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THE FORAGING BEHAVIOUR OF HUMMINGBIRDS THROUGH SPACE AND TIME

María Cristina Tello Ramos

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews

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The foraging behaviour of hummingbirds through space and time

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University of
St Andrews

This thesis is submitted in partial fulfilment for the degree of PhD

at the

University of St Andrews

7th of August 2015

Abstract

Central place foragers, such as territorial hummingbirds, feed from resources that tend to be constant in space and to replenish with time (e.g. nectar in flowers). The ability to remember both where and when resources are available would allow these animals to forage efficiently. Animals that feed at multiple locations would also benefit from forming routes between these multiple locations. Hummingbirds are thought to forage by repeating the order in which they visit several locations following a route called a "trapline", although there are no quantitative data describing this behaviour. As a first step to determining how and if wild free living hummingbirds forage by traplining, I decomposed this behaviour into some of its key components. Through five field experiments, where I trained free-living hummingbirds to feed from artificial flowers, I confirmed that territorial hummingbirds will, in fact, trapline. Birds will use the shortest routes to visit several locations and will prioritize those locations that are closest to a usual feeding site. Additionally, even though hummingbirds can learn to use temporal information when visiting several patches of flowers, the spatial location of those patches has a larger influence in how these birds forage in the wild. Since male and female hummingbirds were thought to forage differently I also tested whether there were sex differences in the types of cues they use when foraging. Contrary to expectation, female hummingbirds will also use spatial cues to relocate a rewarded site. Using the foraging ecology of rufous hummingbirds to formulate predictions as to what information these birds should use has lead me to discover that these birds forage in a completely different way than previously thought.

1. Candidate's declarations:

I, María Cristina Tello Ramos, hereby certify that this thesis, which is approximately 38,000 words in length, has been written by me, and that it is the record of work carried out by me, or principally by myself in collaboration with others as acknowledged, and that it has not been submitted in any previous application for a higher degree.

Declaration of collaboration

I collected all data with the exception of the data reported in Chapter Four. These data were collected by an undergraduate student, Catlin Higgott, under the supervision of me and Dr. Susan Healy.

I was admitted as a research student in September, 2011 and as a candidate for the degree of PhD in May 2012; the higher study for which this is a record was carried out in the University of St Andrews between 2011 and 2015.

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I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of PhD in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.
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Ethical note

All experimental procedures in this thesis were performed with ethical permission from the Animal Welfare Committee of the School of Biology at the University of St. Andrews. The University of Lethbridge Animal Welfare Committee and permits from the Alberta Sustainable Resource Development and Environment Canada were also obtained in order to conduct all research. In the case of the experiments carried out in Mexico, In addition to the ethical approval of the University of St Andrews, I also had permission from the Scientific Station “La Malinche” from the Universidad Autónoma de Tlaxcala, México.

Declaration of publications

The work described in Chapter 3 forms the basis of “Tello-Ramos, Maria C., Hurly, T Andrew & Healy, Susan D. 2015. Traplining in hummingbirds: flying short-distance sequences among several locations. Behavioral Ecology. 26: 812-819”.

The work described in Chapter 4 forms the basis for the paper “Tello-Ramos Maria C., Hurly, T Andrew, Higgott, Caitlin & Healy, Susan D. 2015. Time-place learning in wild, free-living hummingbirds. Animal Behaviour. 104:123-129”.

The work presented in Chapter 6 forms part of “Tello-Ramos, Maria. C., Hurly, T. Andrew & Healy, Susan D. 2014. Female hummingbirds do not relocate rewards using colour cues. Animal Behaviour.93: 129-133”.

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Finally, thanks to the hummingbirds.

The foraging behaviour of hummingbirds through space and time

Abstract

Declarations

Acknowledgements

Mientras,
esperaré a que la locura
llegue cayéndose de sus dos alas,
cuando eso suceda
estaré preparado para emprender
el único vuelo que merece la pena:
El imposible.

Javier Bustamante

There is surely no greater wisdom, than well to time
the beginnings, and onsets, of things.

Francis Bacon

Chapter 1: Introduction

Foraging, the act of searching for food, is an essential behaviour in an animal’s life. Animals need to obtain energy to survive and to reproduce, but foraging behaviour in itself can sometimes consume more energy than that gained. Because optimal foraging theory is based on the assumption that the foraging behaviour of animals is a product of evolution and that the associated fitness has been maximized, animals are expected to forage in a way that allows them to maximise the energy gained (MacArthur and Pianka, 1966; Emlen, 1966; Pyke, Pullin, and Charnov 1977). Take a hummingbird as an example: when a hummingbird finds a new flower patch it obtains nectar quickly because most of the flowers will be full of nectar, but as the bird spends more time foraging in a patch, the nectar will become more scarce. This is true for most resources for foragers regardless of whether the resource is nectar, fruit or a school of fish. Fresh patches will provide food quickly, but the rate of intake declines as the forager depletes the patch (Pyke, 1984). This observation then suggests a trade-off between staying in a patch and searching for a new patch so the question the hummingbird faces is when to abandon the current patch in order to search for a new one.

Simple models predict that a forager should stay in a patch as long as the energy gain in that patch is at least as great as it would gain in another patch, taking into account the energy spent travelling to a second patch (also known as marginal value theorem; Charnov 1976). One of the many assumptions of this optimal foraging model is that the forager can evaluate and rank the profitability (energy gain/ energy used to get the resource) of different types of resources or places. The model also assumes that the forager will remember that information (Krebs and Davis, 1981).

Since optimal foraging theory is used to address why in survival terms an animal behaves in a specific way, research on how energetic demands can make an animal move from one patch to another is abundant (Sih and Christensen 2001). This theory does not, however, deal with the question of how animals use the information gained during foraging to achieve such optimal behaviour.

At the same time the composition of the foraging environment is always changing. For example, the amount and distribution of a resource, the competition for a resource or the time it takes for the resource to replenish may all change as the resource is being depleted. With so many factors that could potentially affect a foraging decision, experience and the information about the status of a resource should be of considerable value to animals. Therefore, the environment in which an animal forages may have an affect on what information an animal should acquire, remember and use.

Determining what information is being used to produce a behaviour is particularly difficult when investigating the natural foraging behaviour of animals in the wild. Knowing the ecology of animals, however, can help us to make testable predictions as to what type of information might be particularly useful for an animal. The success of this kind of approach has been shown by studies on the foraging behaviour of food-storing birds. For instance, food-storing birds have “better” spatial memory and a larger hippocampus (the area of the vertebrate brain that is correlated with spatial tasks) than do closely related non-storing birds (McGregor and Healy 1999; Biegler et al. 2001), because the former rely more heavily in spatial memory to find the food they have hidden.

The cognitive ecology of hummingbirds

Territorial hummingbirds make a useful system for investigating information use by animals foraging in the wild. There are at least two biological advantages of working on the foraging behaviour of hummingbirds. Firstly, there is a lot known about their foraging ecology. Territorial hummingbirds, as do other central place foragers (Lemke 1984; Di Fiore and Suarez 2007; Ohashi and Thomson 2009; Noser and Byrne 2010), feed from resources that are constant in space, albeit irregularly distributed, resources that also will replenish with time. This suggests that hummingbirds should remember the location, morphology or colour of the flowers that provide a reward and return to them. Secondly, as these birds feed multiple times a day it is also possible that they use temporal information to organise their revisits to the refilling rate of flowers. Hummingbirds will therefore benefit from using different types of information available (e.g. visual, spatial and temporal) in order to avoid visiting or defending flowers that have been depleted or found emptied in the first place (Healy and Hurly 2003).

Hummingbirds also make a logistically useful model to examine the cognitive abilities used by animals when foraging in the wild. Due to their high metabolism and costly hovering flight (Kodric-Brown and Brown 1978; Altshuler 2004), hummingbirds need to feed every 10 to 15 minutes (Carpenter et al. 1983) visiting up to a hundred flowers during a single day (Armstrong, Gass, and Sutherland 1987). Since these birds can be trained to feed from an experimental set-up within one to two hours (Healy and Hurly 2003) and because they need to feed at regular intervals, birds can be tested on multiple occasions in a relatively short period of time. Furthermore, male rufous hummingbirds (*Selasphorus rufus*) can be individually marked, males are highly territorial and exclude other birds from the training site and testing apparatus, they are easy to observe because males include open meadows in their territory and experimenters can observe them at very close quarters in the wild (Healy and Hurly 2003).

Cue use in the foraging behaviour of hummingbirds

When hummingbirds' foraging behaviour was first observed it was assumed that these birds had an innate preference to feed from red flowers (Sherman 1913). As early as 1941, however, it was reported that these birds would readily learn the colour of a rewarded artificial feeder irrespective of the colour or appearance of the feeder (Bené 1941). Furthermore, once the hummingbirds had visited the feeder three times the birds would fly directly to the feeder even when its appearance had changed dramatically. This suggests that the hummingbirds did not use the visual features of the feeder when returning to feed but rather its location. Early tests of preference for colour and position cues showed that a captive Mexican violet-eared hummingbird (*Colibri thalassinus*) attended to and learned the location of a reward bottle regardless of its colour (Lyerly, Riess, and Ross 1950). Therefore, the observed preference that hummingbirds have for red flowers during migration might be due to the hummingbirds generalising across red flowers because flowers of that colour tend to offer a reward in high volumes (Grant 1966). Colour cues, therefore are not the only cue hummingbirds use to find a reward.

In addition to colour cues, other types of information could also be used by hummingbirds when foraging. For example, hummingbirds do pay attention to visual features of flowers other than colour. In the laboratory when broad-tailed hummingbirds *Selasphorus platycercus* and rufous hummingbirds were presented with plants that varied in morphology but not in colour, birds preferred to visits often flowers with a wider corolla (*Ipomosis aggregata* rather than *Ipomosis tenuituba*) (Meléndez-Ackerman 1997).

Under different foraging contexts, different types of information may be more reliable than others and therefore the types of cues used might be context-dependent. As territorial hummingbirds become increasingly familiar with the floral resources within their territory, colour or morphological cues may become of less value relative to the location in which the flowers are found. Thus, to avoid returning to the flowers they have recently emptied, then, territorial hummingbirds may preferentially remember spatial information (the location of a flower) over flower colour (Hurly and Healy 1996).

This proposition that some information may be more relevant to rufous hummingbirds when returning to feed from a rewarded artificial flower, was tested using a cue preference test. First, birds were allowed to feed from the one rewarded flower in a four flowers array where all the flowers were of different colours and were arranged in an 80 cm square. The rewarded flower contained enough sucrose solution so that it could not be depleted in one visit and the hummingbird was expected to return to it to feed. After the bird had found and fed from the single rewarded flower the experimenter switched the position of the rewarded flower with one of the other unrewarded flowers so that on its return the hummingbird had a choice between visiting the flower at the correct location or the flower with the correct colour. The birds overwhelmingly chose the flower in the ‘correct’ location in the array (Hurly and Healy 1996).

Further studies confirmed that although rufous hummingbirds appear to weight spatial information very heavily, they will learn to use different types of cues depending on the context. For instance, in some instances the colour of a flower will facilitate the learning of a rewarded flower’s location. This was shown when birds learned with fewer errors to visit a single rewarded flower amongst other nine flowers when all the flowers bore a different colour pattern than when the flowers were all of the same colour pattern (Hurly and Healy 2002). Furthermore, hummingbirds will learn to different spatial cues depending on the distance between rewarded flowers. When flowers in an array were spaced more than 40 cm apart, hummingbirds used the absolute location of the flowers relative to landmarks outside of the array to relocate the rewarded flowers, but if the flowers were spaced closer together then hummingbirds used the relative position of flowers within the array (Healy and Hurly 1998, see also Henderson et al. 2001).

Rufous hummingbirds can remember the location of a rewarded flower so well that they will fly to, and hover at, the location where they had found a rewarded flower previously and will do so even in the absence of a flower (Hurly, Franz and Healy 2010). Only after hovering over an empty space will the territorial rufous hummingbirds then search for the flower at the new location. Territorial male hummingbirds will eventually stop using the spatial cues to return to a rewarded flower, however when a flower has been moved after each of three visits the hummingbirds use the flower as a beacon to directly fly to it in its new location (Flores-Abreu et al. 2012).

There are, then, quite a lot of data to show that territorial rufous hummingbirds use multiple sources of information to learn the locations of profitable flowers in their territories. It is also possible that they represent this information in a systematic fashion, feeding, for example, first from the flowers at the edges of their territory before feeding at flowers nearer to the centre of their territory (Paton and Carpenter 1984). Since the edges of a territory are the most susceptible to intruding competitors, a hummingbird might benefit from emptying the nectar from the edge flowers first, leaving the centre flowers to be defended later in the day. This behaviour will allow these birds both to avoid revisiting depleted flowers and to help them to defend their territory. Indeed, rufous hummingbirds during migration were observed feeding from the edges of their territory during the first bouts of the morning (Gass and Montgomerie 1981). This foraging behaviour, where animals follow repeatable routes around several locations, is called traplining (Janzen 1971) and is thought to be common amongst central-place foragers. Territorial hummingbirds, however, are not typically regarded as trapliners because their foraging behaviour has been categorised as either territorial or traplining (Feinsinger and Colwell 1978).

Taxonomic distribution of traplining

The term traplining was first used to refer to the strategy used by humans when laying fur traps along an area and then returning to check upon them (Thomson, Slatkin, and Thomson 1997). Instead of randomly checking the traps, the efficient fur trapper would allow sufficient time for a prey to be caught in most of the traps by revisiting his traps in the same order. Traplining was subsequently used to describe the foraging patterns of female euglossine bees (Janzen, 1971) and has since been described for a range of species. Many central place foragers, like pollinating insects, birds and mammals or other frugivorous animals are classified as traplining foragers (e.g., bumblebees *Bombus terrestris* and *B. impatiens*: Lihoreau et al. 2013; Ohashi and Thomson 2013, respectively; bats *Glossophaga soricina*: Lemke 1984, pied wagtails *Motacilla alba*: Davies and Houston 1981; capuchin monkeys *Cebus paella*: Janson 1998; baboons *Papio ursinus:* Noser and Byrne 2010; Long Evans rats *Rattus norvegicus*: Reid and Reid 2005), including, several species of hummingbirds (Garrison and Gass 1999; Gill 1988; Temeles et al. 2006; Tiebout III 1991). There is, however, rather little empirical and quantitative evidence for traplining in most of these species. For example, in the case of the traplining hummingbirds, instead of following all the locations and the order of flower visitation of a hummingbird, the periodic return times of birds to focal flowers was taken as sufficient evidence that these birds repeat the order of flower visitation (Gill 1988).

The lack of suitable data is probably due to the difficulty of following animal movements through their natural environment. Since testing whether animals do trapline and how they trapline requires tracking the movements of known individuals as they forage, most of the detailed data come from observations of the Hymenoptera, which are easier to track (Reynolds, Lihoreau and Chittka 2013; Lihoreau et al. 2012; Ohashi and Thomson 2009; but see Heithaus and Fleming 1978 for data from the *Carollia perspicillata* fruit bat).

The detailed study of hymenopteran traplining has led to the identification of multiple advantages to this foraging strategy. For example, by repeating the order of resource visitation, animals can avoid revisiting recently depleted resources. When bumblebees *Bombus impatiens* were given access to a stable array where flowers never change location (rather than one that was changeable) these insects avoided revisiting recently depleted locations more often than did the bees foraging on a changeable array. The stable-array bees also developed stable short routes around the array after as few as 20 bouts (Saleh and Chittka 2007). Traplining animals may also learn to visit multiple locations using shorter routes: with experience of five locations bumblebees, *Bombus terrestris,* develop a shorter trapline than the suboptimal trapline that followed the order in which flowers were first encountered (Lihoreau, Chittka, and Raine 2010). These bees do not use nearest-neighbour movements to “find” the shortest route around several locations (Lihoreau et al. 2012) but are thought to compare the duration or length of previous routes to new, slightly different versions, of a trapline and keep the shorter of the two (Lihoreau et al. 2012). When the flight paths of bumblebees fitted with harmonic radar were compared to simulated paths that were based on an algorithm that found the shortest routes by comparing two routes and keeping the shorter of the two, the simulated paths and the paths flown by the bees did not differ. Traplining behaviour can also result in the prioritization of highly rewarded locations. For example, individual bumblebees trained to feed from five artificial flowers that offered equal rewards first used the shortest possible routes to visit all artificial flowers but when one of the flowers offered six times as much reward as the other four, then the bees re-adjusted their routes to visit the highly rewarded flower first (Lihoreau, Chittka and Raine 2011).. They did this, however, only when visiting the highly rewarded flower first led to the length of the route increasing by 18%. If prioritizing the highly rewarded flower meant that the route was 42% longer, then the bees preferred to fly the previous short route (Lihoreau, Chittka and Raine 2011).

Like the hummingbirds that exploited the peripheral patches first in the morning, other central place foragers may use traplining to outcompete other foragers (Gill 1988; Ohashi et al. 2008). For example, worker bumblebees allowed to visit 16 artificial feeders in an indoor arena took more nectar when experienced with the array than when inexperienced. The experienced bees travelled faster between flowers and the inter-visit intervals were more regular than were those of the inexperienced bees (Ohashi et al. 2007).

I have described this list of examples to show how a central place forager would benefit from traplining. Traplining by territorial hummingbirds has not previously been studied because traditionally hummingbirds' foraging behaviour has been categorized either as territorial or as a trapliner. A hummingbird's ecology has been described based on whether it is classified as a territorial or trapliner hummingbird, however this classification has not been based on empirical data. In the work I describe in my thesis, I investigated whether these foraging strategies need necessarily be mutually exclusive.

The traplining behaviour of hummingbirds

A number of hummingbird species are considered to be trapline foragers (Feinsinger 1976; Gill 1988; Tiebout III 1991; Garrison and Gass 1999; Temeles et al. 2006), a conclusion that appears to be based on the observations of foraging behavior of a few unmarked birds. For instance, in an indoor arena where the foraging behavior of two species of hummingbirds (one a territorial and the other one considered to be a trapliner) was observed, the “traplining” species spent more time flying around the arena, which contained a single feeder, than did the territorial hummingbird species (Tiebout III 1991). With only a single location at which to feed, however, neither these data nor the observation that marked long-tailed hermit hummingbirds *Phaethornis superciliosus* return to feed from artificial flowers at fixed intervals (Gill 1988) provide direct evidence for traplining. Furthermore, although changes in visitation rates to feeders or to natural flowers have also been interpreted as an indication of traplining behavior, such data do not, in fact, constitute explicit evidence that the birds are following a repeated route or sequence around the feeders/flowers (Garrison and Gass 1999; Temeles et al. 2006).

Despite the lack of direct evidence of the foraging routes that hummingbirds may or may not follow, a number of assumptions are often made about a bird’s foraging ecology based on whether it is considered to be a trapliner or territorial (e.g. energy expenditure; Powers and McKee 1994). The morphology of a bird such as the body size, bill length or the wing disc loading have also been used to differentiate between trapliners and territory owners (Feinsinger and Colwell 1978). The data that come closest to evidence for traplining in hummingbirds are the reports of territorial rufous hummingbirds first visiting the edges of their territory and later in the day visiting central patches (Gass and Montgomerie 1981; Paton and Carpenter 1984).

