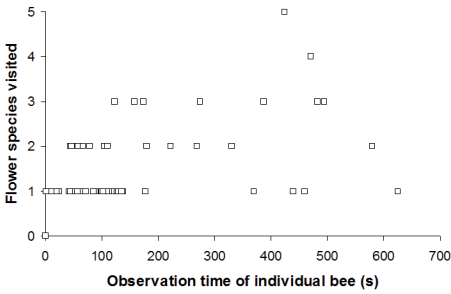
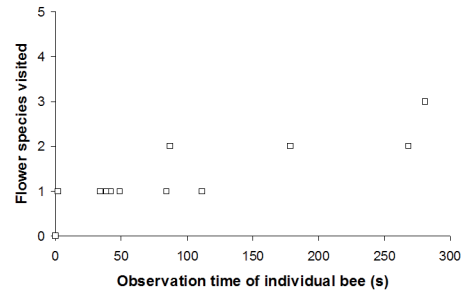
a) *B pascuorum*b) *B veteranus*c) *B lapidarius*d) *B terrestris*

Fig 1: Flower ‘inconstancy’ of the four bumblebee species observed: (a) *Bombus pascuorum* (Scopoli 1763), (b) *Bombus veteranus* (Fabricius 1793), (c) *Bombus lapidarius* (Linnaeus 1758) and (d) *Bombus terrestris* (Linnaeus 1758). Plots illustrate the number of flower species visited as a function of observation time. Each symbol represents data from a different observed foraging sequence: $n = 86$ *B pascuorum*, 57 *B veteranus*, 51 *B lapidarius* and 11 *B terrestris*. Bees plotted with an ordinate (y-axis) value of one indicate individuals that visited only one flower species, ie remained entirely flower constant, during the period in which they were observed.

Thus, the stereotypical view of complete floral constancy is only seen when observing bees for a relatively short time period. Hence, these field data provide a good opportunity to study the rules and mechanisms underlying bumblebee decisions to switch flower species while foraging.

3.2 Constant and transition flight frequencies for all flower species combinations

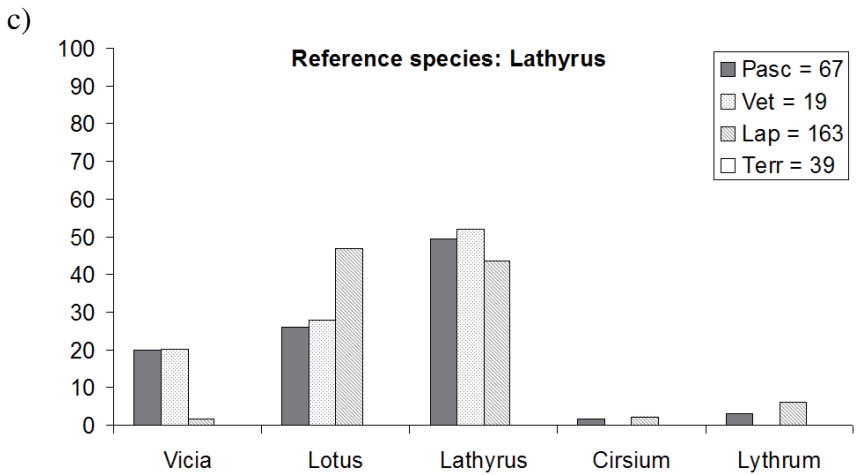
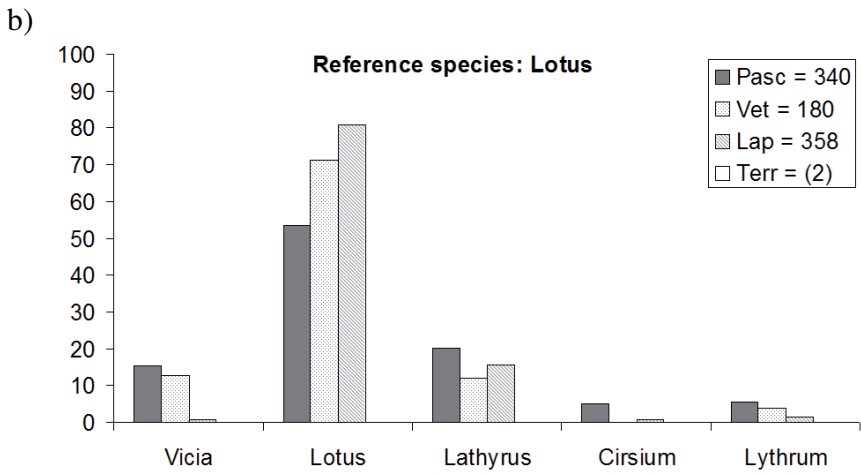
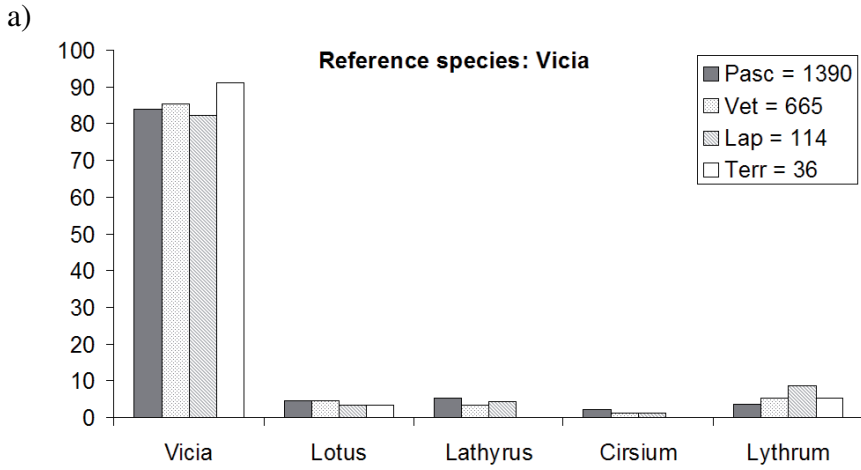
How do the identities of reference and target flower species influence the chances of constancy in foraging bees? The percentages of constant and transition flights for all possible reference \rightarrow target flower species combinations are shown in **Fig 2**. The frequencies of such single flight transitions in the 25 combination categories differ significantly across the four bumblebee species observed (χ^2 goodness-of-fit test; $\chi^2 = 1474$, $df = 72$, $p < 0.001$); hence flight percentages are plotted separately for each bee species. Flight data for all individuals from the same bee species are considered together because the flights are considered from the evolutionary perspective of the plant.