Within species, hummingbirds have also been classified, without quantitative data, as trapliners or territory holders, with females of the species being typically described as trapliners (Carpenter et al. 1991). Here the distinction is recognized as a flexible one, even within the same sex of a species some individuals may be territorial while others are described as intruders or trapliners (Carpenter et al. 1993; Feinsinger 1976; Kodric-Brown and Brown 1978). Here it is worth noticing that the behaviour of a territorial bird that defends its territory by chasing away intruders does not describe how that bird forages around the resource it is defending (i.e., holding a territory and foraging by traplining are not mutually exclusive). It is also worth noticing that territorial hummingbirds are likely to benefit more from traplining than are birds that do not hold a stable territory, because the former have more information on the location, the value and refilling rates of profitable flowers within their territory. Therefore, it seemed timely to investigate whether hummingbirds in the wild can in fact forage by following repeatable routes around several locations and what information they might use to do this.

Following a repeatable route, like the territorial rufous hummingbirds feeding first from the patches at the edges of their territory and then moving to the inner patches (Paton and Carpenter 1984), is similar to learning a sequence of movements. Hummingbirds might, then, develop their traplines by learning the sequence in which they have previously visited flowers. The ability of animals to learn a sequence has been tested in a range of animals under laboratory conditions with the aim of examining cognitive abilities related to counting. The ability to learn a sequence in which a number of items are present has been recorded for a wide variety of animal species (jackdaws *Corvus monedula* (Pfuhl and Biegler 2012), rhesus monkeys *Macacca mulatta* (Terrace, Son, and Brannon 2003), rats *Rattus norvegicus* (Kesner et al. 1985), ants *Gigantiops destructor* (Macquart, Latil, and Beugnon 2008) and of course, honeybees *Apis mellifera* (Schmid-Hempel 1986). Although the ability to learn a sequence is not in doubt for all of these species, the mechanism that enables them to do so is less clear.

One suggestion is that animals link simple discrete associations (Stimulus-Stimulus or Stimulus-Response) to form a complex chain of behaviours, which would not require temporal knowledge. For example, if a hummingbird only learned the inter-location association between two locations of a trapline, for instance, that location 2 follows location 1 and location 3 follows location 2, then if location 2 was taken away (for instance by an experimenter or if the hummingbirds had to skip location 2 to chase an intruder) the hummingbird would not know how to link locations 1 and 3. As territorial rufous hummingbirds do have to chase intruders constantly or perch between bouts, being able to “pick up” a trapline (or sequence) at any one location will be useful. Remembering the order of flower visitation, rather than linking the visits to two locations at a time across a long trapline, might allow hummingbirds to continue their trapline having chased away an intruder.

Learning the order of flower visitation requires animals to know which flowers precede and follow each other such that the ordinal position of that flower will be treated as an intrinsic characteristic of that flower. This is similar to how some animals learn the sequence of items in list (Terrace 2005). It appears that macaques, at least, can not only remember the inter-item associations between items from the same list but also the ordinal position of each item on several lists (Orlov et al. 2006). Learning each flower’s ordinal position within a sequence might allow hummingbirds to “pick up” the sequence at any point, making the foraging behaviour more flexible. Still, it is not known whether hummingbirds can visit several flowers in a predetermined sequence by learning the order in which flowers are rewarded.

Use of temporal information when traplining

In 1988 Gill reported that long-tailed hermit hummingbirds visited *Heliconia* and *Costus* flowers at predictable intervals of about 47 minutes (Gill 1988). He then provided hummingbirds with three artificial feeders that would refill in fixed intervals of either 10 or 15 minutes after the last time a hummingbird drank the nectar from that feeder. If the feeder was not visited in 10 or 15 minutes (depending on the individually fixed interval of each feeder) then the amount of reward was doubled, but if the hummingbirds did not return after 20 minutes, the feeder was emptied. With this experiment, Gill simulated the natural scenario where hummingbirds have to wait long enough until previously visited flowers have produced more nectar but not so long that the likelihood of another hummingbird visiting the flower would increase. He found that in the absence of competition the birds learned in just one morning to time their visits to the different fixed intervals and increased their return times to longer than the fixed intervals, therefore gaining the extra reward. When there was competition, however, the dominant male tended to either return to feed earlier or to lose the reward to another hummingbird (Gill 1988).

Since flowers vary in the time they take to refill their nectar, depending on the time scale, revisiting a flower may or may not be beneficial (Gill 1988; Possingham 1989). After remembering the location of profitable resources then the next foraging decision for these birds would be when to revisit a flower. Gallistel saw traplining as "the hummingbird's ability to forage efficiently depended on its ability to represent time intervals precisely and to extract subtle features of temporal structure from its observations" (Gallistel 1990). It is true that for a hummingbird to benefit from following a trapline, the bird should allow enough time for the flowers in the trapline to refill before it repeats the trapline. Because flowers will refill at a specific rate, it may be useful for hummingbirds to pay attention to temporal information regarding flower refilling.

The use of spatial and temporal information together is common to many species of animals since the availability of food resources, possible mates or the presence of predators often varies systematically across both space and time. The ability to combine both temporal and spatial information is referred to as time-place learning (TPL) and is present in a wide variety of species. Time-place learning has been of interest to psychologists because the ability of animals to associate a reward with more than one cue at a time, in this case a location together with the time of day, also meant that memories could be connections between what, where and when (Gallistel 1990), what was then referred as an episodic-like memory.

The Hymenoptera again played a key role in the early days of the study of time-place learning (honey bees *Apis mellifera* Beling 1929; Wahl 1932). Wahl trained a colony of bees to visit two different places at two different times of day as shown by the majority of honeybees going to feed at the right place at the right time (Wahl 1932). As Wahl only looked at the entire colonies' behaviour, whether one bee learned to visit one correct place at the correct time and then another bee learned to visit the second correct place at the second correct time and subsequently whether those bees then recruited more bees is not known. Unlike the bees however, territorial hummingbirds can be tested individually and so the use of temporal information whilst returning to feed from certain locations can be studied in these birds.

Rufous hummingbirds can time revisits to flowers so that they avoid visiting flowers that have not had time to refill (Hixon, Carpenter, and Paton 1983; Gill 1988; Henderson et al. 2006; Marshall, Hurly, and Healy 2012; Samuels, Hurly and Healy 2014; Jelbert et al. 2014). Furthermore, these birds can use different timing mechanisms. For instance, like the long-tailed hermit hummingbirds, male rufous hummingbirds can time their revisits to artificial flowers that refill at different intervals (Henderson et al. 2006), which means that hummingbirds can use the time interval flowers take to refill to decide when to revisit a flower. Furthermore, rufous hummingbirds use a combination of ordinal timing (where the order of flower reward is learned) and daily timing (also referred to as circadian or phase timing where the time of an internal clock is entrained to the time of day a flower is rewarded) to alternate between two rewarded locations (Marshall et al. 2013; Jelbert et al. 2014) . Whether hummingbirds can learn to revisit more than two different locations at different times of day remains to be tested.

Traplining animals that visit several locations a day will only benefit from repeating a trapline if they have waited long enough between visits so that the resources have had time to replenish. The studies looking at the traplining behaviour of different animals, including those conducted on the Hymenoptera, have only rarely investigated the role that time information plays in this foraging behaviour (Williams and Thomson 1998). Instead, most traplining studies have focused in the animal’s ability to remember the rewarding locations and how they can optimize their traplining routes (Saleh and Chittka 2007; Ohashi and Thomson 2009; Lihoreau, Chittka, and Raine 2011). Since hummingbirds can remember not only the location of rewarded flowers but also remember the time flowers take to refill, these birds might be able to integrate both types of information into their traplines. In order to avoid revisiting empty flowers, traplining hummingbirds could associate different times of day to different patches, they could learn the order of patch visitation or the time interval flowers take to refill. Whether these birds do integrate time information when visiting several locations and which of these timing mechanisms they might use has not been studied.

Cue use by female hummingbirds

Male and female hummingbirds differ in their foraging behaviour. Male hummingbirds are often territorial while female hummingbirds are trapliners, or so common knowledge would have us believe. In several decades of work on hummingbird foraging behaviour in the wild, almost all studies have been carried out using male hummingbirds. In the field, this male bias has a logistical basis because while the males of many different species of hummingbirds tend to monopolise the artificial feeders used in the field to attract hummingbirds to a specific site, females that try to approach the feeders are normally excluded or harassed by the males. Males that are classified as territorial are typically seen defending a particular site, perching in visible branches, chasing away intruders and displaying to females. All other non-territorial individuals, including the females of most of the hummingbird species, are thought to be trapliners. Still, there have been a handful of studies on female foraging behaviour either because they sometimes will defend territories (Healy and Hurly 1995; Temeles and Kress 2010) or because they have been caught with mist nets and studied for a couple of hours under semi-wild conditions (Pérez et al. 2011). The differences in foraging behaviour between males and females have led researchers to believe that most females forage by traplining rather than by feeding within a territory. If male and female hummingbirds do, indeed, have different foraging styles, then the type of information they might use to optimize their foraging might also differ.

The idea that sex differences in cognitive abilities might relate to foraging behaviour provides a connection between behavioural ecology and comparative psychology. In behavioural ecology comparative studies of the memory for spatial locations in hoarders and non-hoarders birds have illustrated how an animal's ecology affects the types of environmental cues it uses to remember a specific location (e.g. Clayton and Krebs 1994). For example, while food hoarding, black-capped chickadees *Poecile atricapillus* preferentially use spatial location to revisit a feeder, the non-hoarding dark-eye juncos *Junco hyemalis* used both the feeder's colour/pattern and its spatial location cue to return to the feeder (Brodbeck 1994). These differences in cue preferences were then also found in memory tests: chickadees remembered spatial information better than the visual information while the juncos remembered the spatial and visual information equally well (Brodbeck and Shettleworth, 1995). However, sometimes non-storing great tits *Parus major* will also prefer to use spatial cues rather than visual ones, a switch in cue preference that appears to depend on the testing context (Hodgson and Healy 2005). These behavioural findings are complemented by the finding that as size of the hippocampal region gets bigger, spatial memory is greater (e.g. Sherry et al. 1981; Sherry et al. 1992; Healy and Krebs 1993; Biegler et al. 2001).

While researchers in cognitive ecology have found that differences in foraging strategies are correlated with variation in spatial abilities, psychologists working either with humans or laboratory animals, have found that between the sexes, spatial abilities may also differ (Gaulin and Fitzgerald 1986; Harris, D'Eath, and Healy 2008; Jozet-Alves, Modéran, and Dickel 2008; Schmidtke and Esser 2011). However controversial this topic might be, nevertheless, sex differences in spatial ability are consistently found across an increasing number of species under a range of test conditions. Although test conditions might not be comparable, and even though it appears that variation in stress may play a role in causing the sex differences (Harris et al. 2007), males often seem to outperform females in different spatial tasks.

For example, men seem to have better mental rotation skills than do women and whenever a rotation task gets more complicated the sooner a difference in performance between men and women is observed (Collins and Kimura 1997; Kimura 1999). On pen and paper map tasks, men seem to use distance and cardinal directions while women prefer to use location of objects (or landmarks) (Galea and Kimura 1993). Somewhat similarly, on a memory task, men used both visual and location information while women relied more on the object’s visual features to solve the test (Jones and Healy 2006). When the proximal causes of these differences have been investigated, sex hormones (oestrogen and testosterone) have been found to have both an organizational and an activational effect on spatial abilities (e.g. Galea et al. 1994; van Goozen et al. 1995; Lacreuse et al. 2001), acting specifically on the hippocampal region of the brain (Kelly, Ostrowski, and Wilson 1999; Spencer et al. 2008). At the same time, an abundance of *ad hoc* evolutionary hypotheses to explain the existence of this sex difference have been proposed, with no definitive or exhaustive testing as yet. While a couple of hypotheses propose female superiority in spatial abilities (*Dispersal* and *Female Foraging Hypotheses*: Silverman and Eals 1992), most hypotheses propose that males have better spatial performance, due to a by product of range size and polygamy, to males travelling long distances to forage, to find mates and/or fight other males, or due to females choosing males with better spatial abilities (*Range size*: Gray and Buffery 1971; *Male foraging*: Silverman and Eals, 1992; Silverman et al. 2000; *Male warfare*: Geary 1995 and *Female choice*: Hawkes 1990, respectively).

Of all of these theories, the one that is best supported by the data is the "Range Size Hypothesis" proposed by Gray and Buffrey (1971). This hypothesis suggests that in order to enhance their fitness, polygynous males have to cover a bigger home range than do their conspecific females and, therefore, better spatial abilities would be favoured in males. Support for this hypothesis came from a correlation between spatial ability and range size when two closely related vole species were compared: male and female prairie voles *Microtus ochrogaster*, which is a monogamous species where both sexes have the same home range size, do not differ in the number of errors it takes them to solve a maze. Polygynous male meadow voles *M. pennsylvanicus*, which have a bigger range size than the females, on the other hand, make significantly fewer errors than do their conspecific females (Gaulin and Fitzgerald 1986; Gaulin and Fitzgerald 1989). Furthermore, the relative volume of the hippocampus differssignificantly between the sexes of the meadow vole but not in the prairie vole (Jacobs et al. 1990). As with the comparative studies of food storing and non-storing birds, variation in ecology is correlated with variation in spatial ability and the size of the hippocampus. Comparative studies looking at differences in spatial abilities, as tested in behavioural tasks, have found that in some cases, but not all, males will outperform their con-specific females (Kavaliers et al. 1996; Seymoure, Dou, and Juraska 1996; Lacreuse et al. 1999; Jozet-Alves, Modéran, and Dickel 2008; Kandori et al. 2009; Schmidtke and Esser 2011)

Although it may seem plausible that sex differences in ecology would result in differences in spatial abilities, whether due to differences in cue use or to differences in memory abilities, it is difficult to disentangle the effects of range size and polygyny from the vole (and other rodent) data. Furthermore, most of the research examining sex differences in cognition has been conducted in the laboratory, where it is at least possible that many of the observed sex differences in spatial abilities might be accounted for by females being more susceptible to acute stress than are males (Beiko et al. 2004; Harris, D'Eath, and Healy 2008; Harris, D’Eath, and Healy 2009). In any case, it is true that in several testing scenarios males can use either visual or spatial cues to locate a target (Jones and Healy 2006; Chai and Jacobs 2010; Rodríguez, Chamizo, and Mackintosh 2011).

Hummingbirds might, then, make a useful model to look at sex differences in cue use because we know that at least the males will use both spatial and visual cues depending on the task. If females forage in a different way to males then it is possible that females will differ in how they relocate a reward. For instance, females could rely only in the visual cues of flowers when deciding from which flowers to feed. Since females can be more elusive than males, the foraging behaviour and types of cues they use has not determined.

Objectives

In this thesis I set out to determine if territorial rufous hummingbirds will trapline when foraging from multiple locations, if these birds will integrate temporal information in their traplines and if female hummingbirds use different types of cues than the males when revisiting a rewarded location.

Firstly, in order to answer the question of whether territorial rufous hummingbirds can repeat the order in which they visit several flowers I presented six male rufous hummingbirds with two artificial flower arrays, with flowers rewarded one at a time in a predetermined sequence, and tested whether they could learn to visit flowers with the correct order. I expected that with experience hummingbirds would visit the different flowers following the predetermined order, alternating between the locations. I present these experiments and the results in Chapter Two.

Secondly, in order to test if these hummingbirds would develop their own repeatable traplines around several locations I trained six male rufous hummingbirds to visit a board containing 2 to 5 artificial flowers added one at a time. Hummingbirds were allowed to visit flowers in any order. I expected that, like the bumblebees (Lihoreau, Chittka, and Raine 2010), hummingbirds would repeat the order in which they visited flowers and that instead of following the presentation order the birds would find the shortest routes around all locations. The experiments and results from this experiment are presented in Chapter Three.

Thirdly, in order to test whether hummingbirds could learn to visit several different locations at different times of the day I adapted a protocol that has been previously used to test time-place learning under laboratory conditions. For this experiment, flowers located in each of four patches contained reward for only an hour each day but the time and sequence in which patches were rewarded was repeated across days. I expected birds to learn to visit the correct patch at the correct time. The results of this experiment are described in Chapter Four.

Fourthly, I also tested whether hummingbirds could integrate the two types of information, spatial and temporal in a single task. In Chapter Five I describe an experiment where I presented birds with three patches of flowers where flowers would refill only after 20 minutes had passed from the last visit. Birds were free to visit the patches in any order or at any time. I expected hummingbirds to avoid depleted flowers by repeating the order of patch visitation.

Finally, to answer the question of whether male and female hummingbirds use different types of cues when relocating a reward I trained female hummingbirds of three different species to feed from an artificial flower. Birds were allowed to feed once from a four-flower array in which only one flower was rewarded and all flowers were of different colours. When the birds returned, the colour of the flower and the spatial cue designating the rewarded flower had been dissociated. I expected females to first visit the same colour flower as the flower they had fed from previously. In Chapter Six I present the results from this experiment.

Chapter Two: Learning a sequence

INTRODUCTION

Animals that follow traplines will move from one rewarded location after another in a repeated sequence from the first location to the second, third and so on (Thomson, Slatkin, and Thomson 1997). In this sense, traplining behaviour can be understood as learning a sequence. Linking rewarding locations in such a sequence might be accomplished by animals memorizing sequences of motor patterns. This was shown to be the case for ants (*Gigantiops destructor*) trained to navigate a maze with different sequential turns. After reaching a training criterion, ants continued to turn in the correct order when extra chambers were added (Macquart, Latil, and Beugnon 2008). Traplining bees too will follow movement rules when feeding within patches of vertical inflorescence, foraging upwards from the bottom flower (Pyke 1984).

There are other ways, however, besides following a sequence of movements, in which animals can learn to repeat a sequence. For instance, under the psychological chaining theory view, behaviour that is organized in a sequence can be understood as discrete Stimulus–Response units, each unit linked to the next in a chain that is learnt by repetition (Terrace 2005). In the case of traplining hummingbirds, birds could be learning the sequence of patch locations one after the other.

Yet another way in which animals can learn a sequence is by learning the exact ordinal position of an item (or a flower patch) within a sequence. Learning the exact order of behaviours or items in a sequence, rather than by chaining pairs of items, would allow animals to know the stage of the sequence at which they are. This would enable them to stop moving around the sequence and to rejoin it when needed. For example, if a hummingbird has fed from the morning patches and then returns to its central perch or chases off an intruder, instead of restarting the trapline, he could skip the already visited patches and go straight to the next patch down its foraging trapline. Hummingbirds that need to keep track of multiple different locations at which they have fed should benefit from knowing which locations have been visited and which can be visited next.

Territorial male rufous hummingbirds will learn to move from at least one artificial flower to another in a sequence (Marshall et al. 2013; Jelbert et al. 2014), which suggests that hummingbirds can repeat the order in which they visit several locations in a trapline. Therefore, as a first approach in determining whether hummingbirds do trapline I tested whether these birds would learn to visit three or five flowers in a predetermined order. To do this I presented six male rufous hummingbirds with two artificial flower arrays, with flowers sequentially rewarded, and tested whether they could learn to visit flowers with the correct order.

GENERAL METHODS

Rufous hummingbirds migrate from Mexico back to Canada during the summer months (May to mid July) for their breeding season. During this time male hummingbirds defend territories of hundreds of flowers while also displaying to passing females. In Canada, artificial feeders with 14- 20% sucrose weight by weight (w/w) are hung two meters from the ground in early May so that by the middle of May male territorial hummingbirds will be defending feeders and can be seen perching close to their feeder. At this point I was able to train hummingbirds to go in and out of a trap so that I could mark them. Birds were marked on their breast feathers with nontoxic waterproof ink (Jiffy Eco-marker Ink) to enable individual identification. One day after a bird was marked I returned to his territory and trained him to feed from artificial flowers. This procedure was followed for all experiments presented in this thesis.

Subjects and experimental site

The subjects in this first experiment were six male rufous hummingbirds, five of which had had experience of feeding from artificial flowers in two previous unrelated experiments and therefore did not need to be trained to feed from my flowers. One hummingbird did not have previous experience so he was first trained to feed from an artificial cardboard “flower”. Flowers consisted of a 700µl Eppendorf tube surrounded by a colour cardboard circle (6cm in diameter) containing 120µl of 25% sucrose solution mounted on a 60cm wooden stake. After each visit I moved the flower in a radius of 5 meters from the location of the feeder. When the bird was readily visiting the flower anywhere in this area, the sequence training began.

Trials were run between 0800 and 1800 hours Mountain Standard Time from June to mid-July 2012.

Sequence training

I trained and tested birds using a sequence of three and a sequence of five flowers. All birds were presented with both sequences and the order in which they were trained and tested was pseudo-randomly assigned, such that three birds were trained and tested on a sequence of three flowers followed by a sequence of five and the other three birds were trained and tested in the reverse order.

A sequence consisted of three or five flowers placed in an array where only one flower at a time, in a predetermined sequence order, contained 120µl of 25% sucrose solution. The sequence of three flowers was arranged in a shape of a 1 meter-sided equilateral triangle while the array for the sequence of five flowers was arranged in a 1 m x 1 m square with a centre flower 72 cm from all other corner flowers (Figure 2.1). In order to control for possible bird biases to visit flowers in a certain position (periphery vs. central flowers) or in a certain direction, two possible spatial arrangements of each array were used and hummingbirds were pseudo-randomly assigned to one of these for each of the two sequences.

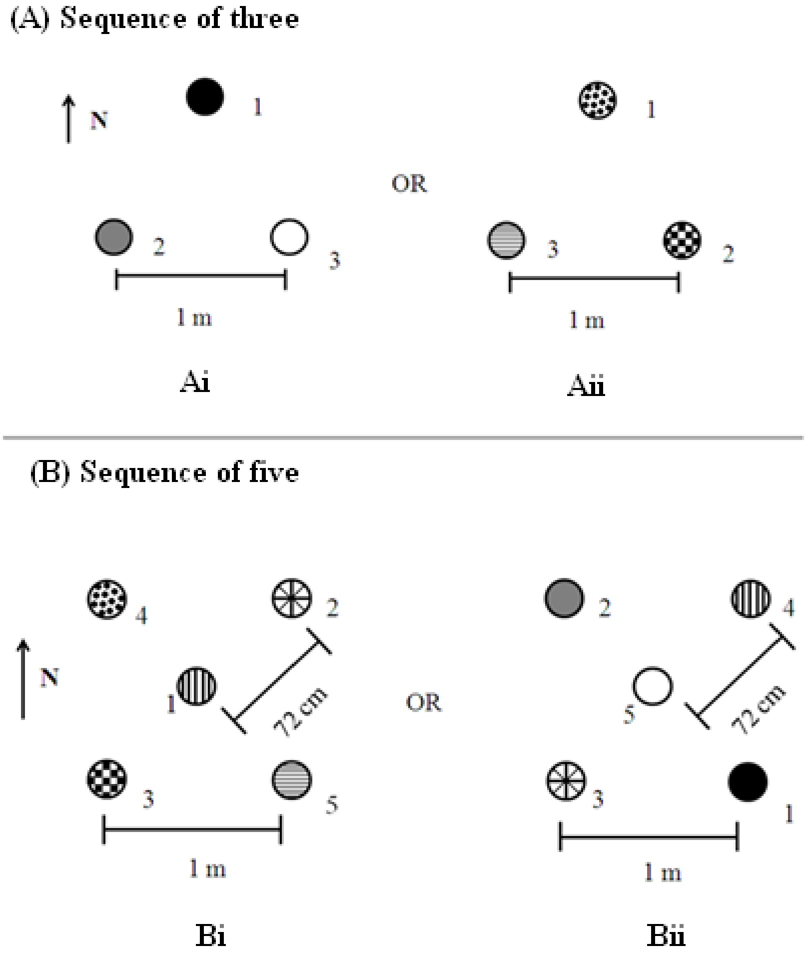

Figure 2.1. Diagram of the three- and five-flower sequences I used to train birds to find a rewarded flower in sequence. A hummingbird was only trained and tested in one of the two possible flower arrays for a sequence of three and a sequence of five (Ai or Aii and Bi or Bii). The circles represent the cardboard flowers and the numbers beside them denote the sequential order in which each flower was rewarded. Each flower with in an array and between the two different sequences had a distinctive colour, represented in the diagram by shades of grey and different textures. All flower arrays were oriented towards the North.

All flowers in both arrays were of a distinctive colour unique to each sequence (although not unique across birds). The colour, position and sequence order of each flower was also pseudo-randomized. The arrays, as well as the flowers within the arrays, always occupied the same spatial location during the training and testing of a sequence. In order to differentiate each of the 20 training trials, the feeder was returned at the end of each sequence of three or five flowers and the hummingbird was allowed to feed from the feeder once before starting the sequence again. At the start of every sequence the array was placed in the same location as the previous session and the feeder was taken away. Each hummingbird was given one pre-sequence session followed by 20 consecutive training sessions before being tested. On the single pre-sequence training session the flowers were presented one by one in sequence so that the bird would feed from each flower in the correct order at least once. On each sequence-training session all flowers were presented simultaneously but only one was rewarded at any one time, requiring the hummingbird to feed from each flower in a sequence across sequential visits to the array. For instance, after a bird had fed from the rewarded flower that was first in the sequence and flown away, I emptied and cleaned the flower and then put 120µl of sucrose in the second flower, waited until the bird had fed from that flower, then emptied and cleaned that flower, then put the sucrose in the third flower and so on. For each training session, I recorded the order in which the hummingbird visited the flowers on the array.

Experimental trials

To determine whether or not a bird had learned the sequence of three flowers I presented it with a single probe test in which all three flowers were presented but none was rewarded. If the bird had learned the sequence, he was expected to fly from Flower 1 to Flower 2 to Flower 3. I again recorded the order in which the bird visited the flowers on the array.

After the training on the sequence of five flowers was completed, I carried out three different tests, each followed by an additional two training sessions. Test “A” was a probe test in which all flowers were presented and all were empty. If the bird had learned the sequence, it was expected to fly from Flower 1, to Flower 2, to Flower 3, to Flower 4 and to Flower 5.

For test “B” I presented birds with the whole array of five flowers and allowed to feed first from Flower 1 and then Flower 2. However, before the bird returned to feed from Flower 3, I removed that flower. If the bird had learned the sequence, it was expected to fly directly to Flower 4.

Finally, for test “C” I presented only Flowers 2 and 4, both of which were empty. If the bird had learned the sequence, he was expected to fly first to Flower 2 and then to Flower 4.

For all tests the order of visit was recorded. The order of tests for the sequence of five was counterbalanced across the birds.

Data analysis

Three variables were extracted from the order of all visits a bird made during each training session for analysis. First, I calculated the combined proportion of all the correct choices the birds made in each training session, using the visits made to all flowers. If the sequence was learned one would expect the proportion of correct choices to increase with increasing training sessions. Second, I looked at the specific number of first visits made to each flower during each flower’s turn in the sequence, I expected that during the first training sessions the birds might first approach the previously rewarded flower or possibly the flower that would next be rewarded, but as the training progressed, the birds would increasingly direct their first visits to primarily the correct flower. Third, I calculated the proportion of correct visits made to each flower across training to determine whether some flowers within the array were more readily learned than others. For the tests, I analyzed the first visits the hummingbirds made to the array.

I compared performance with chance expectation using a one-sample proportion Pearsons X^2 test or an Exact Binomial Test with R Studio Package.

Results

Sequence of three flowers

Two of the six hummingbirds visited the array in the appropriate sequence during two occasions only. The overall performance of the hummingbirds, measured as the mean proportion of correct first choices in each sequence was worse than predicted by chance across the 20 training trials (Pearson's χ^2 tests with an expected proportion of 0.333 and 0.666: Trials 1-5: 31/179 $\chi^2 = 19.94$, $P < 0.05$; Trials 6-10: 30/179 $\chi^2 = 21.38$, $P < 0.05$; Trials 11-15: 39/161 $\chi^2 = 5.60$, $P < 0.05$; Trials 16-20: 37/165 $\chi^2 = 8.35$, $P < 0.05$; Figure 2.2).

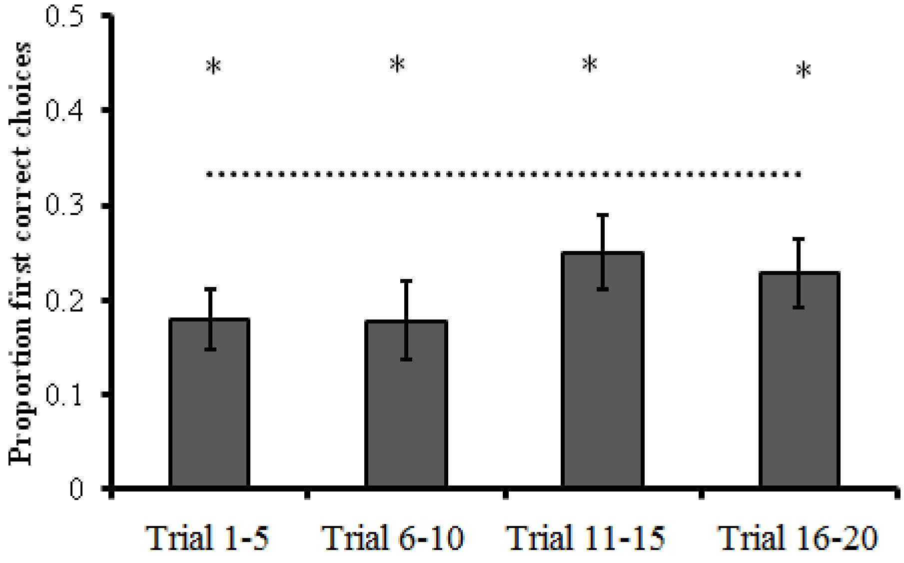

Figure 2.2. Proportion of correct first choices by six hummingbirds visiting a sequence of three flowers. Mean ±SE proportion of correct first choices across the 20 trials divided in four blocks. Significant difference from chance level was analysed using a one-sample proportion Pearsons X^2 test *P < 0.05. The dotted line represents chance level P= 0.333.

The proportion of first visits to Flower 3 was above chance level when the sequence of rewarded flowers was Flower 1, Flower 2 and Flower 3 (Pearson's χ^2 tests with an expected proportion of 0.333 : Proportion of first visits to Flower 3 when Flower 1 was rewarded χ^2 =10.20, $p < 0.05$; when Flower 2 was rewarded χ^2 =17.33, $p < 0.05$; when Flower 3 was rewarded χ^2 =37.20, $p < 0.05$). Also, Flower 2 was always visited significantly less often than expected by chance when Flower 1, 2 or 3 was rewarded (Pearson's χ^2 tests with an expected proportion of 0.333 and 0.666: Proportion of first visits to Flower 2 when Flower 1 was rewarded χ^2 = 20.70 $p < 0.05$; when Flower 2 was rewarded χ^2 = 6.83 $p < 0.05$; when Flower 3 was rewarded χ^2 =14.25 $p < 0.05$; Figure 2.3).

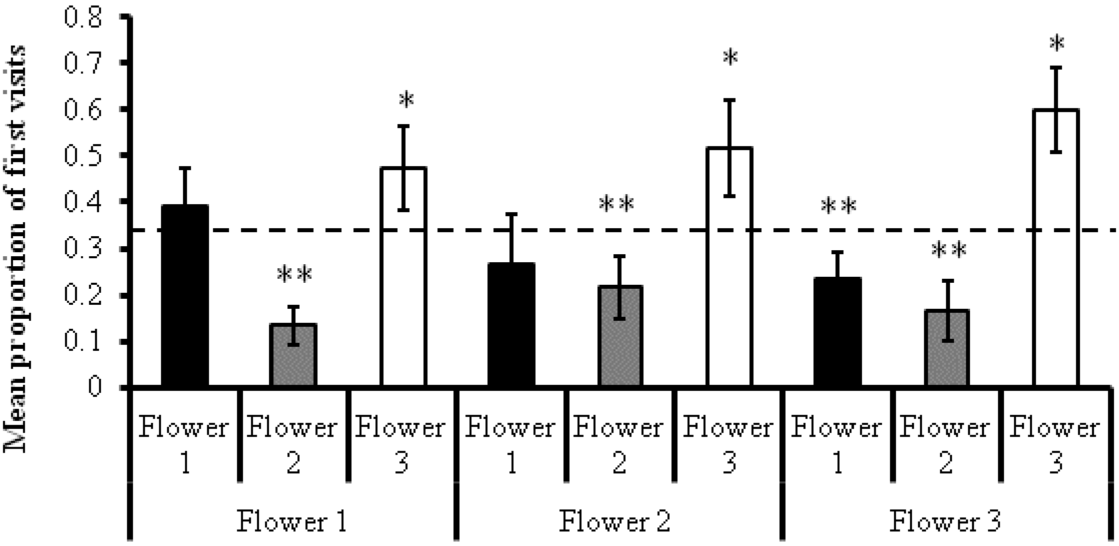

Figure 2.3. Mean ±SE of the proportion of first choices to each of the three flowers when each flower was rewarded throughout all twenty trials. The dotted line represents chance level P = 0.333. * *P* < 0.05 ** *P* < 0.01.

For the first ten sessions with a sequence of three flowers the hummingbirds visited the first and last flower in appropriate order. By the last five sessions, however, most birds started to visit the array with a visit to Flower 3. Throughout the 20 sessions Flower 2 was visited less often than expected by chance. During the first five training trials three of the hummingbirds visited either Flower 1 or Flower 3 more often than expected by chance (Exact Binomial Test with an expected proportion of 0.333 : Flower 1, Birds 3 and 4 p < 0.05; Flower 3, Bird 5 p < 0.05; Figure 2.4a). By the last five training trials, however, four out of six birds were correct when first visiting Flower 3 (Birds1, 2, 4 and 5: p < 0.05; Figure 2.4b). For the majority (4) of the birds, the proportion of correct visits to Flower 2 was lower than for the other two flowers during the first and last training trials.

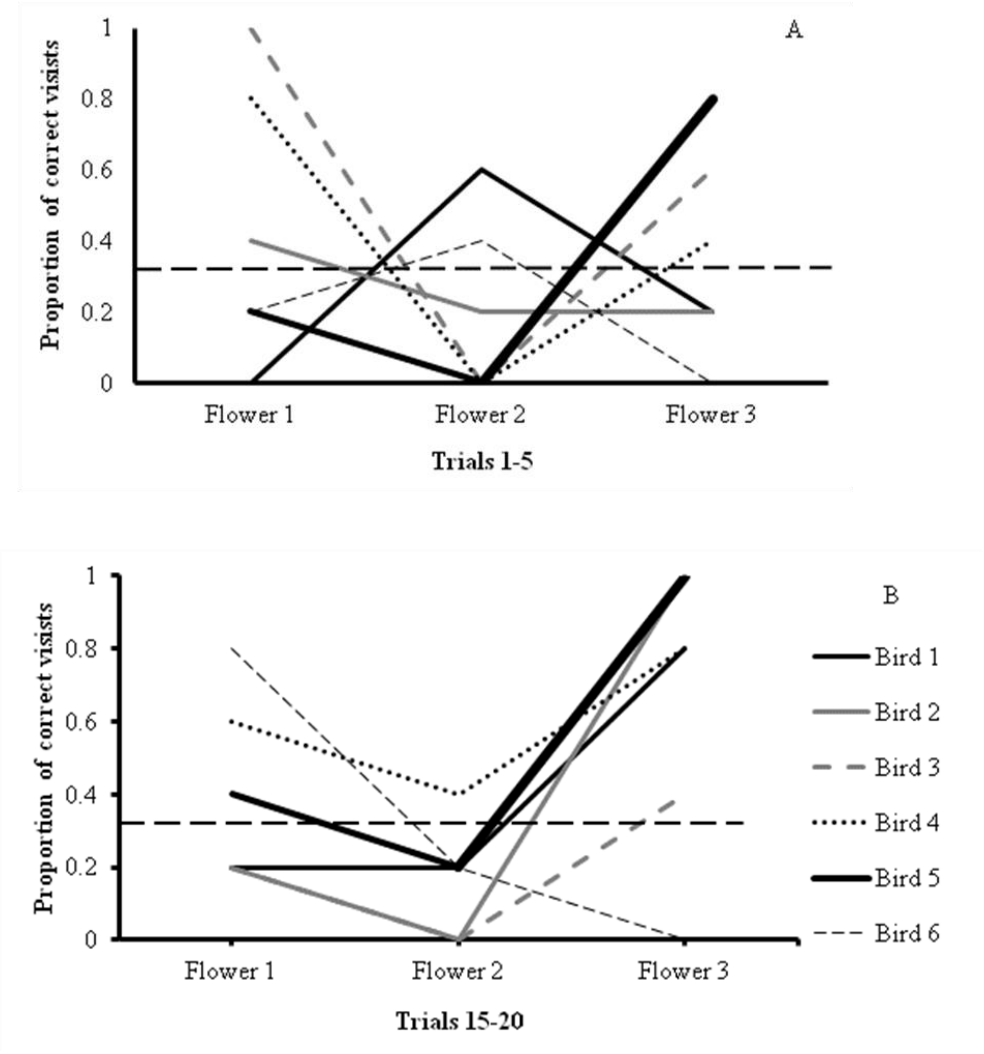

Figure 2.4. Proportion of correct choices made by six male rufous hummingbirds to each of the three rewarded flowers in trials 1-5 (a) and (b) trials 16-20. The dotted line represents chance level P = 0.333.

Sequence of three flowers: test

On the test trial, bird 4 visited the array in the correct sequence, while birds 3 and 6 began visiting the array at Flower 1. Conversely, birds 1, 2 and 5 began visiting the array at Flower 3. None of the birds started visiting the array by Flower 2 (Figure 2.5).