The identity of individual flower visitors is not important to the plant, but the identity of the flower species from which the visitor comes (bringing pollen), and will go on to visit next (carrying pollen) are.

Constant flights (flights between conspecific flowers) are considerably more common than switches to another species (transition flights) for all four bumblebee species irrespective of which flower is considered as the reference species. Interestingly, generally this was even the case for the two least common flower species in the meadow (*Cirsium* **Fig 2d** and *Lythrum* **Fig 2e**), although *B veteranus* was almost equally likely to switch to *Vicia* as remain constant to *Cirsium* (**Fig 2d**). However, one might expect that switches from less to more common species are more frequent than vice versa. To test this possibility, the relative frequency of constant and transition flights were compared for the commonest (*Vicia* **Fig 2a**) and rarest (*Lythrum* **Fig 2e**) reference species by means of a χ^2 goodness-of-fit test (i.e. *Vicia* → *Vicia* : *Vicia* → X versus *Lythrum* → *Lythrum* : *Lythrum* → X), for the two bumblebee species for which most data was available (*B pascuorum* and *B veteranus*). Bees were significantly more likely to switch (rather than remain constant) when they have just visited the rarest (*Lythrum*) rather than the most common (*Vicia*) flower species; *B pascuorum*: $\chi^2 = 79$, *df* = 1, *p* < 0.001 and *B veteranus*: $\chi^2 = 18$, *df* = 1, *p* < 0.001. The same pattern was seen when comparing flights from *Vicia* (the most common: **Fig 2a**) and *Cirsium* (the 2nd rarest: **Fig 2d**) flower species; *B pascuorum*: $\chi^2 = 89$, *df* = 1, *p* < 0.001 and *B veteranus*: $\chi^2 = 36$, *df* = 1, *p* < 0.001. However, this relationship becomes less clear when comparing flights from *Vicia* with those from the intermediately abundant flower species, *Lotus* and *Lathyrus* (2nd and 3rd most abundant respectively: **Fig 2a-c**). In line with the expectation of a higher frequency of flights to the most common species, it is found that the frequency of flights to *Vicia* (originating from either *Lotus* or *Lathyrus*) is mostly higher than the frequency of flights ended on *Cirsium* or *Lythrum* flowers (**Fig 2b, c**). However, flights from *Lathyrus* to the most similar species (*Lotus*), and vice versa, are even more common than those to *Vicia* (**Fig 2c**). Furthermore, the percentage of constant flights originating from *Lathyrus* flowers is only around 50% – the lowest of all plant species in the study. Overall, when considering a single transition between flowers there is a high probability of constancy, irrespective of the reference flower or bumblebee species considered. However, when species switches do occur bees are generally more likely to move to a common, rather than a rare, target flower species.

3.3 Flower handling times do not increase after bees switch species

Do floral handling times increase when bees switch from one species to another? In order to test this, it was essential to establish benchmark handling times for each flower species (*Vicia*, *Lotus*, *Lathyrus*, *Cirsium* and *Lythrum*) when bees exhibited flower constancy, to which the handling times following a switch in the species visited could then be compared. Due to the high degree of variation in the handling skills shown by individual bees when visiting the same flower species (**Fig 3**) it was necessary to relate changes in handling time following a species switch to performance for each individual bee. The number of handling times for all transition flights which were above (or below) the median value for constant flights were counted for each individual bee, and each flower reference species. The sums of handling time differences from the individual median, following a species switch, were then examined to see whether they differed significantly from a random distribution. If handling times did increase after a species switch, it was hypothesized that any effect should be strongest after a prolonged absence from visiting the reference flower species.