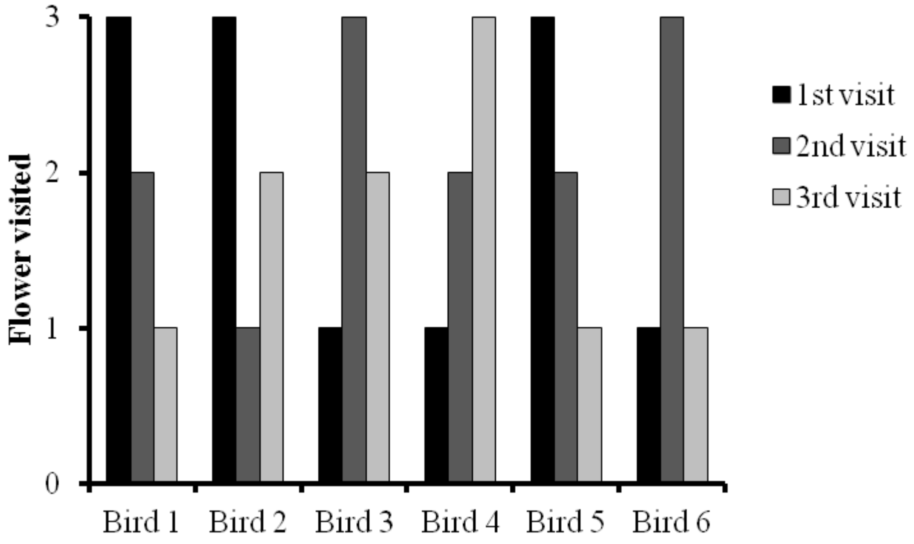

Figure 2.5. Order in which each bird visited the three flowers during the probe test where no flower was rewarded. The bars represent the first, second and third visit while the "Y" axis references to the identity of the flower visited on each of those three first visits. Bird 4 performed correctly while birds 1 and 5 visited the flowers in reverse order.

Sequence of five flowers

Across the 20 training trials, the mean proportion of correct first choices made by the hummingbirds was significantly lower than predicted by chance (Pearson's χ^2 tests with an expected proportion of 0.2 and 0.8: Trials 1-5: 34/462 $\chi^2 = 5.35$, $p < 0.01$; Trials 6-10: 30/435 $\chi^2 = 45.86$, $p < 0.01$; Trials 11-15: 47/416 $\chi^2 = 19.14$ $p < 0.01$ and Trials 16-20: 33/421 $\chi^2 = 38.16$, $p < 0.01$; Figure 2.6).

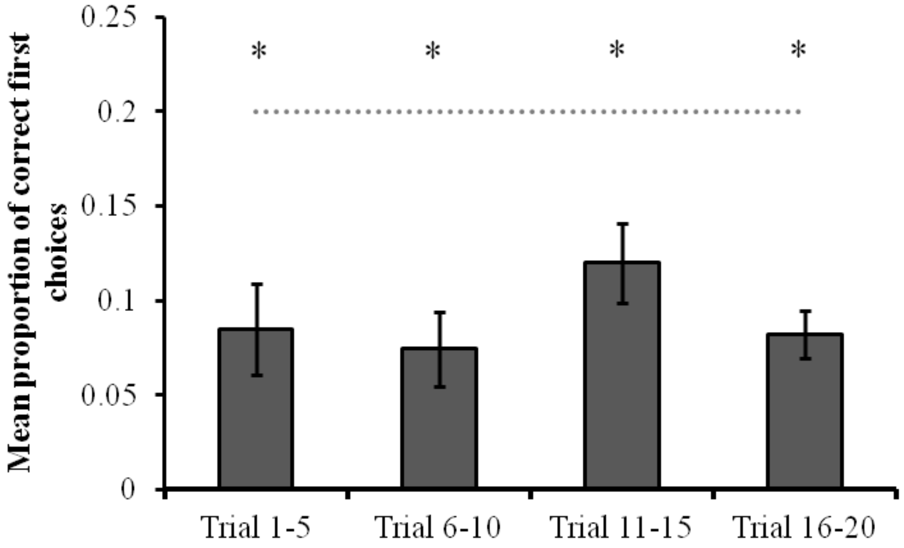

Figure 2.6. Performance of six hummingbirds trained on a sequence of five flowers. Mean ±SE proportion of correct first choices through twenty trials divided in four blocks. *P < 0.01. The dotted line represents chance level P = 0.2.

The proportion of first visits the hummingbirds made to each of the five flowers was no better than chance throughout the sequence with the exception of two flowers: birds visited Flowers 2 and 5 in the correct order significantly more often than would be expected by chance (Pearson's χ^2 tests with an expected proportion of 0.2 and 0.8: Proportion of first visits to Flower 2 when Flower 2 was rewarded: 35/120 $\chi^2 = 5.74$, p = 0.01; Proportion of first visits to Flower 5 when Flower 5 was rewarded: 41/120 $\chi^2 = 14.17$, $p < 0.01$).

Although birds did not seem to learn the sequence of five flowers, they tended to visit sequences of two flowers: birds 1 and 4 repeated the sequence 5-1 (Flower 5 then Flower 1) a total of 52 times (between the two birds), birds 3 and 5 tended to start at Flower 4 and then continue to Flower 1, repeating that sequence 35 times, bird 6 visited Flower 1 first 35 times while bird 2 did not repeat a particular sequence.

Sequence of five flowers: tests

During the probe test "A" when the five flowers were present but all were empty, none of the birds visited the flowers in the correct sequence (Figure 2.7). Only one hummingbird started visiting the array at Flower 1, while three others began at Flower 2.

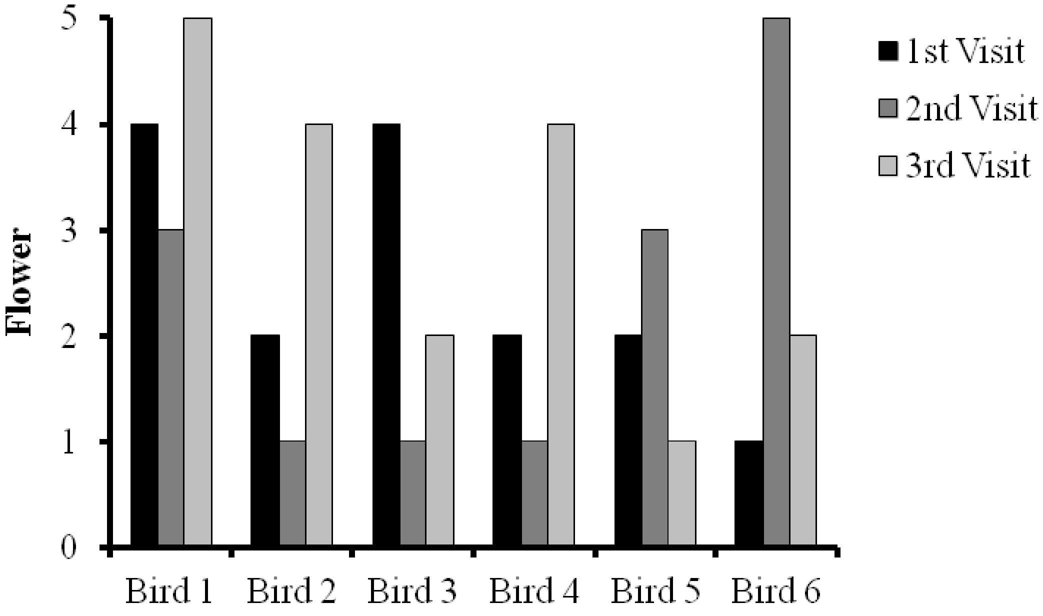

Figure 2.7. The order in which the birds visited the five-flower array during test "A". The bars represent the first, second and third visit while the "Y" axis references to the identity of the flower visited on each of those visits. Only bird 6 first visited Flower 1 first.

During the test "B" when the array was presented after the birds visited Flower 2 without Flower 3, three of the birds visited Flower 4 (Figure 2.8).

Finally, during test "C" when only Flowers 2 and 4 were presented, four out of the six birds visited the correct flower first, which was Flower 2 (Figure 2.9).

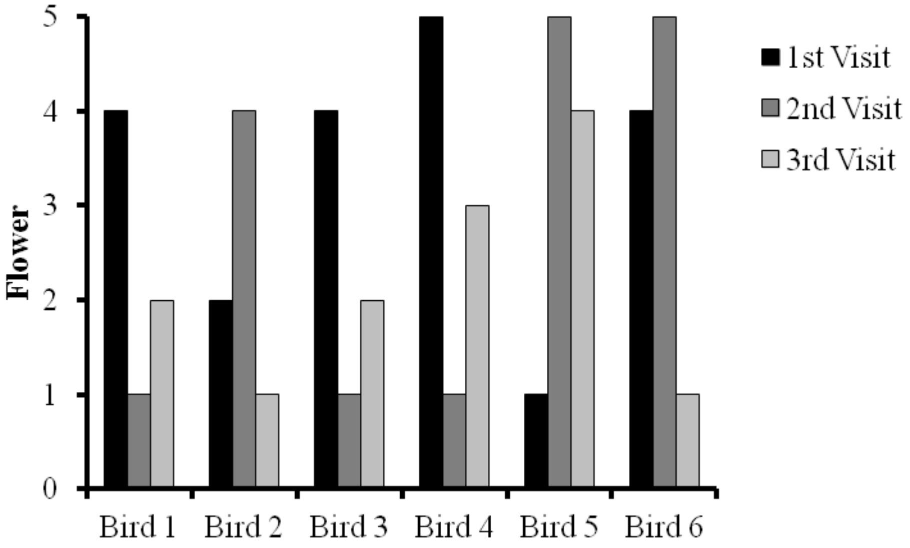

Figure 2.8. The order in which each bird visited the five-flower array during test “B”. The bars represent the first, second and third visit while the “Y” axis references to the identity of the flower visited on each of those three first visits. Birds 1, 3 and 6 first flew to flower 4.

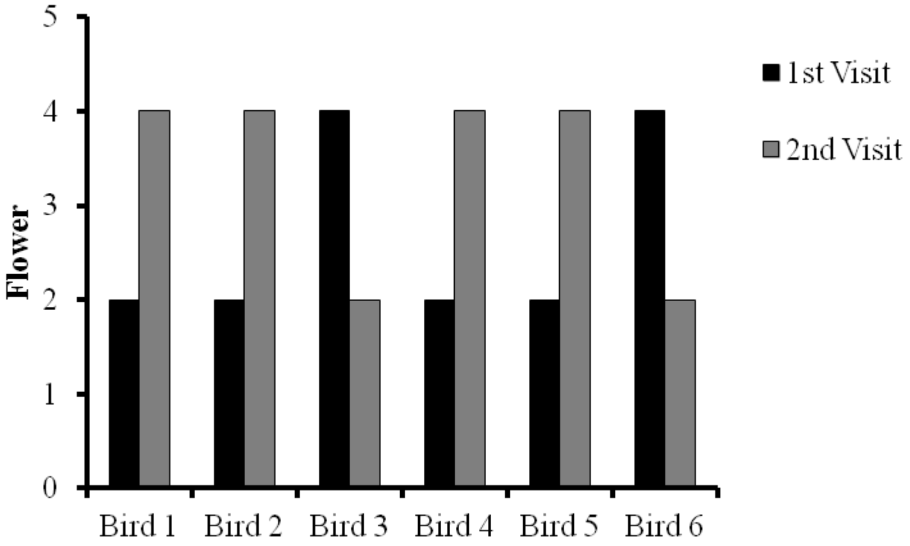

Figure 2.9. Order with which each bird visited the flower array during test “C”. The bars represent the first and second visit while the “Y” axis references to the identity of the flower visited on each of those three first visits. Birds 1, 2, 4, 5 flew in the correct order.

Discussion

In this experiment I attempted to train hummingbirds to follow a sequence of either three or five rewarded flowers. Once trained, I conducted probe tests to determine what it was that the birds had learned. During the probe test in the three-flower array in which all the flowers were empty, one of the birds visited the array in the correct sequence, two birds started to visit the array at Flower 1, while the other three birds visited Flower 3 first. For the five-flower array, I had three probe tests, in each of which at least one bird behaved as if he had learned the sequence.

Although the birds did not appear to learn the whole of the sequence, these data are consistent with the sequence literature, where subjects normally respond correctly to the first and last items of a list, while items in the middle of the list are remembered less accurately (Crystal and Shettleworth 1994). These effects are referred to as a primacy and recency effect, respectively. Relative to the number of trials that subjects often take to learn a sequence, which can vary from hundreds to thousands (Chen, Swartz, and Terrace 1997; Orlov et al. 2006; Pfuhl and Biegler 2012), the hummingbirds took rather few sessions to begin to show these primacy and recency effects. These effects, however, were apparent only for the first few sessions and as the experiment progressed, the hummingbirds increasingly began searching the array by first visiting the flower nearest to the location of their feeder. For four of the hummingbirds this meant visiting Flower 3 first. Since the flowers on the array were only one meter apart from each other, it is possible that there was little cost to checking whether a flower is empty flower while en route to a known rewarded flower. This might suggest that the birds’ failure to learn the sequence of rewarded flowers may have been due to the spatial layout of the flowers, rather than to a paucity of experience.

The methodology for training the birds to the sequence differed in at least two ways from most laboratory sequence learning experiments in which the subject is required to complete the entire sequence correctly before they receive a reward (Terrace 2005; Orlov et al. 2006; Pfuhl and Biegler 2012; Yamazaki et al. 2012). Sequence training is also typically built up from two to several items, adding further items only when the animal has learned the shorter sequence (Pfuhl and Biegler 2012). For the hummingbirds in my experiment, however, the entire array was presented simultaneously rather than one flower at a time, which allowed hummingbirds to visit flowers even when they were not rewarded. Secondly, there was no punishment for visiting an unrewarded flower en route to the rewarded flower as birds were allowed to continue to search for the rewarded flower.

When hummingbirds visited the three-flower array the proportion of correct visits to the first and third flower was higher than the proportion for the second flower (Figure 2.4). Unlike the data from the three-flower sequence, in the trials with a five-flower sequence, I found no evidence for a primacy effect although most birds did visit correctly Flower 5 i.e. the last flower in the sequence, which may constitute evidence for a recency effect. A recency effect while solving a sequence or list has been linked to the use of working memory, rather than long-term memory, because information about the ordinal order of the sequence is reinforced while subjects go through the sequence (Hampton et al. 1998; Winter and Stich 2005; Terrace 2005). It might be the case that as hummingbirds continued to visit the array, they were also remembering which flowers they had already visited and so, when Flower 5 was rewarded there was only one option remaining. Although the birds did not learn the sequence of five flowers, five of the six birds did repeat short, appropriate sequences of flowers e.g. Flower 5 followed by Flower 1. It is possible that with more appropriate training, therefore that these birds would be able to learn a spatial sequence of five flowers.

The key assumption for this experiment was that because they trapline, hummingbirds should readily learn to follow a spatial sequence of rewarding flowers. Although they did not readily learn the sequence as I trained them, the birds did begin to visit the flowers in their own sequence, typically by first visiting the flower that was closest to their feeder before moving on to other flowers. In between feeding bouts, hummingbirds chase intruders, display to and harass passing females, but mostly, the territorial male hummingbirds spend their time perching at a nearby tree branch. Hummingbirds might use one or two branches to perch, switching from one to another, probably to get a view from different perspectives, but common to all perching branches is that they seem to allow the hummingbird to see if an intruder approaches the feeder. As a result of the perching behaviour, hummingbirds tend to fly towards the feeder or flower array from one or two directions only. If hummingbirds behave in the same way when they are feeding from patches of flowers, it might be the case that the birds do not forage in traplines but follow a familiar flight path. Once in the patch, they might forage by visiting flowers that are along a chosen flying direction. This “direction effect” was also observed when the hummingbirds’ flight path was restricted by blocking three out of four possible entries to a covered arena with an array of 16 flowers (Henderson 2004) hummingbirds made fewer errors on the array when they were trained to fly in through a single entrance to the arena than when they could access the arena through any one of four entrances. Future sequence training should take into account the bird’s tendency to approach a flower array by the same direction.

The data from this experiment allow for the design of an improved protocol to test whether hummingbirds can learn a sequence of rewarded flowers, which would support the assumption that these birds forage using traplines.

Chapter 3: Traplining at a small scale

INTRODUCTION

Territorial male rufous hummingbirds need to feed from hundreds of flowers every day and since these birds defend the same territories over weeks it is likely that they revisit flowers within their territory. We do not know, however, how or what “system” these birds use to avoid revisiting depleted flowers or whether they repeat the order in which they revisit profitable locations. The data from Chapter Two suggest that these birds may at the very least repeat the order in which they start to visit a patch of flowers. Although the hummingbirds did not visit the flower arrays following the predetermined order, these birds did establish their own order as they increasingly started at the same flower when they visited the flowers. Therefore, it is possible that these birds may develop their own sequence or traplines when foraging freely around several flowers.

To the best of my knowledge, however, no description of the actual foraging paths or order of flower visitation of a wild territorial or traplining hummingbird hitherto has been documented. Here, then, I set out to determine whether wild, free-living rufous hummingbirds (*Selasphorus rufus*) would develop traplines when I presented birds with 2-5 artificial flowers (one flower added at time). This foraging behaviour is not, however, exclusive to hummingbirds, so here I use a framework that has been developed for investigating whether an individual develops and maintains repeatable routes around several rewarded locations in the Hymenoptera (Thomson et al. 1997; Ohashi and Thomson 2009; Lihoreau et al. 2013). Based on that research, I used a procedure that had two main features. First I have presented flowers sequentially, adding new flowers one at a time (Lihoreau et al. 2010) to test whether the order of presentation would have an effect on the possible sequences used by the hummingbirds. Second, I used a pentagon-shaped flower array (e.g. Lihoreau et al. 2012) to allow me to determine whether hummingbirds would adopt a route dependent on the order of flower presentation or on the nearest neighbouring flower(s).

Based on experimental evidence from traplining behaviour in bumblebees (Reynolds et al. 2013), if male rufous hummingbird could trapline, I would expect to observe at least three key features: (1) the birds would fly few, repeated sequences around the flowers, (2) those sequences would be near to optimal (shortest or with few directional changes; Ohashi et al. 2007; Lihoreau et al. 2012; Lihoreau 2012), and (3) the optimization of the trapline would not depend on the order in which the birds first encountered the flowers (Lihoreau et al 2010).

METHODS

Subjects and experimental procedure

The subjects were six free-living territorial male rufous hummingbirds that had been flower trained in other unrelated tests. Trials were run between 0700 and 1800 hours Mountain Standard Time from June to July 2013.

Each bird was marked as described in the General Methods in Chapter Two. One day after a bird had been marked, I trained him to feed from a single artificial flower (a syringe cap surrounded by a 6 cm diameter, orange cardboard circle) attached to a white foam board (37.5 x 51 cm), which was mounted at a 45° angle on a metal stake (75 cm). At this stage the flower contained 600µl of 25 % (w/w) sucrose solution. Once a bird was feeding regularly from this flower, I added a second flower and recorded the order in which the bird visited them.

I used one of two criteria for the bird's behaviour before I added another flower to the board: the bird had to visit either (1) the flowers in the same order for 9 out of 10 consecutive bouts or (2) the board 60 times. Once a bird had reached one or other of these criteria, I then added an extra flower to the board and repeated this procedure until there were five flowers in total. Each time another flower was added to the board I also reduced the amount of sucrose solution contained within each flower to encourage the bird to visit all of the flowers on the board. When there were two flowers on the board each flower contained 40 µl, when there were three flowers each contained 25µl, 20 µl when there were four flowers and 15 µl in each of the five flowers (Figure 3.1.).