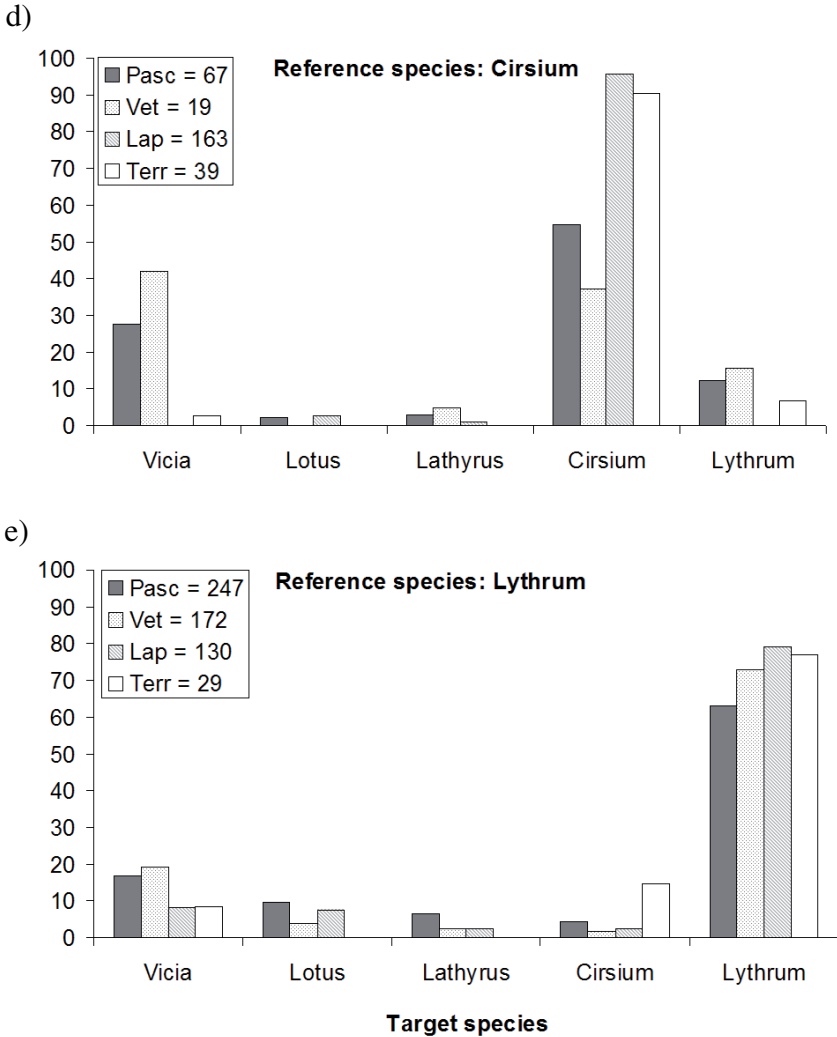


Fig 2: Percentages of constant flights (e.g. *Vicia* → *Vicia*), and all possible combinations of transition flights (e.g. *Vicia* → *Lotus*), made by the four bumblebee species: *Bombus pascuorum* (Scopoli 1763) = Pasc, *Bombus veteranus* (Fabricius 1793) = Vet, *Bombus lapidarius* (Linnaeus 1758) = Lap and *Bombus terrestris* (Linnaeus 1758) = Terr. Flights starting from each reference flower species are plotted separately, in decreasing order of their abundance within the study area: (a) *Vicia cracca* (Linnaeus 1753), (b) *Lotus corniculatus* (Linnaeus 1753), (c) *Lathyrus pratensis* (Linnaeus 1753), (d) *Cirsium oleraceum* ((Linnaeus) Scopoli 1769) and (e) *Lythrum salicaria* (Linnaeus 1753). The target species (ie the species to which bees fly from the reference flower) are given along the x-axis in decreasing order of abundance from left to right. The numbers associated with the bumblebee species abbreviations indicate the total number of flights made by each bumblebee species starting from each of the reference flower species. The frequencies of transitions between the 25 reference → target flower species combinations are significantly different across the four different bumblebee species (χ^2 goodness-of-fit test; $\chi^2 = 1474$, $df = 72$, $p < 0.001$); hence flight percentages are plotted separately for each bumblebee species. NB: because *B terrestris* was only observed to make a total of two flights originating from the reference species *Lotus*, these data are not presented here (**Fig 2b**).

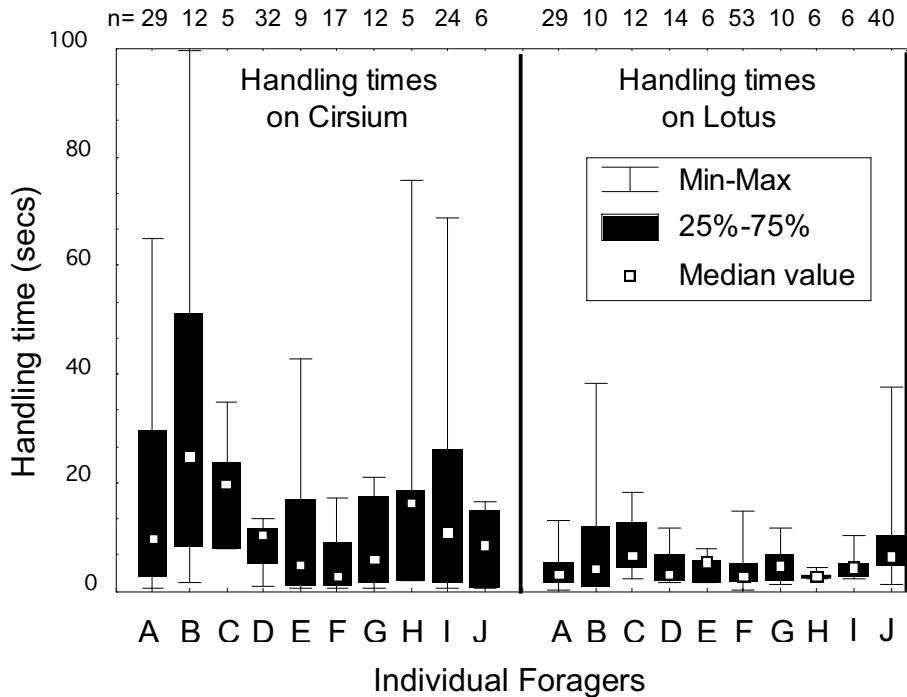


Fig 3: Variation in handling times shown by ten *Bombus lapidarius* (Linnaeus 1758) bees on flowers of both *Cirsium oleraceum* ((Linnaeus) Scopoli 1769) and *Lotus corniculatus* (Linnaeus 1753). Each column denotes the variation in handling time for an individual forager (labelled A-J) on one flower species. The white square indicates the bee's median handling time for the flower species, the upper and lower edges of the box represent the 75% and 25% quartiles respectively, and whiskers the range (maximum and minimum values). The number of flower visits observed per forager is indicated above each column (n). Foragers were selected at random from those observed to visit both flower species at least 5 times. Handling times differ appreciably among individuals handling the same flower species, both in terms of median and range of performance. In general, the handling times on *Cirsium* were considerably longer than those on *Lotus* flowers.

Therefore it was tested whether handling times were more likely to exceed the individual median if the bee had been absent from the reference species for a sequence of (a) 1–4, (b) 5–8 and (c) more than 8 consecutive flower visits using χ^2 squared goodness-of-fit tests.

Flower handling times did not increase following a switch for any reference species. There was also no statistically measurable effect on handling times depending on how long bees had not visited the species in question (**Fig 4:** shaded columns). Even if a bee had not visited the reference species for more than 8 previous choices, the first post-switch handling time was not significantly longer than its median handling time for that flower species. As bees handled the flowers of all three fabaceous species (*Lotus*, *Lathyrus* and *Vicia*) in a very similar way, there may be no increase in handling time if bees switch amongst these species, but rather if they switch from either *Cirsium* or *Lythrum* to any of these three species. To exclude this possibility, the median handling time for each bee was recalculated, classifying the three fabaceous species as a single 'functional' species – hence bees flying between *Lotus*, *Lathyrus* and *Vicia* flowers were considered 'flower constant' for this analysis.

However, bees handled all of these three fabaceous flower species equally fast irrespective of whether they arrived from another fabaceous flower, or switched from either *Cirsium* or *Lythrum* (Fig 4: open columns). These results support the view that bumblebees switching among the five species tested here did not incur handling time costs.

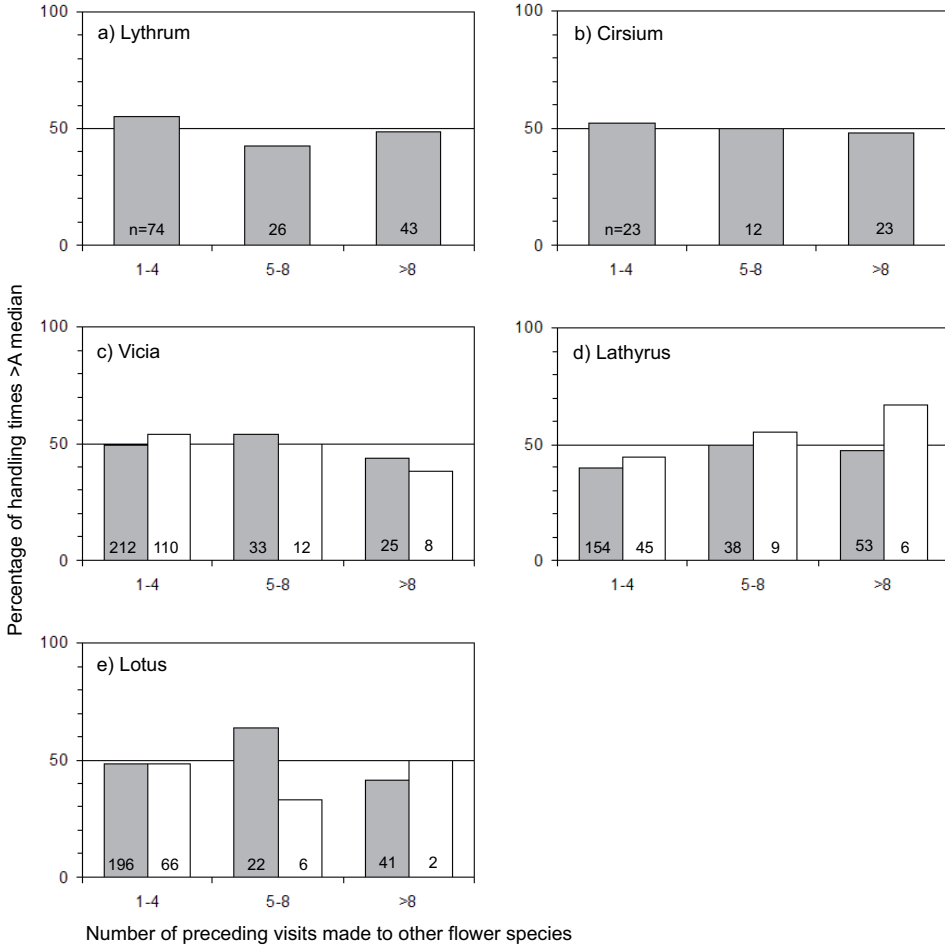
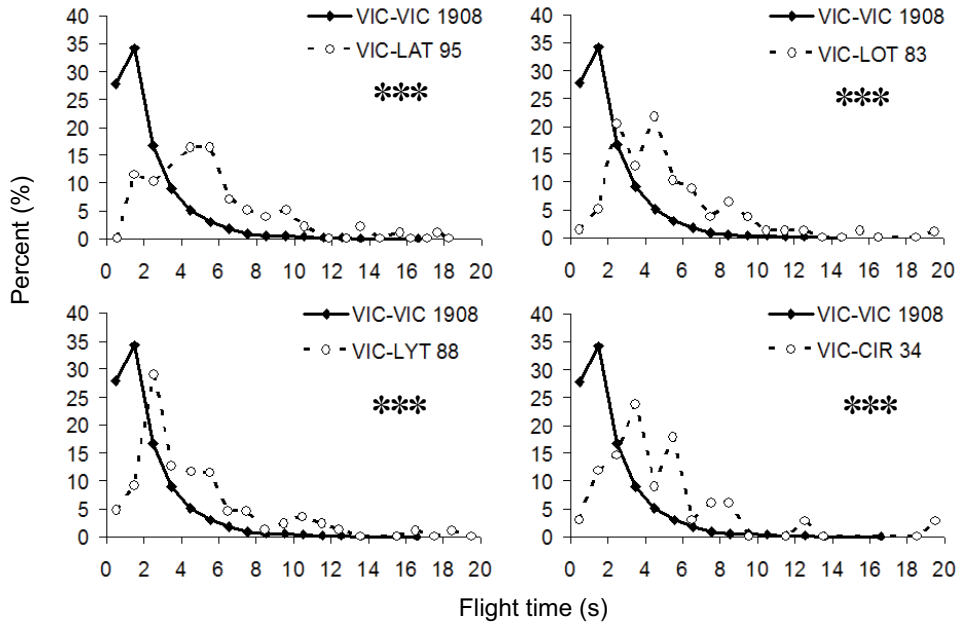
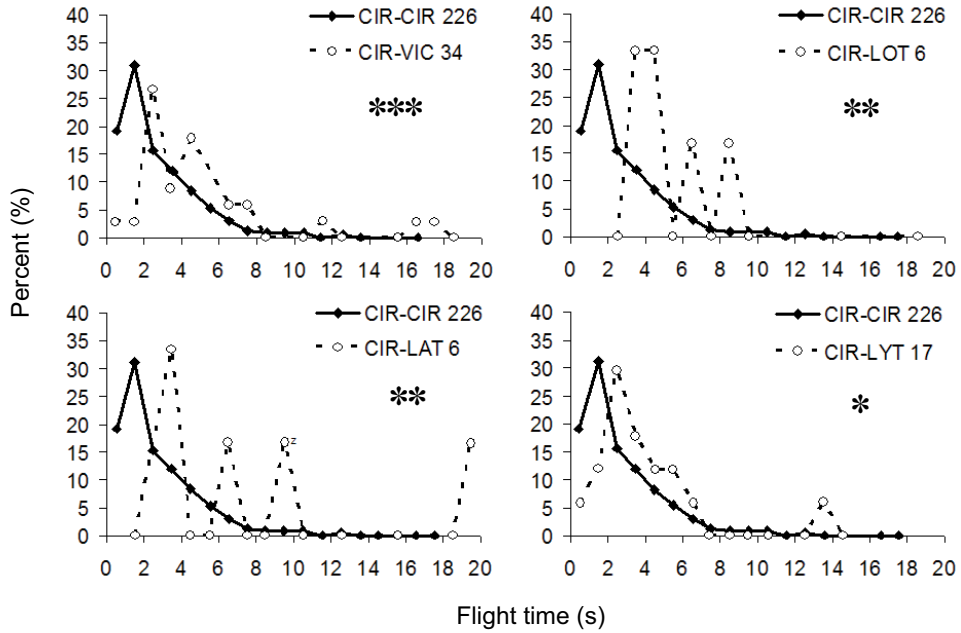