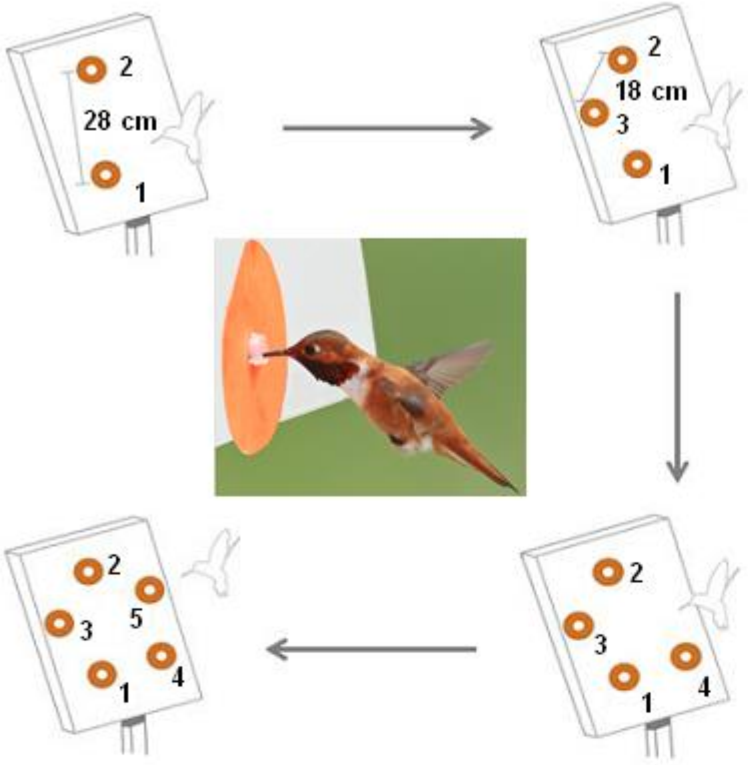

Figure 3.1. Diagram of the flower array. In the middle of the diagram there is a photograph of a male rufous hummingbird visiting one of the training flowers attached to the edge of the board. Flowers were added to the sequence only once the hummingbird had completed 60 visiting bouts or visited the flowers in the exact same order 9 out of 10 consecutive visits to the board. Whenever a flower was added to the array the amount of 25 % (w/w) sucrose solution contained in each flower was reduced so that the total amount of the reward remained at 75-80µl. Photo taken by Dr. Andy Hurly.

A foraging bout was defined as any time the bird visited the board, feeding from as many flowers as he wished and then flying away. Between bouts birds spend most of their time perching in a nearby branch (up to 75 % of their wake time; Hixon et al.1983) and came down to feed every ten minutes. Every time a bird came to visit the board all flowers visited by the bird were refilled. In the rare case that a bird did not finish all the solution available in one flower, I emptied the remaining sucrose and refilled that flower. Every time a bird came to feed all flowers contained reward.

All flowers were the same colour (orange) and the position of the flowers on the board was the same for all birds. The first two flowers that we presented were separated by 28 cm (Figure 3.1). Flower 3 was presented between Flowers 1 and 2, Flower 4 was added next to Flower 1 and Flower 5 was placed between Flowers 2 and 4. When all flowers were present on the board, the flower array formed a pentagon where each flower had its two nearest neighbour flowers at a distance of 18 cm (Figure 3.1). Since the order in which I added flowers meant that flowers were not contiguous, if birds were to fly around the flowers in the order in which we presented the flowers the total distance flown would be greater than if the birds flew from each flower to its nearest neighbour. I used a single board throughout the experiment as flowers could be attached and detached to the board for each stage of the experiment. All birds used the same board and birds were tested in their individual territories. Birds took in average 4.6 days ± 0.61 (mean ± S.E., n = 6) to complete all stages (from 2 to 5 flower on the board).

For each bout, I recorded the sequence in which the hummingbird visited the flowers.

RESULTS

The six hummingbirds made a total of 1333 visits (or bouts) to the board of which 59.7 % were complete bouts where birds visited all available flowers once, 32.5 % were incomplete bouts where the birds left the board before visiting all flowers available and 7.8 % were extended bouts where birds fed from all the flowers and then revisited one or more of the empty flowers. I included the incomplete sequences in further analyses since the percentage of these sequences was equally distributed across the four stages of the flower array (X^2 test; 2 flowers: 32.7 %; 3 flowers: 32.5 %; 4 flowers: 34.3 %; 5 flowers: 30.6 %; $X^2_3 = 0.2118$, $P = 0.975$) but as the extended bouts were rare for all four stages of the flower array, I excluded those sequences from further analyses.

Sequences used and repeated

In order to address the first feature of traplining, I first asked whether hummingbirds visited the flowers using all or most of the possible routes and whether the birds repeated some of those routes more often than others. Depending on the number of flowers on the board at one time, there was a different number of possible sequences in which birds could have visited the flowers: for 2 flowers 2! = 2, for 3 flowers 3! = 6, for 4 flowers 4! = 24 and for 5 flowers 5! = 120. I capped this latter number, however, at 60 sequences because, given the experimental criterion of a maximum of 60 bouts, the maximum number of possible sequences in which a bird could have visited the five flowers was 60. Here it should be noted that this estimate of the number of possible sequences the birds could have flown is an underestimate because it is based only on the number of possible complete sequences. However, this did not affect the analysis of the results since overall hummingbirds did not use all the possible complete sequences.

Additionally, they repeated at least one sequence more often than was predicted by chance (Binomial tests with chance probability set as the number of different sequences flown by each bird $P < 0.05$; also see the description below for each stage of the experiment; Figure 3.2, Appendix 3.1). The number of sequences repeated more often than expected by chance did not change significantly as more flowers were added to the board (Friedman's repeated measures ANOVA $X^2_3 = 7.68$, $P = 0.053$). Nevertheless, the number of different sequences flown by each hummingbird did increase significantly as the number of flowers on the board increased (Friedman's repeated measures ANOVA $X^2_3 = 17$, $P < 0.001$). *Post hoc* tests with Bonferroni corrections showed that the number of sequences flown did increase significantly from when there were two flowers on the board to four flowers (*difference* = 13) and from two flowers to five flowers (*difference* = 17; Figure 3.2). In both cases the critical difference ($\alpha = 0.05$ corrected for the number of tests) was 11.8 (Figure 3.2).

When there were two flowers on the board, including the bouts when birds did and did not visit all flowers (i.e. 1-2, 2-1, 1 only, 2 only), the average number of different sequences in which birds visited the flowers was 3.8 ± 0.16 (mean ± S.E., n = 6). There was no difference between the total number of sequences that started at flower 1 and the sequences that began at flower 2 (Binomial test with an expected proportion of 0.50 for the sequences starting at flower 1: 134/301, $Z = 1.90$, $P = 0.064$). Of the different routes the birds flew around the flowers, two of those sequences formed the majority (average = 73.61 % ± S.E. 3.34 of all sequences, n= 6). For all birds, the distribution of the number of times each sequence was flown differed significantly from that expected by chance. I used multinomial tests to compare each bird's distribution of the number of times they flew each sequence with the total number of sequences flown by that bird as the random probability (for all birds $P < 0.05$). For example, if a bird had used four sequences during 60 visits to the board, the expected distribution of the number of times each of those four sequences was repeated would be 15 each.

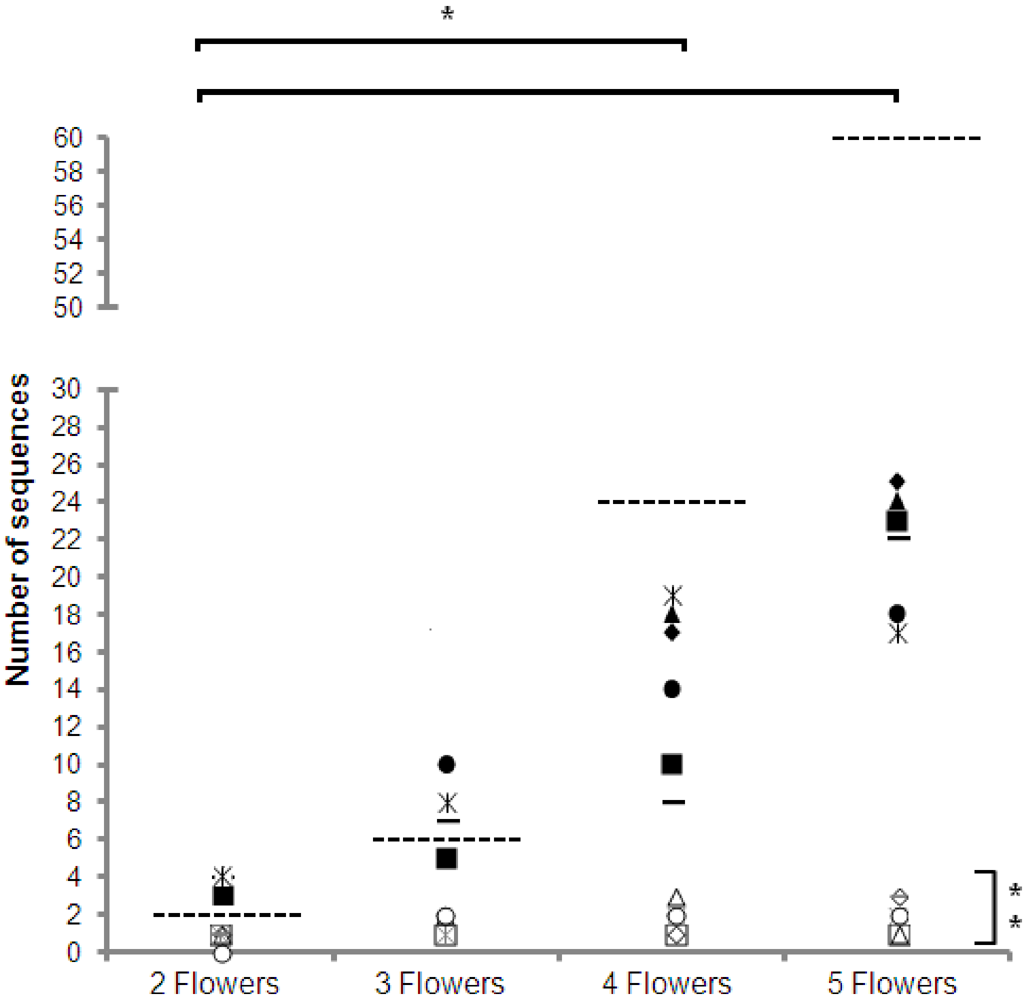

Figure 3.2. The number of sequences of flowers flown at least once by each hummingbirds (n = 6; black symbols). * *P* < 0.001 as tested by a Friedmans's repeated measures ANOVA. The empty grey symbols represent the number of sequences that each bird repeated more often than expected by chance as tested by a Binomial test with chance probability set as the number of different sequences flown by each bird. ** *P* < 0.05. The dotted lines represent the total number of possible different sequences the birds could have flown depending on the number of flowers on the board if birds were to visit all flowers once.

Since the distribution of the sequences that the birds flew was different than expected by chance, I also then looked at whether one or more sequences was repeated more often than the others. For each bird, the number of different sequences used and the total number bouts made by that bird set the probability of repeating a sequence. For example, if a bird flew four different sequences (probability = 1/4) and visited the board on 60 bouts, then any one sequence that was repeated more than 23 times was flown more often than predicted by chance. When there were two flowers on the board, five out of the six birds repeated one of the sequences more often than expected by chance (Binomial tests with the probability set as the number of sequences used on the total of bouts made to the board, $P < 0.05$; Figure 3.2).

When there were three flowers on the board the hummingbirds flew an average of 8 ± 0.77 (mean ± S.E., n = 6) sequences (including incomplete sequences). Of all visits to the board, the two most common routes formed on average 59.09 % ± S.E. 4.34 (n = 6) of the total and the distribution of the number of times the sequences were repeated was significantly different than expected by chance (For all birds; multinomial test: $P < 0.001$). Two of the birds repeated one sequence more often than predicted by chance while the other four birds repeated two sequences more often than was predicted by chance (Binomial tests with probability set as the number of sequences flown by each bird at $P < 0.05$; Figure 3.2). To test whether a bird flew around the flowers on the board in a preferred direction I compared the number of complete sequence (when the bird visited all flowers) in a clockwise direction (sequence ‘132’, ‘213’or ‘321’) to the total of complete sequences. The analysis of the complete sequences showed that birds 1, 2 and 4 preferred to fly sequences in a clockwise direction (Binomial test with an expected proportion of 0.50 bird 1:18/20, $Z = 3.57$, $P < 0.001$; bird 2: 17/23, $Z = 2.29$, P

= 0.034; bird 4: 25/35, $Z = 2.53$, $P = 0.01$), but that birds 3, 5 and 6 had no preference for either direction (Binomial test with an expected proportion of 0.50; clockwise, bird 3: 27/45, $Z = 1.34$, $P = 0.23$; anticlockwise, bird 5: 25/ 42, $Z = 1.23$, $P = 0.25$, bird 6: 24/45, $Z = 0.44$, $P = 0.766$),

When the board held four flowers the hummingbirds flew on average 14.5 ± 1.92 (mean ± S.E., n = 6) sequences out of the 24 possible. The distribution of the number of repeated sequences was significantly different from that expected by chance (for all birds, multinomial test $P < 0.001$) for all birds. On average, birds repeated 1.8 (± S.E. 0.30, n = 6) sequences more often than predicted by chance (Binomial tests $P < 0.01$; Figure 3.2). The two most common sequences formed 45.35 % ± S.E. 5.8 (n = 6) of the total. In order to assess direction preferences we counted the number of sequences birds flew in a clockwise or anticlockwise direction including the incomplete sequences that did not skip contiguous flowers (e.g. a clockwise sequence would be “1 3 2” or “4 1 3 2”). Bird 1 was the only bird that showed a significant preference for flying sequences in clockwise direction (Binomial test with an expected proportion of 0.50 clockwise 28/31, Z = 4.49, P < 0.001). Birds 2 and 3 flew more sequences in a clockwise than in an anticlockwise direction but the difference was not significant (Binomial test with an expected proportion of 0.50 clockwise flights: bird 2: 21/31 $Z = 1.97$, $P = 0.07$; bird 3: 14/27, $Z = 0.19$, $P > 0.99$). Birds 4, 5 and 6 did fly more sequences in an anticlockwise direction but again this difference was not significant (Binomial test with an expected proportion of 0.50; anticlockwise, bird 4: 15/24, $Z = 1.22$, $P = 0.30$; bird 5: 21/39, $Z = 0.48$, $P = 0. 74$; bird 6: 31/49, $Z = 1.85$, $P = 0.08$).

When the board held all five flowers, the birds flew on average 21.6 ± 1.38 (mean ± S.E., n = 6) of the total of 60 possible sequences. During this stage, the two most common routes formed 39.20 ± 5.11 % of the total (n = 6). The distribution of the number of repeated sequences was different than that expected by chance (For all birds, multinomial test, $P < 0.001$). Of the sequences that the birds flew, 2 (± S.E. 0.36, n = 6) of them were flown more often than expected by chance (Binomial tests with probability set as the number of sequences flown by each bird at $P < 0.05$; Figure 3.2). Including incomplete and complete sequences that did not skip contiguous flowers birds did not show a preference for a clockwise or an anticlockwise direction (e.g. of sequences with a clockwise direction: “1 3 2”, “4 1 3 2”, “5 4 1 3 2”) as tested by Binomial tests with an expected proportion of 0.50 (bird 1: 14/27, $Z = 0.19$, $P > 0.99$; bird 2: 21/39, $Z = 0.48$, $P = 0.74$; bird 3: 22/36, $Z = 1.33$, $P = 1.33$; bird 4: 29/51, $Z = 0.98$, $P = 0.40$; bird 5: 25/45, $Z = 0.74$, $P = 0.55$; bird 6: 26/51, $Z = 0.14$, $P > 0.99$).

Continuous use of the preferred sequence

As an additional assessment of the birds’ traplining behaviour I also looked at whether the sequences used became more similar to each other as the birds made more visits to the board. I did this by comparing consecutive sequences, following an analysis first proposed by Thomson et al. (1997) where the authors examined traplining in bumblebees. To determine if the similarity between sequences increased with the number of visits to the flowers when there were three, four and five flowers, I first aligned the start point of every consecutive sequence (including for this analysis the extended sequences as well) and then created a matrix for each pair of sequences. I then calculated a similarity index by dividing the number of matches by the total number of cells along the diagonal. The similarity index of two consecutive sequences could range from 0 (where consecutive sequences are completely different) to 1 (where consecutive sequences are identical). I did this for each bird, averaged the scores across all six birds and compared the similarity indexes to a 95% threshold of similarity indexes calculated from 1,000 random simulated sequences. If the birds' similarity indices surpassed the 95 % threshold then the birds' similarity indexes were considered to be significantly different from chance (at the 5% level).

When there were three flowers on the board, the similarity index increased across the first 20 visits to the board but then decreased and oscillated as the birds made more visits to the flowers, there was no significant difference between the average SI of birds across the sixty bouts (compared in six bins of 10 bouts; Friedman's repeated measures ANOVA, $X^2_5 = 7.94$, $P = 0.15$). Nevertheless, 87 % of the birds' similarity indices were above the random threshold of the simulated sequences similarity indices (0.33; Figure 3.3). When there were four and five flowers on the board, the birds' sequences did not get more similar to each other as they visited more the board, as there was no significant difference in the average SI (of ten bouts) of birds across the six bins (Friedman's repeated measures ANOVA, Array with four flowers: $X^2_5 = 3.62$, $P = 0.604$; Array with five flowers: $X^2_5 = 7.11$, $P = 0.212$). Only 8.9 % of the similarity indices between consecutive sequences were above the threshold of the random simulated sequences (0.5 and 0.4, for the four-flower array and the five-flower array, respectively; Figure 3.3). Although birds did repeat one or two sequences more times than expected by chance, they did not tend to fly these preferred sequences consecutively. Rather, the birds interspersed their two most common sequences with sequences that they flew only once or twice.