Fig 4: Do handling times depend on the number of previous visits made to other flower species? Columns indicate the percentage of flower handling times (for each target species) following a species switch, that exceed the median handling time for the same individual bee when exhibiting flower constancy on the same flower species (the target species are indicated in the top left corner of each graph). The x-axis categories indicate the number of previous visits made by the bee to flowers other than the target species before switching (i.e 1–4, 5–8, or >8 visits). The number of handling times included within each of these categories (n) is given in each column. None of these column categories differed significantly from chance (50%: indicated by horizontal lines) as tested using χ^2 squared goodness of fit tests. Shaded columns include data from bees switching to the target from any of the four reference flower species. Open (white) columns are presented for the three Fabaceous flower species (c: *Vicia*, d: *Lathyrus* and e: *Lotus*), and take into account only switches to each of these three target species from either (a) *Lythrum* or (b) *Cirsium*.

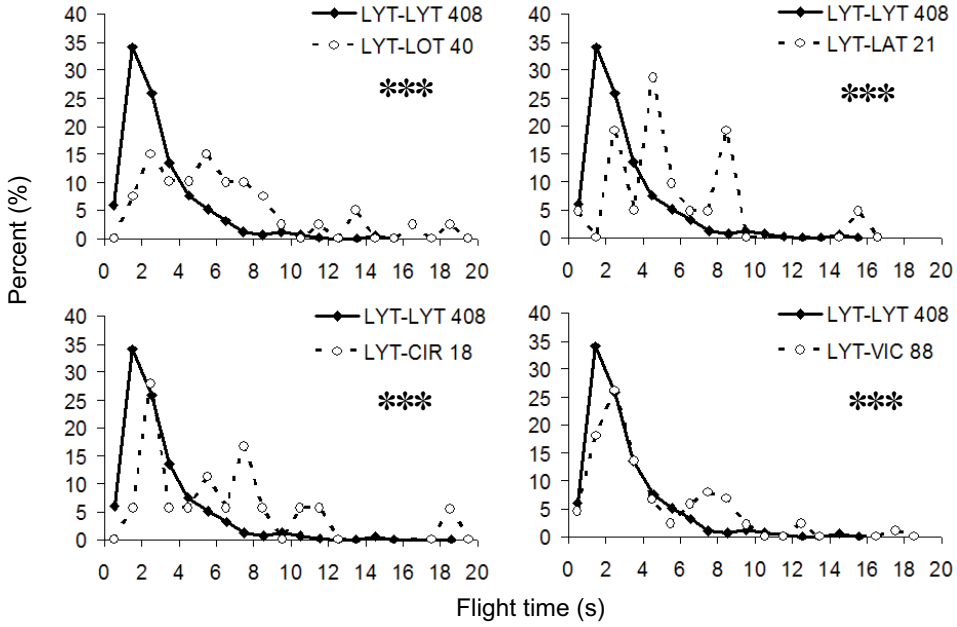
a) reference species: *Vicia*



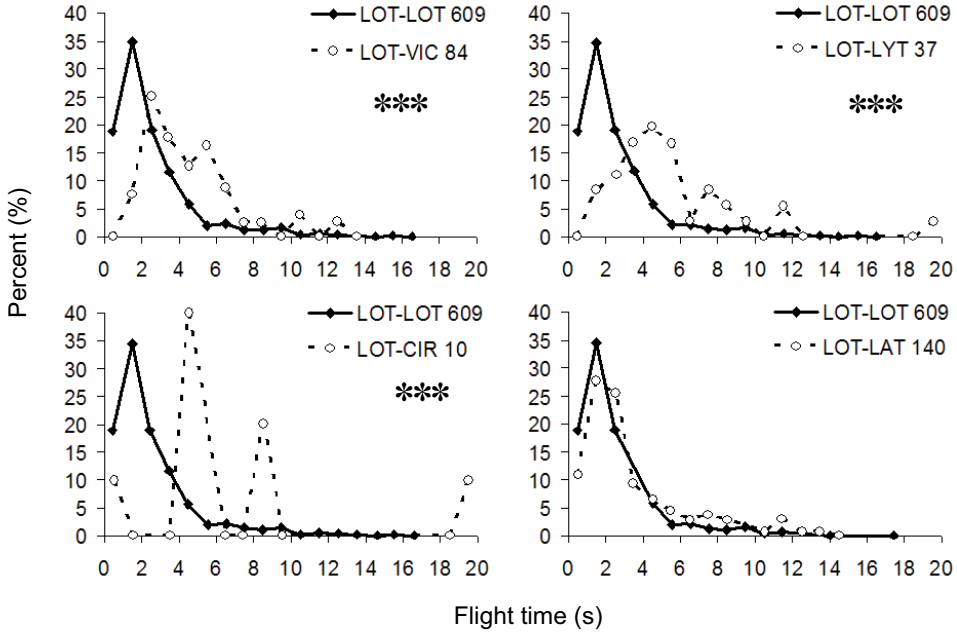
b) reference species: *Cirsium*



c) reference species: *Lythrum*



d) reference species: *Lotus*



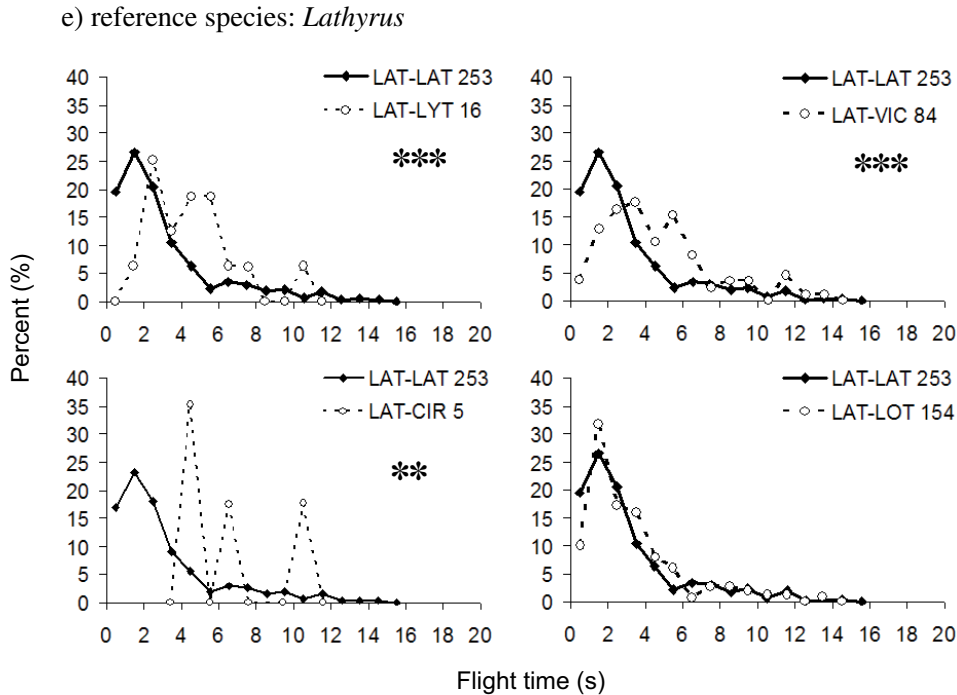


Fig 5: The frequency distributions of flower constant flights (continuous lines) and transition flights (dashed lines) for each reference species, given as percentages calculated for each 1 second interval. The numbers of flights evaluated for each species pair are given in the key for each panel of the figure. Asterisks indicate the significance level of differences between constant and transition flights ($* < 0.05$, $** < 0.01$, $*** < 0.001$). The continuous line is the same for each of the four graphs pertaining to the same reference species, because it shows the same distribution of flower constant flights. In all but two of the graphs, flight times lasting only a few seconds are markedly dominated by constant flights, whereas the frequency of transition (switch) flights peak at longer time intervals. The two exceptions are found in the combinations of species whose signals are similar, *Lotus* and *Lathyrus* (**Fig 5d, e**; right hand panels). In contrast to all other flower species combinations, these distributions of constant and transition flights are almost perfectly matched to each other.

3.4 Transitions are longer than constant flights, independent of flower spatial distribution

In order to examine the distribution of flight times between flowers species, it was first ascertained whether times differed across individuals (within each bee species), and/or bee species, to establish whether data from different observations could be pooled. The distribution of flight times for individual bees were compared within each bee species by means of the Kruskal-Wallis-H test for each of the possible transitions combinations ($n = 25$) between the 5 flower species. The results of each of these individual tests were combined using Fisher's test for combining probabilities ($p = 0.325$), allowing us to conclude flight time distributions of individuals in each bumblebee species can be legitimately pooled.

The flight time distributions for all possible transitions combinations ($n = 25$) between flower species were compared across the four bee species.

Each bee species was tested against the other three using Mann-Whitney U tests (6 pair-wise comparisons in total). 51 of the possible 150 (6 x 25) combinations could not be tested because bees of one (or both) species being compared were never observed to fly between the two flower species in question in the required direction. Fisher's combined probability value for the 99 tests performed was non-significant ($p = 0.426$), allowing us to pool the flight time distributions across the four bee species.

Constant flights (*Vicia* → *Vicia*) were significantly shorter in duration than switch flights originating from *Vicia* flowers (**Fig 5a**). As *Vicia* was the most common species in the study meadow, average distances between *Vicia* flowers were much shorter than distances to other flower species [CHITTKA et al 1997]. Thus, the shorter flight times of constant (*Vicia* → *Vicia*) compared to switch (*Vicia* → X) flights originating from *Vicia* could be explained by the spatial distribution of flower species. In contrast, this is not true for the rarer reference species, *Cirsium* and *Lythrum*. In both these species, distances to all other species were not significantly longer than intraspecific distances [CHITTKA et al 1997]; thus if flight times are governed by the spatial distribution of flowers we would expect the average duration of constant and transition flights originating from these species to be equal. However, as with *Vicia*, all switch flights originating on either *Cirsium* or *Lythrum* were significantly longer than constant flights (**Fig 5b, c**). Given that there were no differences in intraspecific spacing between these two reference flower species, these observed flight time distributions could be explained by the dynamics of bee decision making processes. This observation is confirmed by comparing the flight distributions originating from *Lotus* and *Lathyrus* (as reference species): in all but two cases (flights between the almost indistinguishable flowers of *Lotus* and *Lathyrus*), transition flights were significantly longer than constant flights. This would not be expected based on the spatial distribution of these two flower species, as there should be no difference in flight times from a less to a more common flower species, such as *Lotus* → *Vicia* (**Fig 5d**) or *Lathyrus* → *Vicia* (**Fig 5e**). Any potential for an overall correlation between inter-flower distances and flight times for all flower species pairs was tested, and found to be highly non-significant ($r_s = 0.289$; $n = 25$; $p = 0.159$: [CHITTKA et al 1997]).

3.5 Probability of switching increases with flight duration

Does the chance of switching change with the time bees spend flying between flower visits? In order to address this question, the durations of all bee flights were rounded to the nearest second. The proportion of constant and transition flights were then calculated for each 1 second category, for each reference flower species. Flights made by all four bee species were again pooled for this analysis. A consistent pattern was observed across all five reference species showing that the longer the flight from the reference flower lasts, the greater the chance of switching becomes (**Fig 6**). The switch probability increased most sharply over the first few seconds (1–3 seconds), and the rate of increase then began to level off between 5–8 seconds after leaving the reference flower. This is compatible with the hypothesis that the floral signal of the reference flower fades relatively rapidly from working memory once the bee leaves the reference flower. The dynamics of the observed increase in switch probability agree closely with those found by ZHANG et al [2005] in their pattern matching trials. Their results showed an exponential decay in choice accuracy with increasing flight time after observing the informative stimulus (sample pattern).