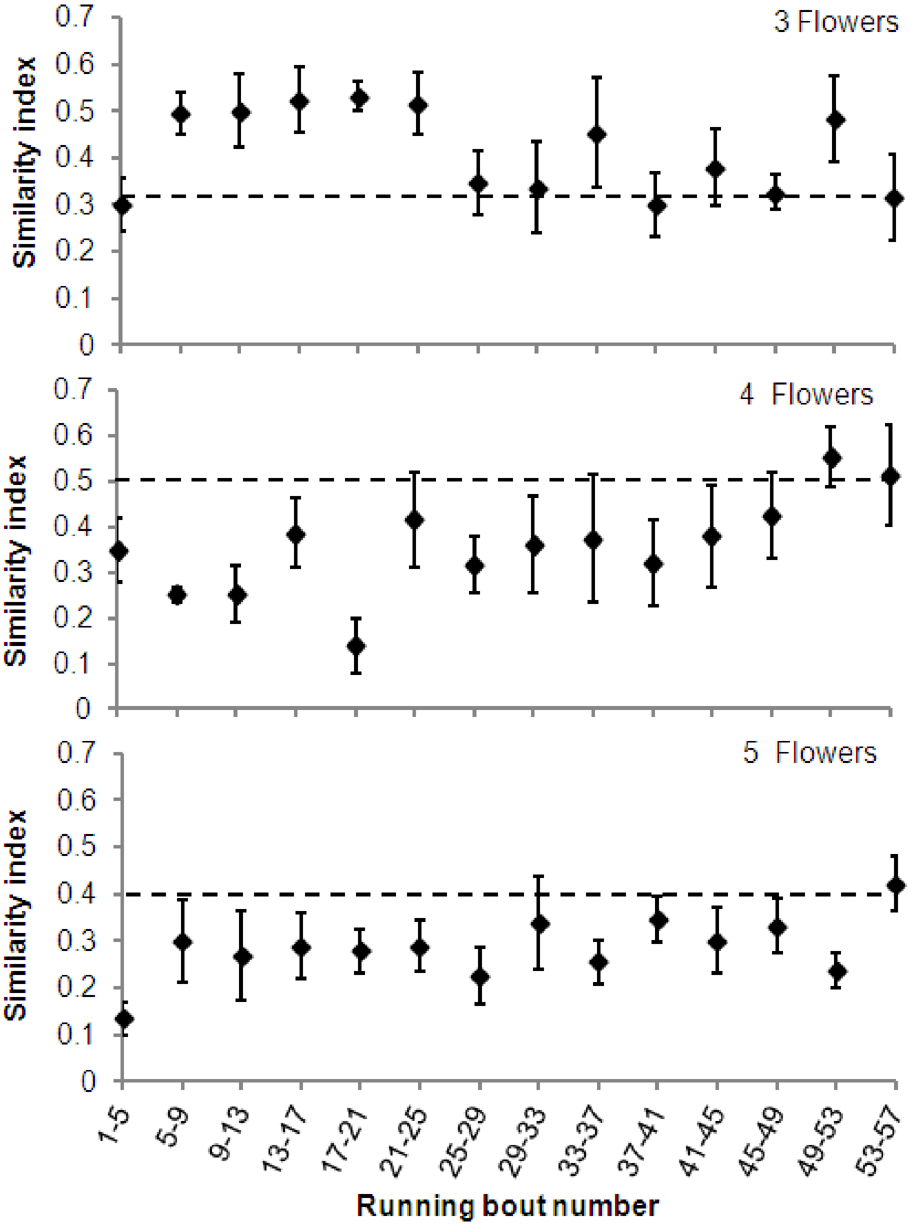

Figure 3.3. The mean (± SE) similarity index of consecutive sequences flown by all birds (n = 6) grouped in a running average of five bouts. The top panel shows the similarity index of sequences flown when there were three flowers. The middle panel shows the similarity indexes of the sequences the hummingbirds flown when there were four flowers and the bottom panel shows the similarity index when there were five flowers on the board. The dotted line in each panel represents the 95 % of similarity indexes derived from the random simulated sequences (n = 1,000). Points above the

dotted line represent a similarity index that is significantly greater than expected by chance (at the 5 % level).

Distance of the preferred sequences

Because animals foraging by following traplines are also predicted to develop optimal routes, I tested the second feature of traplining by assessing the distance of the sequences the birds used. To determine whether birds flew the shortest routes around the flowers, I categorized sequences into short-distance sequences (sequences where birds visited all flowers without skipping neighbour flowers, e.g. sequence 3-1-2-4-5 or sequence 2-3-1-4; Figure 3.1) and long-distance sequences (where birds flew between non-neighbouring flowers e.g. sequence 1-2-3-4-5 or sequence 3-2-4-1; Figure 3.1, Appendix 3.1).

Including the incomplete and complete sequences, the birds made a total of 763 short bouts and 234 long bouts along the three last stages of the board. The number of flowers on the board from three flowers to five flowers did change the percentage of short and long distance sequences each bird made (Friedman’s repeated measures ANOVA $X^2_2 = 8.33$, $P = 0.015$; Figure 3.4). *Post hoc* tests with Bonferroni corrections showed that the number of short sequences flown when there were five flowers on the board did increase significantly, while the number of long sequences decreased, compared to the sequences flown when there were four flowers on the board (*difference* = 10) with the critical difference ($\alpha = 0.05$ corrected for the number of tests) being 8.29 (Figure 3.4).

I analyzed the percentage of short and long distance sequences the birds made when there were three, four and five flowers on the board with Generalized Estimate Equation or GEE analyses, with a Poisson distribution and an auto-regressive correlation structure. I chose to use this model because GEE models allow the use of count data of repeated measures (Zuur et al. 2009). I used the geepack in R as described by Halekoh et al. (2006). Birds flew more short-distance sequences (including both the incomplete and complete sequences) than they flew long-distance sequences (GEE: Main effect of Distance: $X^2_1 = 36.3$, $P < 0.001$) irrespective of the number of flowers on the board (GEE: Main effect of Array: $X^2_2 = 39.3$, $P < 0.001$). Furthermore, the interaction between the distance and the type of array was also significant (GEE: Interaction Distance × Array: $X^2_2 = 99.4$, $P < 0.001$): only when there were four flowers on the board, there was no significant difference between the percentage of short-distance sequences and long-distance sequences flown by the birds (GEE: Array 4: Wald = 1.67, $P = 0.19$; Figure 3.4).

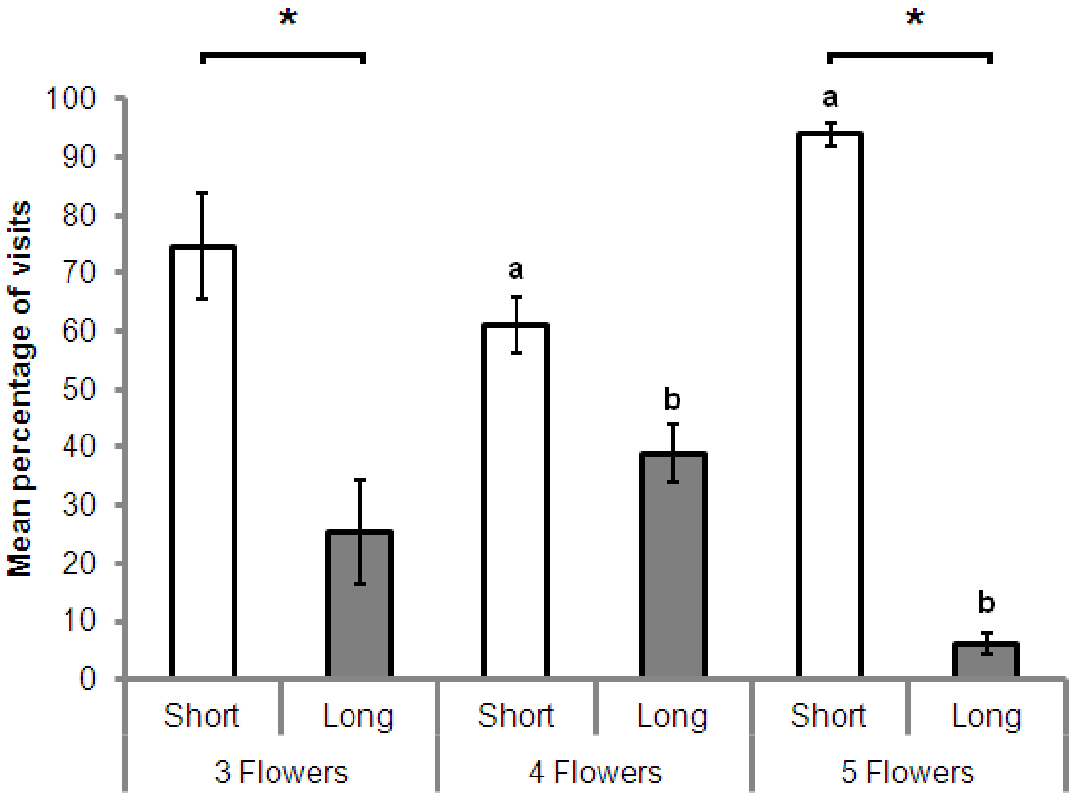

Figure 3.4. The mean (± S.E.) percentage of flights for which the hummingbirds flew a short-distance sequence or a long-distance sequence (n = 6) when there were three, four and five flowers on the board. * represents $p < 0.01$, as tested by a Generalized Estimate Equations (GEE) test. Columns with the same letter are significantly different from each other (Friedman's repeated measures $P = 0.015$).

Effect on flight of order of flower presentation

The third key feature of traplining would be that, when I added a flower to the board, the route the birds flew around the flowers would not follow the order of flower presentation. One way to determine if this was the case was to look at whether the flower at which birds began their routes differed as I added more flowers. When there were two flowers on the board there was no difference between the number of sequences that started with flower one or flower two (Wilcoxon test, W= 13.5, $P = 0.52$). Nevertheless, once the third flower was added to the board, the majority of birds' sequences started with flower three (mean ± S.E. 41.4 % ± 9.2, n = 6) and the difference in the distribution between the sequences that started with flower one, two or three differ significantly from a random distribution ($X^2_2 = 13$, $P = 0.001$). When I added flowers four and five, most of the birds' bouts began by flower three (mean ± S.E. 45.96 % ± 9 and 36.61 % ± 9.41; for the four and five flower array, respectively, n = 6). The difference in the percentage of sequences that began at flower number three rather than at the other flowers was significant ($X^2_3 = 36.6$, $P < 0.001$ for the array with four flowers and $X^2_4 = 30$, $P < 0.001$, for the array with five flowers). Therefore, by the time there were three flowers in the array, birds tended to begin their sequence at the same flower and not by the presentation order of the flowers. The reason why hummingbirds preferred to start the majority of their visits to the board by flower three can only be speculated at this point.

DISCUSSION

In this experiment, the behaviour of male rufous hummingbirds met three of the key features that constitute traplining: (1) birds repeatedly flew a small subset of the many possible sequences around an array of flowers and all the hummingbirds repeated one or two sequences more often than others; (2) birds were much more likely to fly the shortest routes around the flowers; and (3) the sequence of flowers the birds visited did not reflect the order in which we presented the flowers. Furthermore, once there were at least three flowers in the array, hummingbirds began their sequence from the same flower.

I contend that these data provide the first quantitative confirmation that at least some hummingbirds are traplining foragers. These data also show that this foraging style is used even by territorial birds: each of these hummingbirds was fiercely maintaining a foraging territory centred around a single feeder on which they fed almost exclusively. I can confirm, therefore, that territoriality and foraging by traplining are not mutually exclusive (Feinsinger 1976). Furthermore, birds developed traplining even at the relatively small spatial scale I used: the flowers were spaced 18 cm apart with the greatest distance between any pair of flowers of 28 cm. This might suggest that hummingbirds will trapline among flowers both around their territory and/or at smaller spatial scales, such as flowers on the same bush even though they can learn the locations of a number of rewards, apparently without flying a regular route around them (Healy and Hurly 2013).

Hummingbirds flew regular routes around the flowers but also continued to alternate between their commonly-used sequences and novel sequences that they used only rarely. This “sampling and shifting traplining” behavior is expected and advantageous when the environment is not stable (Ohashi and Thomson 2005). In an environment where flowers cease to produce nectar or when competition is increased, hummingbirds, like other pollinators, should try to optimize their routes (Lihoreau et al. 2010) and sampling novel sequences is a method by which these birds might achieve this.

Although the traplining I observed in my hummingbirds shared a number of features with the same behaviour observed in the Hymenoptera (Reynolds et al. 2013), one of the most obvious differences was that, unlike the strong individual directional biases that bumblebees show when traplining (Lihoreau et al. 2011), our hummingbirds showed little preference as to the direction in which they flew around the flowers. It is possible that this was due to the very small scale over which we tested our birds but further experimentation is required to confirm this.

Given the relatively short distances between the flowers, the fact that the birds were much more likely to fly the shortest distances around the flowers might suggest that flight costs even over these comparatively short distances are relevant. And yet, across the different numbers of flowers, 30 % of the visits birds made to the board were incomplete (i.e. they did not visit all of the available flowers). Since I refilled emptied flowers every time a hummingbird visited them, this means that birds were frequently drinking only around 60 µl of the available 75-80 µl. As even 80 µl is less than the amount a male rufous hummingbird normally drinks in each bout (the average meal size of a territorial male rufous hummingbirds is 100 µl of sucrose solution per bout (Karasov et al. 1986), which is about a sixth of its' crop size (Hainsworth and Wolf 1972), given the reliability of food at the board, the birds were prepared to trade off more sucrose for carrying less weight. Carrying less weight in their crops would allow them to perform their flight displays and to chase conspecifics at greater speeds than if their crops were filled to a greater extent (Carpenter et al. 1991). This possibility gains some support from the observation that non-territorial males drink far more sucrose when they manage to steal a foraging opportunity at a feeder than do the owners of those feeders (Powers and McKee 1994; Gass and Garrison 1999). Intruders have to take as much as they can when they can because energetically rich patches are rare. By returning to flower patches at regular intervals, territorial hummingbirds that trapline may gain an energetic advantage over the intruders. Intruders depend on occasionally encountering energetically rich patches that have not been visited recently, while territorial males that systematically visit the patches in their territory can "harvest" the nectar as it renews. Just as for bees and bumblebees, traplining birds do not need to depend on finding the rare rich patch but instead; these birds can both feed steadily from the renewing resource and outcompete intruders at the same time (Possingham 1989; Ohashi and Thomson 2005).

It is less clear that the explanation for birds flying the same routes repeatedly or for their initiating most flights at the same flower, irrespective of the number of flowers available, is because these strategies are energetically more efficient. It seems unlikely, for example, that the first flower of each foraging flight was closer to the bird's perch than were the other flowers, as I placed the board was so that it was facing the bird's most commonly used perch such that all of the flowers were essentially equidistant from that perch. Rather, it seems plausible that few, regular routes reduce the cognitive load of the decision making as to which flower to visit next: the typical rule, then, may be simply 'fly to the flower immediately in front' and in that way, the animal remembers to avoid empty flowers. Although these hummingbirds can and do remember emptied flowers in order to avoid revisiting them (Hurly 1996), it would appear that they will also readily adopt a movement pattern via which they need not remember each flower. This behavioural response seems especially efficient given the typical duration of a foraging bout for these birds (< 10 seconds) and that they have flown more than four thousand kilometres for breeding to be completed within six weeks: these birds spend the vast proportion of their time in looking out for conspecifics, females for mating and males against whom they defend their feeders. Key to the adoption of this foraging pattern, even at such a small scale, is the reliability of the rewards. If the flowers had different refill rates, for example, it seems unlikely that the birds would trapline around them in the way we observed. Rather, I would expect the birds to learn the refill rates associated with each location and then to visit the flowers accordingly.

In nature, flowers take much longer to replenish than was the case in my experiment. In order for hummingbirds to benefit from foraging by using a trapline, therefore, birds would actually have to wait for longer before they revisit particular flowers i.e. before they fly around the same route. If the bird has exclusive access to the flowers, then, they can afford to delay their return indefinitely. If, however, the longer flowers are left unvisited the more likely it is that an intruder will visit one or more of the flowers, then hummingbirds would benefit from learning not just the spatial arrangement of the flowers but also both the time at which they emptied the flowers and the time the flowers take to refill. Because hummingbirds can learn the time at which flowers refill (Henderson et al. 2006; Marshall et al. 2012; Samuels, Hurly and Healy 2014) as well as what, where and in which context flowers are rewarded (Marshall et al. 2013; Jelbert et al. 2014), it seems plausible that they could integrate temporal and spatial information into their trapline. Indeed, I would suggest that traplining animals in the ‘real’ world all need to pay attention to both temporal as well as spatial information. Whether this is the case is the subject of the next chapter where I have tested if these hummingbirds can learn the different times at which different patches are rewarded.

Chapter 4: Time-place learning in hummingbirds

INTRODUCTION

Having shown that territorial male rufous hummingbirds will repeat the order in which they visit several artificial flowers, I then wanted to know if these birds could also time their visit to different locations. As suggested by Paton and Carpenter (1984), it is possible that hummingbirds feed from different locations at different times of the day. Learning both the time and place of that event is known as time-place learning and has been studied in a wide range of species.

Typically in the laboratory a time-place learning protocol involves animals being trained to receive a food reward in one place at a specific time of day and a food reward in another place at another time. A number of species from a wide taxonomic range can do this (garden warblers *Sylvia borin* Biebach, Gordijn, and Krebs 1989; Krebs and Biebach 1989; pigeons *Columba livia* Saksida and Wilkie 1994; ants *Ectatomma ruidum* Schatz, Lachaud, and Beugnon 1999). In the wild animals appear to use this kind of memory to return to rewarding locations at the right time: animals will arrive from distant locations at the place and the time when food resources are available predictably (honey bees *Apis mellifera* Beling 1929; Wahl 1932; oystercatchers *Haematopus ostralegus* Daan and Koene 1981; amakihis *Loxops virens* Kamil 1978; kestrels *Falco tinnunculus* Rijnsdorp, Daan, and Dijkstra 1981; marine iguanas *Amblyrhynchus cristatus* Wikelski and Hau 1995). For instance, scavenging birds anticipate food provided by people that eat their daily lunch al fresco as the birds appear one to two hours before people gather (pigeons, gulls *Larus sp.*, starlings *Sturnus vulgaris* and crows *Corvus caurinus* Wilkie et al. 1996). Nevertheless, some species seem to be better at learning the temporal information in a time-place learning task than are others (Thorpe and Wilkie 2006). For example, when only the time of day predicted the availability of a reward rats did not learn when to expect a reward, but when rewards were available at different locations at different times of the day, the rats readily learned when to go to each of the two rewarded locations (Deibel et al. 2014).

Because flowers often refill over time, it seems plausible that nectar-feeding animals would benefit from learning not only the location of profitable flowers but also the time when these locations are profitable. For example, the Hawaiian honeycreeper amakihi appears to wait until flower clusters have had enough time to refill before returning to forage (Kamil 1978). Similarly, the nectar-feeding tropical ant *Paraponera calvata* could learn the time and place where nectar was provided (Harrison and Breed 1987) whereas fourteen species of European ants that do not feed on nectar did not show any evidence of time-place learning (Dobrzański 1956). To the best of my knowledge, however, the only field experiment on time-place learning has been conducted on the nectar-feeding Mexican ant *Ectatomma ruidum*: after eleven days, the ants of two different colonies had learned which of two sites contained a honey reward at two different times of day (Schatz et al. 1999).

Like *E. ruidum,* hummingbirds too, should benefit from learning both the time and place of profitable flowers as they need to feed from hundreds of flowers every day to maintain a positive energy budget (Armstrong, Gass, and Sutherland 1987; Gass and Garrison1999; Kodric-Brown and Brown 1978). Information that would allow them to avoid wasting energy by visiting unprofitable flowers should, therefore, be especially useful. Furthermore, for those hummingbirds that forage by traplining, time-place learning would seem especially advantageous.

Although there is, surprisingly, little compelling evidence that hummingbirds trapline, some hummingbirds can learn both the location of profitable places and the time intervals between reward deliveries (Healy and Hurly 2004; Henderson et al. 2006; Sutherland and Gass 1995). They also seem to do so with few experiences. For example, rufous hummingbirds *Selasphorus rufus* will learn rewarded spatial locations in a single visit (Flores-Abreu, Hurly, and Healy 2013) and they will learn refill intervals within a single day of foraging experience (Marshall, Hurly, and Healy 2012; Samuels, Hurly, and Healy 2014). It is not clear from these data, however, whether these birds will also learn an association between a rewarded location and the time of day at which it is rewarding.