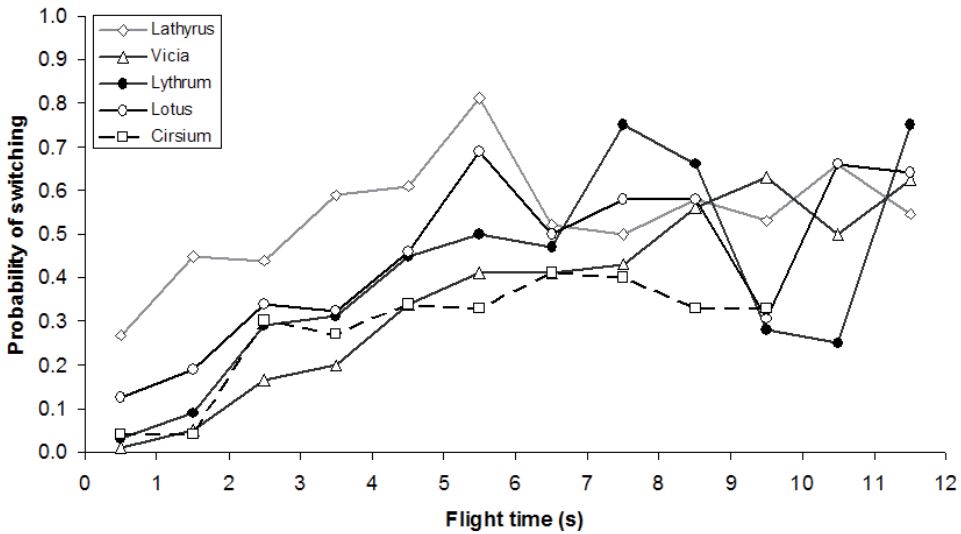


Fig 6: The probability of switching flower species as a function of the flight time duration between flower visits for each reference species (see key to plotting symbols). The longer a bee spends flying after leaving the reference flower the greater the chance of switching becomes. Over the first few seconds after take off from the reference species the probability of switching rises sharply, after which it levels off between 5–8 seconds. The durations of all observed flights between flowers were rounded to the nearest second. The proportion of constant and transition flights were then calculated for each 1 second category, for each reference species. Data from all four bumblebee species were pooled for this analysis.

3.6 Probability of switching decreases after sequences of constant flights

Is a bee's decision to switch affected by its recent flower choices? To answer this question the frequency of constant and transition flights were compared for sequences of flower choices made by *B. pascuorum* foragers for each reference flower species. This was expressed as a switch probability (defined as the number of switches/total number of flights observed) after each sequential flower visit (from 1–6 visits) to the reference species. A general trend was observed across all five reference species, showing that the switch probability decreased steadily as bees made an increasing number of visits to the reference species (Fig 7). This general trend, a decrease in switch probability associated with an increase in the number of flower constant choices in a visitation sequence, is consistent with the hypothesis that periods of flower constancy consolidate the reference flower signal in working memory. Interestingly, the switch probabilities for bees making sequences of visits to each of the two species whose flowers are almost indistinguishable, *Lotus* and *Lathyrus*, were almost identical. This suggests that the very similar appearance of these flower species are held, and reinforced, in working memory in a similar way.

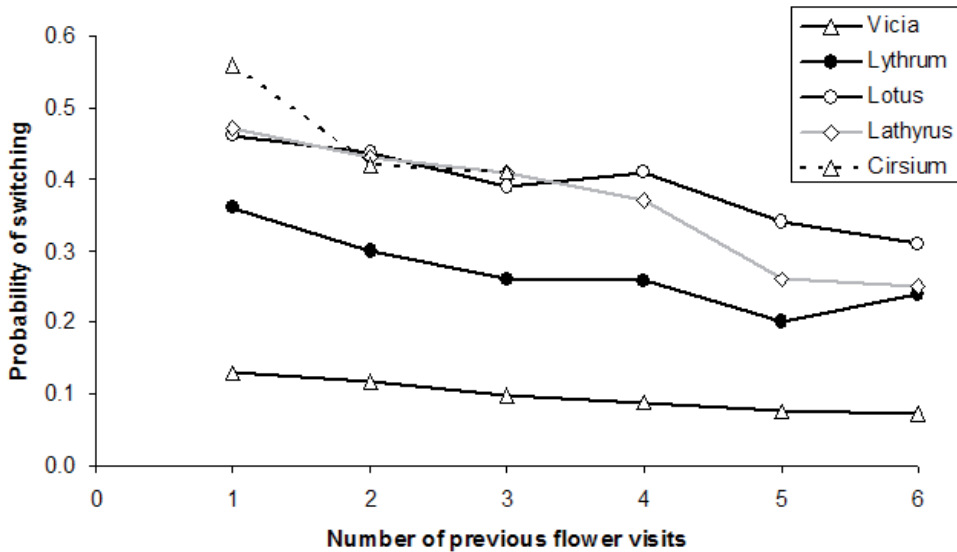


Fig 7: The probability of switching flower species as a function of the number of previous flower visits made to the reference species. The switch probability drops steadily as the number of visits to the reference species increases. Data presented show relative frequency of constant and transition flights (expressed as a probability of switching) made by *Bombus pascuorum* (Scopoli 1763) after successive (from 1–6) visits to the reference flower species.

4 Discussion

The flower choices of bees, and in particular flower constancy behaviour, remain a source of fascination reflected by the wealth of research in this area [e.g. BENNETT 1884, GRANT 1950, CHITTKA et al 1997, CHITTKA, INGS & RAINE 2004, RAINE & CHITTKA 2005, RAINE et al 2006a]. The focus of this paper is the role played by memory dynamics in the decision making processes of foraging bees. Here the significance of our results is discussed in this context, drawing on both the original paper by CHITTKA et al [1997] and this reanalysis of those data.

Is floral constancy related to a limited capacity of bee memory to store the necessary motor patterns involved in efficiently extracting rewards from different flower species? Results from this study show that median flower handling times did not increase as a result of a species switch, even under conditions in which the switch was preceded by up to eight visits to different flower species. The consistency of this pattern across all five plant species is interesting because bees foraging on these flowers must learn and remember at least three different sets of handling skills (assuming that the three species, *Vicia*, *Lotus* and *Lathyrus* (Fabaceae), with very similar flower morphology are handled by bees using similar motor patterns). Thus the four bumblebee species in our study are clearly able to store and recall the appropriate motor skills required to handle multiple flower species on demand without a loss of handling efficiency. These results agree with LAVERTY's [1994] study on flowers with relatively simple morphological complexity, like the flower species in our study. However, there may still be costs to switching associated with delays in retrieving the sensory cues and/or motor skills required to handle flower species with more complex, or unusual, morphology. Indeed, bees switching between plant species with very different floral morphologies can show significantly increased flower handling times [WOODWARD & LAVERTY 1992, CHITTKA & THOMSON 1997].