To test whether wild, free-living hummingbirds would learn a time-place association I presented territorial male rufous hummingbirds with four patches of artificial flowers whereby each of the patches was rewarded one hour at time (e.g. Patch 1 contained reward from 8-9 am, the flowers in Patch 2 contained a reward from 9-10 am and so on for the four patches). Additionally, to determine which of two possible timing mechanisms birds might use to visit the correct flower patch at the correct time, after six days of training I presented the birds with a test. During the test day I presented the patches only at the fourth hour (with all flowers empty). If birds learned the order in which they encounter rewarded patches they should go first to the flowers in the first patch of the day to contain reward. If, however, the birds learned the specific times of day at which each patch contained a reward birds should fly directly to flowers in the fourth patch.

METHODS

Experimental site and Subjects

The experiments took along the same valley as described in previous chapter and with renewal of all ethical approvals. The test subjects were eight male rufous hummingbirds six of which had no previous experimental experience while the other two birds had been tested before during the traplining board test described in Chapter Three.

Experimental training and array

For the experiment, the birds were required to visit four different patches of artificial flowers over a four-hour period with only one patch rewarded per hour (Figure 4.1). Each of the four patches contained six flowers each separated from its nearest neighbours by 40 cm, forming a rectangular patch. Patches were separated from each other by 3 m, measured from the edges of the closest flowers. The flowers in each patch were all of the same colour but those of the different patches were different colours (blue, pink, purple, orange). Across birds, the order in which each of the four patches was rewarded and the colour of patches was pseudo-randomly assigned. The patches of flowers were of different colours as although colours cues can facilitate the use of spatial and temporal information (Hurly and Healy 2002; Samuels, Hurly and Healy 2014), hummingbirds prefer to use spatial over feature cues when both types of cues indicate reward (Miller et al. 1985; Hurly and Healy 1996) and will only use feature cues when the spatial cues are unreliable (Hornsby et al. 2014; Flores-Abreu et al. 2013).

Since patches were presented for only four hours a day I increased the number of birds used in this experiment by presenting half of the individuals with patches in the morning (from 0800 to 1200hrs, Mountain Standard Time) and the other half of the birds during the afternoon (from 1300 to 1700 hrs).

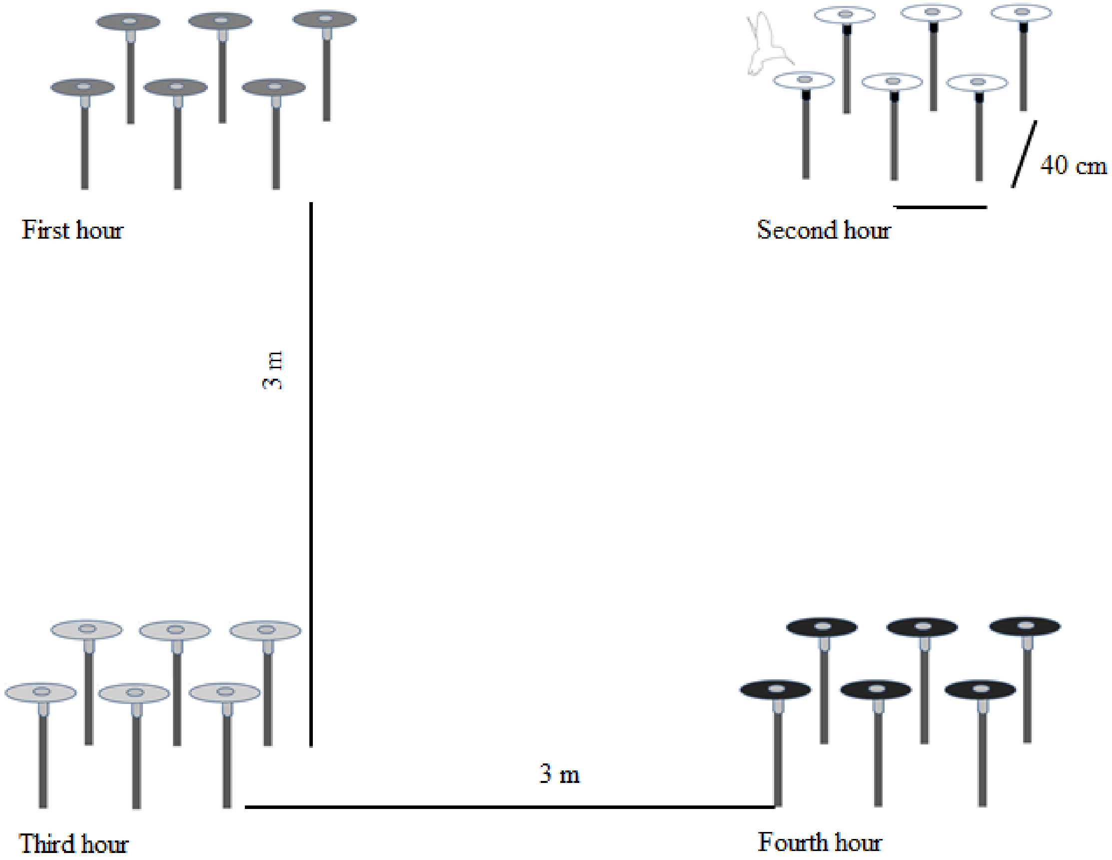

Figure 4.1. A schematic diagram of the flower array. The flowers were the same colour within patches but differed between patches. The patches were three metres apart and the flowers within a patch were 40 centimetres from their nearest neighbour(s).

For the four-hour training and testing period each day of the experiment, I removed the feeder at the beginning of the first hour and returned it at the end of the fourth hour. On the first day of the experiment (Day 0), after removing the feeder, I presented just the flowers of the first patch in their location and allowed the bird to feed from them for an hour. Each rewarded flower contained 50µl of 25% sucrose solution. I refilled any flower that the bird emptied as soon as he had left the patch. At the end of the hour, I removed that patch of flowers and put out the second patch at the second location. At the end of the second hour, I put out the third patch at the third location and similarly for the fourth patch at the fourth hour. I determined that the bird had ‘visited’ a flower when he had inserted his tongue into that flower and defined a bout as all of the visits the bird made to flowers in any patches before he left the area where the flowers were located.

The day following this sequential patch presentation (Day 1) I presented a bird with all four patches simultaneously placed at the same locations as for the previous day at the hour designated as the first hour for that bird. Only the flowers in the patch designated as Patch 1 for that bird were rewarded for that first hour and they each contained 40 µl, which between the six flowers provided a total of 240 µl of 25% sucrose. This amount of sucrose was sufficient for the bird not to be able to empty all of the flowers in the patch in one bout. All of the flowers in the other three patches were empty during that hour.

For each bird, the order, time of day and location in which the patches were rewarded remained the same across the five days of the experiment. After each bout I refilled the sucrose in the flowers visited, so that for that hour, the flowers on the rewarded patch always contained a reward. At the end of each hour, I emptied all of the flowers in the rewarded patch and added 40 µl of 25% sucrose to each of the flowers in the next patch. Across birds, the relative position and the order of reward across the patches differed (note that each bird was tested within its own territory). For half of the birds contiguous patches were rewarded one after the other, while for the other half of the birds the subsequent patches were located in opposite corners.

I presented the patches in this way for five consecutive days. During each four-hour session I recorded all visits made by a hummingbird, noting both the time and location.

Test

To determine whether birds used the time of day or the sequence in which they visited patches to locate the rewarded patch, I carried out a test on the day after training had finished. During this test, I presented the patches only at what had been the fourth (last) hour of the normal sessions, skipping the three first hours of the normal training session. This type of test, where the first session(s) are skipped, allowed us to determine whether animals use a daily ordinal timing strategy. If the birds were to first visit the first patch regardless of the time of day (in this case, the correct patch according to the time of day was the fourth patch) that would mean that the hummingbirds used the order in which patches were rewarded daily and not the time of day. All of the flowers in all four patches were empty during the test and I allowed birds to make up to three visiting bouts before I removed all of the flowers and returned the feeder to its usual location.

Analysis

I used the first visit hummingbirds made during each bout as the response variable. Using a binomial test, I compared the number of correct first visits hummingbirds made with the number I would have expected if the birds had visited the flowers at chance (25%).

I then used Wilcoxon tests to (1) determine whether the choices made by birds tested in the morning differed from those made by the birds tested in the afternoon and, (2) compare the number of correct first choices made by the birds on the Day 1 and Day 5 of the experiment. I used related Friedman’s ANOVAs to compare the percentage of first visits made to each patch during each of the four hours. I also used a binomial test to determine whether the percentage of visits to the correct patch as a second choice during the first bout of each hour differed from that expected by chance (33.3%). I used chi-square goodness-of-fit tests to determine whether there was a difference between the patches in the number of birds that first visited each patch during the test. Whenever the expected number was smaller than five, I used a randomization test with 10 000 permutations to assess the probability of obtaining the observed and more extreme results (Sokal and Rohlf 1995). Again, all the analyses were carried out using the statistical software R (version 2.13.2, R Development Core Team 2012).

RESULTS

Training

The percentage of correct first choices (visits where the bird first probed flowers in the patch that was rewarded at that time) made by the hummingbirds did not differ significantly between the morning and afternoon groups (Wilcoxon 2-sample signed ranks test, $W = 13$, $P = 0.2$). I, therefore, pooled the data from both groups for all further analyses.

Even on Day 1 the birds, as a group, made more correct first visits than would be expected by chance (25%): 57 total correct first choices out of a total of 141 first visits to the array made by all hummingbirds (Binomial test with an expected proportion of 0.25 correct choices 57/141, $Z = 4.13$, $P < 0.001$). Individually, however, only three of the birds made more correct first choices than would be expected by chance during Day 1 (Binomial test with an expected proportion of 0.25 correct choices, Bird 2: 13/21, $Z = 3.66$, $P < 0.001$; Bird 4:10/22, $Z = 1.97$, $P = 0.024$; Bird 5: 14/26, $Z = 3.17$, $P < 0.001$; Figure 4.2). As the experiment proceeded, 6 out of 8 birds increased the percentage of correct choices they made and by Day 5 the mean percentage of correct choices had increased significantly compared to the first day (the mean percentage of correct visits on Day 1 was 36 ± 0.05 % (± S.E. n = 8) versus 54.1 ± 0.06 % on Day 5; Wilcoxon test, *W* = 1, *P* = 0.015). On Day 5, only birds 7 and 8 did not make more correct choices than expected by chance (Binomial test with an expected proportion of 0.25 correct choices Bird 7: 1/7, *P* = 0.311; Bird 8: 4/10, *P* = 0.1459; all other birds *P* < 0.01, Figure 4.2). These two birds were tested shortly before they started migrating back to Mexico. As a result , Birds 7 and 8 visited the patches half as many times as the other six birds did (mean ± S.E number of visit to the patches; Birds 7 and 8: 8.8 ± 0.2; Birds 1 through 6: 20.9 ± 1.08).

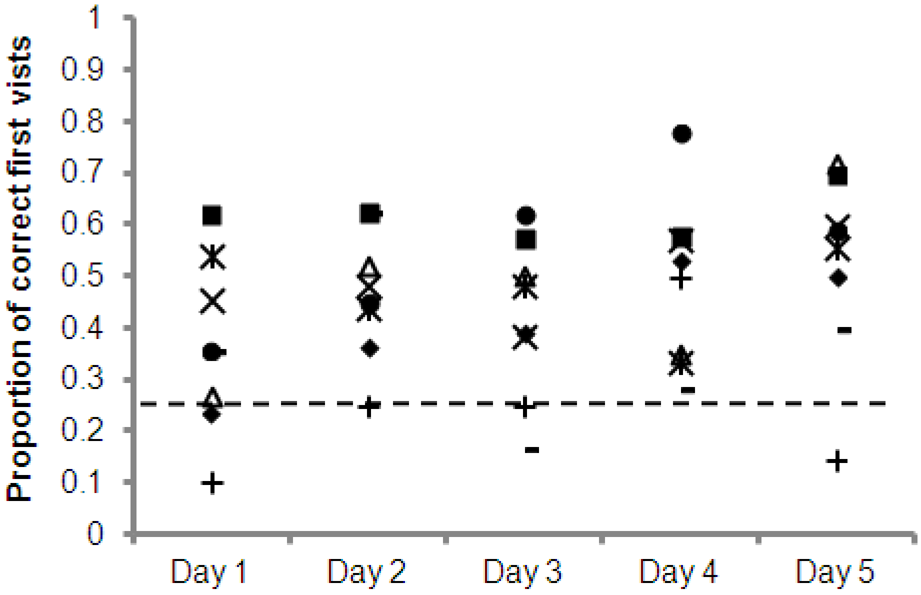

Figure 4.2. Proportion of correct first choices made by each of the eight birds (different symbols) on days 1 through 5 of the training stage. The dashed line at 0.25 represents chance performance.

For all four patches and across all five days, the birds' first visits were mostly to the correct patch at the correct time (related Friedman's ANOVA first hour: X^2_3 = 15.85, *P* = 0.001; second hour: X^2_3 = 13.23, *P* = 0.004; third hour: X^2_3 = 16.4, *P* < 0.001; fourth hour: X^2_3 = 15.07, *P* < 0.001; Figure 4.3).

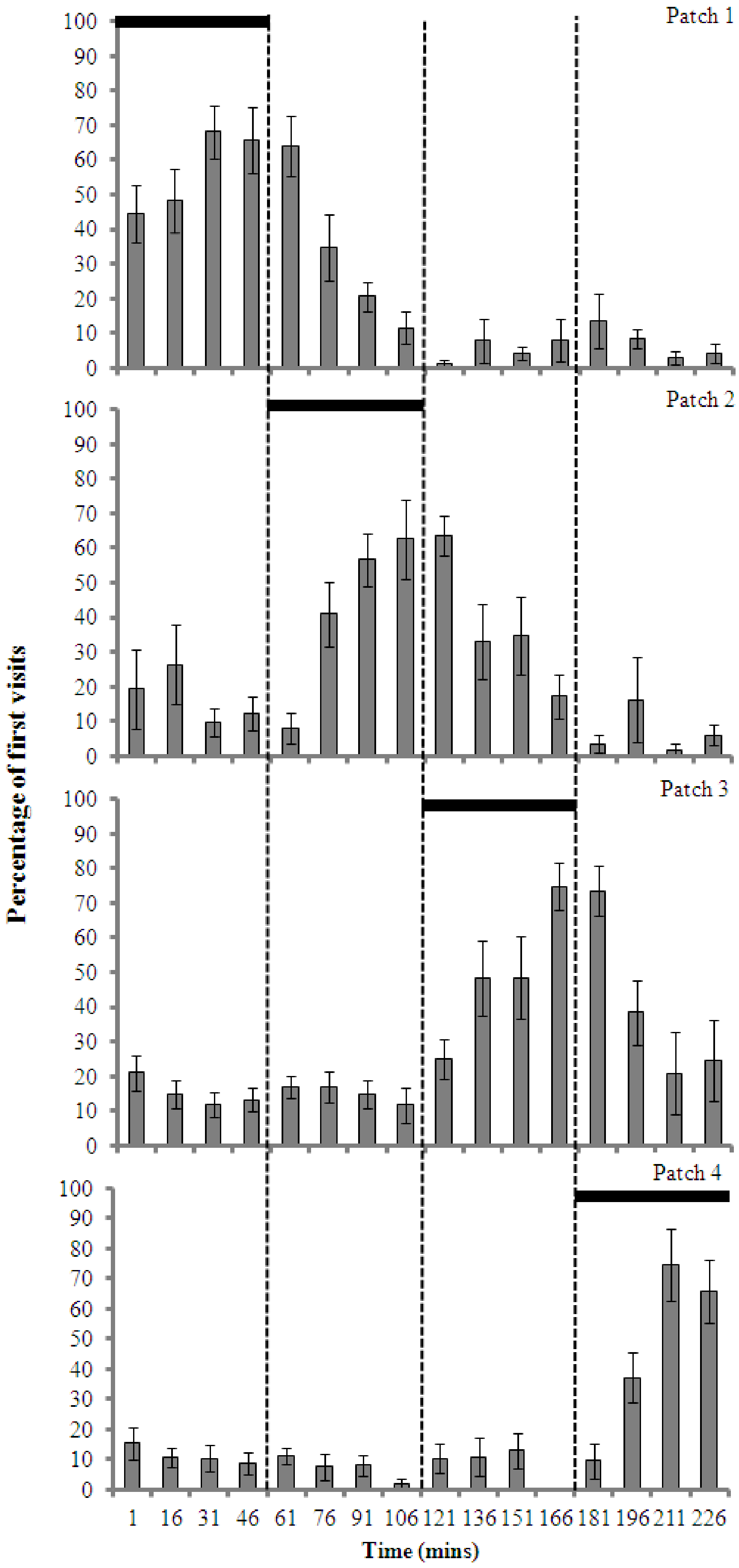
Patch 1
Patch 2
Patch 3
Patch 4
Percentage of first visits
0
10
20
30
40
50
60
70
80
90
100
1
16
31
46
61
76
91
106
121
136
151
166
181
196
211
226
Time (mins)

Figure 4.3. All first visits (mean ± SE) over the five days (N = 8 birds) shown for each patch at each time bin. Each part of the figure shows the visits to each of the patches over the four periods during which the experiment took place. Each hour is divided into 15-minute intervals starting from the first minute. The vertical lines running through the graphs indicate the times at which a patch became empty and the next patch contained reward. The horizontal black bars represent the duration over which the flowers in that patch contained reward.

At the beginning of each day birds directed their first visits largely to the correct patch, Patch 1. As the hour progressed the birds continued to visit this patch and did not switch to the Patch 2 until Patch 1 was empty. I observed a similar pattern of visits for all subsequent patches: the birds did not appear to anticipate when to begin visiting flowers in the next rewarded patch. Instead, the birds continued to direct their first visits to the previously rewarded patch for at least the first visit of the first bout of that next hour (Figure 4.3).

To examine whether the birds used a win-stay/lose-shift strategy to visit the correct patch at the correct time, I looked at which flowers the birds visited following their discovery that the rewards had changed. If the birds used a lose-shift strategy I would expect that, having visited an empty patch, they would move away from that patch and visit the other patches at random until they found the now rewarded patch. If they did this the probability of visiting the correct patch as a second choice at chance would be 33.33%. And on Day 1, this is what it appears the birds did: the mean percentage of visits the birds made to the correct patch having discovered that the previously rewarding patch was now empty was equal to chance (data = the second patch the birds visited during the first bout of each hour; binomial test with an expected proportion of 0.33: 8/31, $Z = 0.66$, $P < 0.45$). By Day 4, however, after visiting an empty patch birds moved directly to the new rewarded one more often than expected by chance (binomial test with an expected proportion of 0.33 for the second patch visited during the first bout of each hour; Day 4: 18/32, $Z = 2.61$, $P < 0.01$; Figure 4.4). On Day 5 birds continued to move directly from the previously rewarded patch to the correct patch more often than expected by chance (data = the second patch the birds visited during the first bout of each hour; binomial test with an expected proportion of 0.33: 21/33, $Z = 3.56$, $P < 0.001$).