In honeybee sensory learning, massed trials yield different acquisition (learning) curves than spaced trials [McCLELLAN & BITTERMAN 1988, MENZEL 1990]. If the same applies to motor learning, this may well also influence the degree of constancy exhibited by foragers with different levels of experience [LAVERY 1980].

Whilst there appear to be no costs to switching species in our study with respect to flower handling times, there do seem to be costs incurred when flying between flowers of different species. On average flights between different flower species take longer than those between flowers of the same species. Constant flights most commonly last 1–2 seconds, whereas transition flights of all kinds most frequently last 3–6 seconds (**Fig 5**). GREGGERS & MENZEL [1993] recorded honeybee choice behaviour in an array of four feeders spaced 1m apart. The flight time dynamics between their feeders are strikingly similar to those (between flowers) in our study, even though in their investigation the conditioned stimulus is a location, whereas in this case it is a compound (floral) signal whose spatial co-ordinates are much less clearly defined. GREGGERS & MENZEL [1993] found that the decision to return to the same (reference) feeder was made earlier during an inter-feeder flight (predominantly within 3 seconds) than decisions to fly to a different feeder (for which flights most commonly lasted 3–5 seconds). In common with our results, it is not possible to explain these choice dynamics in terms of the spatial arrangement of flowers. Thus, the intrinsic dynamics of the bees' choice process must determine the probability of constancy and switching. These conclusions are also strongly supported by recent work on honeybee working memory dynamics in delayed matching to sample trials [ZHANG et al 2005]. In these experiments, honeybees were presented with a sample pattern as they flew through a tunnel at the end of which they had to choose one of two chambers to receive a reward. Honeybees would choose the correct chamber, and receive the reward, if they matched the sample seen whilst flying through the tunnel with one of the test patterns presented at the subsequent decision point. By manipulating the position of the sample pattern presented in the tunnel the experimenters controlled the length of time for which the bee had to remember the pattern to be able to solve the task. Bee choice accuracy was highest during the first 2–3 seconds after encountering the sample pattern, and recall of this pattern had decayed from working memory to levels indistinguishable from chance between 6.5–8.9 seconds [ZHANG et al 2005]. The similarity between the memory dynamics found by ZHANG et al [2005] under highly controlled laboratory conditions and those from this field study provide strong support for the idea that memory dynamics govern the foraging behaviour of bees under natural conditions.

The duration of flights between flowers appears to be predictably linked to the probability of switching species: the longer a flight lasts, the greater the chance of switching becomes. The rate of increase in the probability of switching was highest during the first few (1–3) seconds after leaving the reference flower, then began to drop between 5–8 seconds, before the probability of switching reached a fairly constant level after around 8 seconds (**Fig 6**). This pattern was consistent across all five reference flower species in our study, and is compatible with the hypothesis that the signal of the reference flower fades relatively rapidly from working memory after the bee departs. The dynamics of the observed increase in switch probability are very similar to those found by ZHANG et al [2005] in their pattern matching trials. A similarly consistent general trend was found across all five reference flower species when investigating whether a bee's decision to switch is affected by its recent flower choices. The probability of switching decreased steadily with an increase in the number of consecutive flower constant choices (from 1–6) made by the bee. These observations are consistent with the hypothesis that periods of flower constancy consolidate the signal of the reference flower (species) in working memory.

It was also particularly interesting that the switch probabilities for bees visiting sequences of *Lotus* or *Lathyrus* flowers were almost identical. These two flower species are very hard for humans to tell apart, and are also predicted to be virtually indiscriminable for bees [CHITTKA et al 1997]. These results suggest that the similarity in appearance of these two flower species leads to them being processed and reinforced in a very similar way by bee memory.

How then, should the influence of these intrinsic memory dynamics in determining the probability of constancy versus switching be interpreted? One possible interpretation is that the most recently visited flower species (stimulus) is passively held in a transient form of short-term, or working, memory for around 1-2 seconds [HONIG 1978, MENZEL et al 1993]. While the bee is in flight, incoming stimuli (images of flowers) then interact with the contents of this working memory [GREGGERS & MENZEL 1993, MILLER & DESIMONE 1994]. Should the sensory system indicate the encounter of a floral signal that matches the one held in working memory, the probability might be greater that the bee will fly toward this (flower species) signal. After this transient form of short-term memory subsides (after more than 3 seconds), the probability of switching species increases, because the decay of working memory may lead to a change in the relative weighting between working and reference memory. As a result of this change in weighting any floral stimuli addressing the reference memory may be more effective than during the first few seconds following the last flower visit. Since the reference memory holds a record of reward probability over a long period of time [HONIG 1978], the effect of an encountered floral signal may then depend on the reward expectancy for that particular flower species [GREGGERS & MENZEL 1993, CUTHILL, HACCOU & KACELNIK 1994].

An alternative explanation is that bees ‘deliberately’ decide to switch from one species to another, for example in response to a sequence of low rewards encountered in flowers of a particular species [DUKAS & REAL 1993, GREGGERS & MENZEL 1993], and that the retrieval of the memory for another signal (flower species) takes longer than that for the species most recently visited. Yet another possibility is that bees combine the switch from one species to another with a longer flight, to escape local areas of reward depleted flowers [PYKE 1978, SCHMID-HEMPEL 1984, DUKAS & REAL 1993, KEASAR, SHMIDA & MOTRO 1996]. However, no differences in the dynamics of transition flights were found depending on whether these occurred after short or long previous handling times [CHITTKA et al 1997], indicating that ‘deliberate’ and ‘passive’ switch flights follow similar temporal dynamics.

The results of this study show that the degree of constancy may indeed be determined by memory limitations as has been previously suggested [DARWIN 1876, LEWIS 1986, WASER 1986, LAVERTY 1994b]. However, the limitations are neither a lack of capacity to store the signals of multiple flower species, nor their handling procedures, in long-term memory. Rather, the explanation may be the relative weighting of working and reference memory at different intervals following a flower visit, and the resulting low probability of retrieving the memory for a different flower species (than the species just visited) within a very short time interval of this most recent flower visit.

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