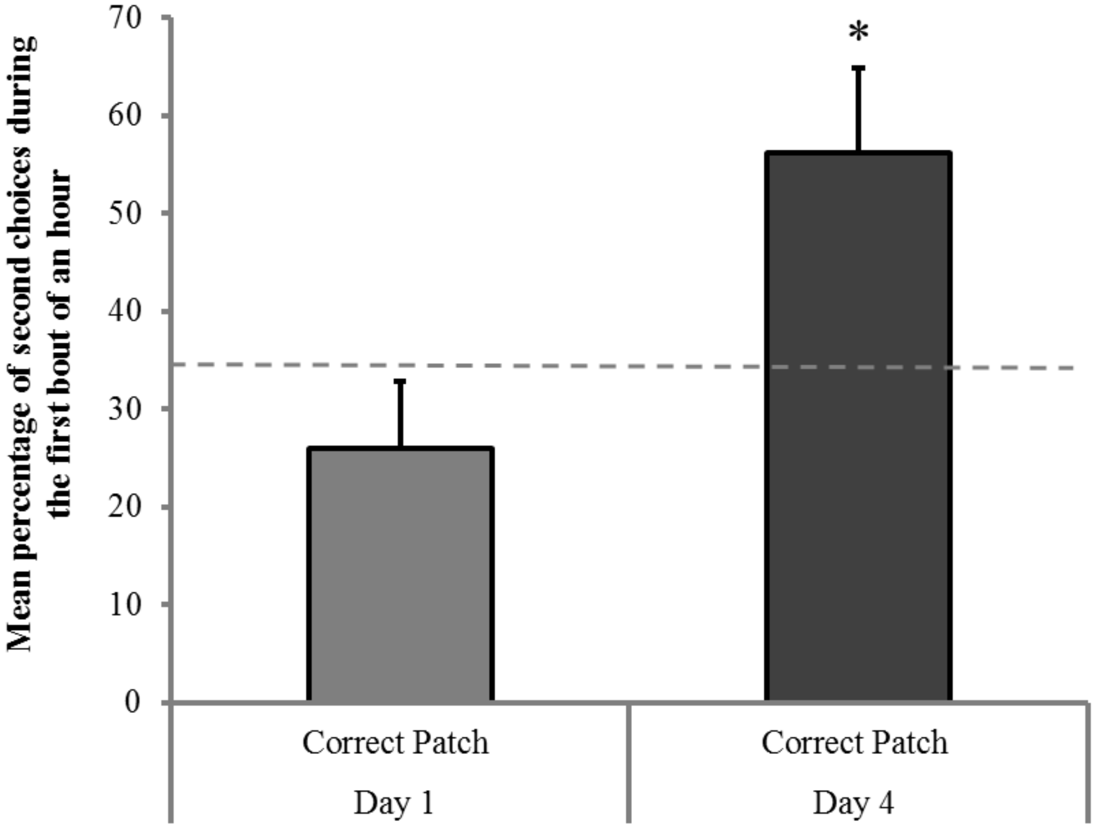

Figure 4.4. The mean (± SE) percentage of visits made to the correct patch during the second choice of the first bout of each hour. Birds' visits are compared between day one and day four (N = 8 birds). The line at 33.33% represents chance.

Test

To test whether birds used ordinal information or the time of day to visit to the correct patch at the correct time, the day after training finished I presented the four patches only during the fourth (last) hour of that day. As birds 7 and 8 did not perform better than at chance on the last day of training I excluded their visits from the following analyses. The distribution of the birds' first visits on this test day did not differ from that expected by chance ($X^2_3 = 0.66$, $P > 0.88$, with randomization $P > 0.73$). As on the previous days hummingbirds first visited a previously rewarded patch before moving to the correct patch, I also analysed the distribution of the second visits made during the first bout. The distribution of second visits by the remaining six birds did not differ from chance either ($X^2_3 = 3.33$, $P = 0.34$, with randomization $P = 0.53$). Finally, as birds visited more flowers in some patches than in other patches, I also compared the percentage of visits to flowers of each patch made during the test. Again, the mean percentage of visits made to each patch did not differ from that expected by chance ($X^2_3 = 1.97$, $P = 0.57$).

DISCUSSION

Wild, free-living male rufous hummingbirds learned the different times and locations at which four patches of artificial flowers contained a reward and some of the birds did this after a single day of training. On the test day when I presented the patches only at the fourth time period the birds were no more likely to visit one patch than any other.

I predicted that these birds might learn the time-place associations. Nonetheless, in spite of spending most of their time chasing other males and displaying to females, the birds learned these associations after many fewer trials (total number of visits to the patches during Day 1: mean ± SE: 17.37 ± 1.85) than is typically required for animals learning time-place associations in the laboratory. For example, golden shiners *Notemigonus crysoleucas* take 3-5 weeks to learn two times and two food delivery locations (Reebs 1996) while pigeons take a total of 40 sessions over four weeks to learn to peck two keys in either the morning or the afternoon (Saksida and Wilkie 1994). I suggest two, not mutually exclusive, explanations for such ready time-place learning in our hummingbirds. First, it is possible that the methodology used facilitated acquisition of the associations. Specifically, that by presenting the patches alone on Day 0, I may have helped the birds to learn each of the associations in isolation. Second, it is possible that this time-place task mimicked the key features of the way in which these birds typically forage: in nature once a patch is no longer profitable hummingbirds move to another location and only return when flowers have had time to refill (Henderson et al. 2006; Marshall et al. 2012; Samuels, Hurly and Healy 2014).

Other birds, however, also readily learn the time and place of an event (Biebach et al. 1989; Krebs and Biebach 1989; Saksida and Wilkie 1994), although there appears to be interspecific variation in the details of what they learn. For example, although both an insectivorous black-necked weaverbird *Ploceus bicolor* and the closely-related fire-crowned bishop *Euplectes hordeaceus* (a granivorous species) learned to visit four different places at different times of day, the two species responded differently to subsequent tests. One test involved a phase shift, in which the birds could visit the rooms six hours earlier than in training and the other, a blocking test, in which the birds were prevented from visiting any room for four hours in the middle of day (Falk, Biebach, and Krebs 1992). In both tests, the insectivorous birds shifted their visits to the appropriate locations consistent with the time manipulation while the granivorous species did not. Falk et al. suggested that the species differences could be explained by the degree in which time information is useful for these two species: whereas the insectivorous species feeds from a resource that is limited to a specific time of day, the granivorous species feeds from a resource that is available throughout the day and therefore the time information is less relevant for the granivorous bird.

It certainly seems unlikely that time-place learning is a bird-specific ability as species from other taxa will also use time-place information while foraging (ants, Schatz et al. 1999; marine iguanas, Wikelski, and Hau 1995; honeybees, Moore et al. 1989; Pahl et al. 2007). This might mean that time-place learning is, as Falk and collaborators (1992) suggested, an example of an adaptive specialisation whereby animals that feed from resources that have a temporal component (i.e. resources are restricted to certain times of day or that are available cyclically) are better at time-place learning than are animals that feed from resources that are constant in time or are available only once. It might also mean that experience of time-place learning through an animal's normal foraging routine leads to that animal learning a time-place task more readily than does an animal that has not had that experience. Dissociating these two explanations will not be easy not least because in the laboratory, where animals are maintained under constant light and dark conditions, phase-shift tests (where the external cue used to switch on the interval timer is either advanced or delayed) are used to exclude or to confirm the role and mechanistic basis of any timing ability the animal may show. In the field, such tests are not possible.

At least in one respect, the tested hummingbirds did not behave as have animals in other time-place learning experiments (Biebach et al. 1989; Saksida and Wilkie 1994; Schatz et al. 1999) as they did not appear to anticipate the time-dependent switch between rewarding patches. On the contrary, the hummingbirds continued to visit first the previously rewarded patch for at least the first bout of the new hour. This suggests that the birds needed to find empty flowers in order for them to stop returning to a rewarding patch and move to the new, rewarding patch. By Day 4, however, once a bird had discovered that the patch that had been delivering reward was empty he flew directly to the correct patch. This pattern of behaviour would suggest that the timing mechanism the birds used to solve the task was the order in which patches were rewarded. Using the order of patch reward combined with waiting until a patch no longer contained reward was an effective strategy, as most birds made few unrewarded visits. This outcome is similar to that obtained from mid-session reversal tasks that are used to test how quickly an animal learns to switch from one previously rewarded stimulus to a previously unrewarded stimulus. Over the course of many trials, pigeons *Columba livia* learn to anticipate the switch, which leads them to make unrewarded choices prior to the switch. Rats *Rattus norvegicus*, on the other hand, do not appear to anticipate but rather use the more effective win/stay, lose/shift strategy, to make fewer unrewarded choices (Rayburn-Reeves et al. 2013).

If the hummingbirds used the order of patch reward in order to decide where to move next, however, they should have visited the first patch first during the test. They did not do this and they did not make this decision on their subsequent choices. There are at least two possible explanations for their behaviour in the test. The first is that the hummingbirds used a combination of both the order of patch reward as well as the time of day. This combination would be consistent with previous results where rufous hummingbirds learned the replenishment interval of flowers apparently by combining both the order of flower presentation and the time of day to select the rewarded flower (Marshall et al. 2013). They may do this by anchoring the sequence of events (ordinal timing) to the time of day (Pizzo and Crystal 2002; 2004). If hummingbirds do integrate temporal and ordinal information in this way to make decisions about the possible rewarding state of flowers, then, in the test our birds should have known that it was the fourth hour. This would not, however, have provided enough information with regard to which patch was rewarding, hence their random choices.

The second explanation for the birds' behaviour in the test was that they recognised that something was different from the preceding days such that their learned choices were no longer useful. To be able to do this, however, also implies that they recognised that the patches had been presented for the first time at a different time of day. This, too, suggests that they may use time of day as an anchor.

Combining the two types of timing information may also explain how rats learn the duration with which levers provide food (Thorpe and Wilkie 2002) whereby the expiration of an internal timer for the first lever could serve as a cue to start the timer for the second lever and so on. In the case of the hummingbirds, a recently depleted patch could serve as the cue to move to the next patch. Whether hummingbirds can learn to use only the order of visitation or the time of day to visit the correct place at the correct time remains unknown. Because in nature the order of visitation and the time of day are most likely to be paired, the value of just using one strategy might be irrelevant.

With this experiment I have shown that wild hummingbirds can learn a time-place learning task and that they do so with little training. It is not yet clear, however, what timing mechanism they use to accomplish this.

Chapter 5: Traplining around patches of flowers

INTRODUCTION

The results from Chapters Three and Four demonstrated that hummingbirds will repeat the order in which they visit rewarding flowers. Furthermore, most birds learned this sequence after only one day of training. The data from both of these experiments suggest that hummingbirds will use both the spatial information and temporal information (a combination of the order and daily timing) to choose to move from a recently depleted location to a profitable one. In nature, however, temporal information regarding the availability of nectar in flowers may be gained not only by learning the time of day or the order of visitation, but also by learning the interval over which flowers refill.

In the wild, hummingbirds are likely to encounter different species of flowers that refill after different intervals and may, then, learn the refill intervals of different flowers. For example, hummingbirds that were presented with flowers that refilled either after 10 or 20 minutes from the last visit learned to match the refill schedules of the different flowers (Henderson et al. 2006; Marshall, Hurly, and Healy 2012; Samuels, Hurly, and Healy 2014). The ability to time multiple refill intervals might be useful for hummingbirds when traplining around multiple flowers in a day. Rather than remembering the time of day hundreds of individual flowers are rewarded at, or the order of individual flower visitation, birds might use interval timing to time their flower visits to multiple locations.

Interval timing differs from daily and order timing in the scale of the time period learned (Carr, Tan, and Wilkie 1999) and in the type of association formed (Thorpe and Wilkie 2006). For instance, in daily timing (also known as phase timing or circadian timing) animals learn to associate an event with a time of day. The event is cyclic and occurs with a fixed periodicity once a day. In the case of the experiment described in the previous chapter, for example, hummingbirds might have learned that a patch was rewarded from 8 to 9 o'clock. On the other hand, with ordinal timing animals learn the order of events, so that without "knowing" the time of day, animals can still learn what event will come next. Ordinal timers can be reset and can incorporate sequential information (Crystal 2009) so that an animal can learn that once an event has ended another one will follow. In the previous experiment, then, as the hummingbirds learned to switch to the correct patch when they found a patch no longer contained reward, it is possible that at least in part those birds had learned the order in which patches were rewarded. In the case of interval timing, however, animals learn the duration of events or the duration between events that might occur several times within a day. In a traplining context, interval timing could be useful for animals that need to learn how long to wait until revisiting a refilling flower. Whether hummingbirds can use interval timing to time their consecutive visits to different locations in a trapline has not been tested previously. Furthermore, it is still not clear whether hummingbirds can combine spatial and temporal information when developing a trapline that will help them avoid depleted flowers.

Having shown that hummingbirds can develop traplines around several flowers and that they can also learn to visit one patch at a time I wanted to know if these birds would also develop a trapline when flowers only refilled after a time interval. In order to record the development and use of a trapline around patches that refill after a certain interval I tried to mimic a "natural" foraging scenario in the following way. Within a male's territory I placed three patches, each with 'flowers' that collectively containing more sucrose than a bird usually consumes in single bout. A bird was allowed to feed from the patches freely, following any order of visitation, visiting one, two or three patches at a time but I refilled the flowers only after 20 minutes had elapsed from the last visit. In this way, unlike the experimental set up I described in Chapter Four, the birds' own flower visits determined the time at which a flower would contain reward. As territorial male rufous hummingbirds feed approximately every 10 minutes, a hummingbird that visited one patch in each bout and only returned to feed in a patch after having visiting the other two, should avoid the most recently visited patch and visit one of the other two patches. A bird that returns after 20 minutes would find only one patch containing reward. In this way, to fly directly to a patch containing food, the bird needed to learn to associate the time at which he had emptied a patch and the time that would elapse before that patch would refill.

METHODS

Subjects and experimental site

For this experiment I tested eight free-living territorial male rufous hummingbirds and trials were run between 0700 and 1800 hours Mountain Standard Time from June to July 2014. As for my earlier experiments, I marked birds on their breast feathers with nontoxic waterproof ink (Jiffy Eco-marker Ink). One day after a bird was marked I returned to his territory and trained him to feed from an artificial flower. As described in Chapter Four, the artificial flowers I used in this experiment consisted of a syringe tip surrounded by a coloured cardboard circle (6 cm in diameter) mounted on a 60 cm wooden stick. Once the bird was feeding regularly from the training flower I started to pre-train the bird to feed at the different locations.

Pre-training

To ensure that hummingbirds would search for sucrose at all the locations at which I planned to present patches of flowers, I presented one of the four flowers of a patch in its eventual location. The flower contained enough sucrose for a single bout. I removed the flower once the bird had fed from it and then presented a flower of another patch in its eventual appropriate location.

Experimental procedure

At the beginning of the experiment I allowed the bird to feed from the feeder for 20 minutes from the time he last visited the last flower in the pre-training stage. Following a male’s visit to his feeder, I presented him with all three patches in their respective locations. The three patches were presented simultaneously and were separated by 3 m forming a regular triangle. Flowers within patches were separated by 40 cm forming a square (Figure 5.1). The colour of flowers within a patch and the location of patches with respect to the hummingbirds’ main perch were counter-balanced across birds. The possible five colours used were: pink, green, blue, purple and orange. To prevent birds from always visiting the closest patch to their preferred perch or near to where the feeder would usually hang, two of the patches were placed equidistant to that perch or feeder location.

I then filled all flowers with 25 µl of 25 % sucrose solution (w/w). When patches were first presented, all flowers in all patches were rewarded but once a hummingbird had visited a flower that flower was refilled only after a 20-minute interval. If a bird only sampled or did not drink all the sucrose in a flower the remaining sucrose was emptied and the flower was refilled 20 minutes later. Patches were presented for at least 175 bouts, but some birds (4) completed as many as 200 bouts to the patches.

As before a bout was defined as any time the hummingbird came to feed from any of the flowers in a patch and ended when he then left the area. The bird was free to visit the patches throughout the day. At the end of each day the feeder was returned to its usual location and I removed the patches. I marked the locations of the flowers in each patch with tape so that the next day I could return the flowers to the same locations.

Birds took on average 4 days ± 0.59 (mean ± S.E., n = 8) to complete all the bouts. For each bout I recorded the order of visitation and the time each flower was visited.

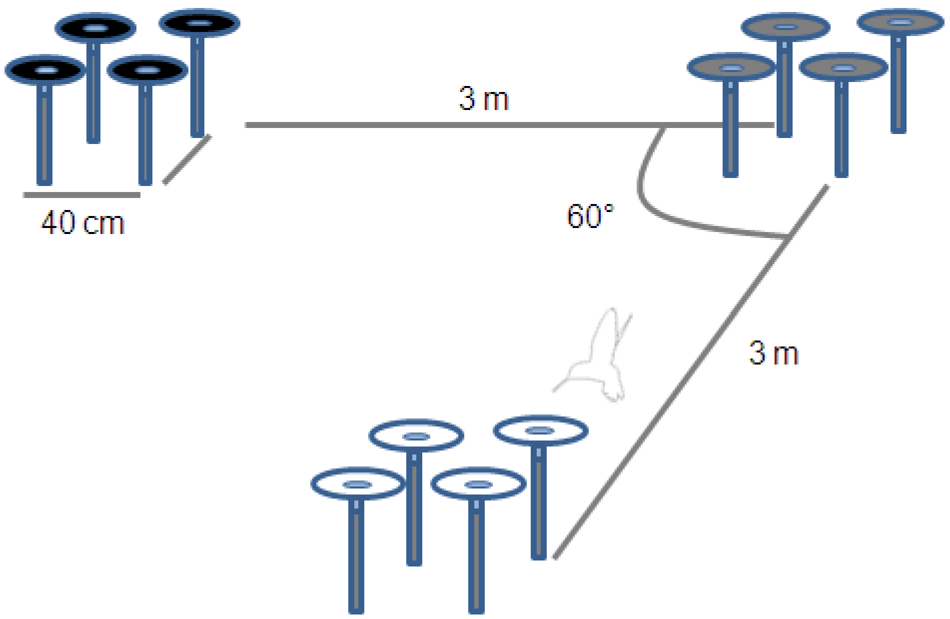

Figure 5.1. Diagram of the three-patch flower array. Patches of flowers were separated by 3 m and flowers within patches were 40 cm apart. Birds fed freely from the artificial flowers but these were refilled (25 µl of 25 % sucrose solution) only after 20 minutes had passed from the last visit.

RESULTS

Timing of patch visits

The eight hummingbirds made a total of 1563 bouts to the flower array. The average interval at which the birds visited the array at the beginning of the experiment did not differ from that at which they visited the array at the end of the experiment (Wilcoxon matched-pairs test: $W = 16$, $P = 0.79$; Figure 5.2). During the first 50 bouts birds visited the array on average every 09:10 ± 0:55 minutes (mean ± S.E., n = 8) and by the last 50 bouts birds visited the array on average every 10:08 ± 1:32 minutes (mean ± S.E., n = 8). As expected, then, hummingbirds visited the patch array about every 10 minutes.

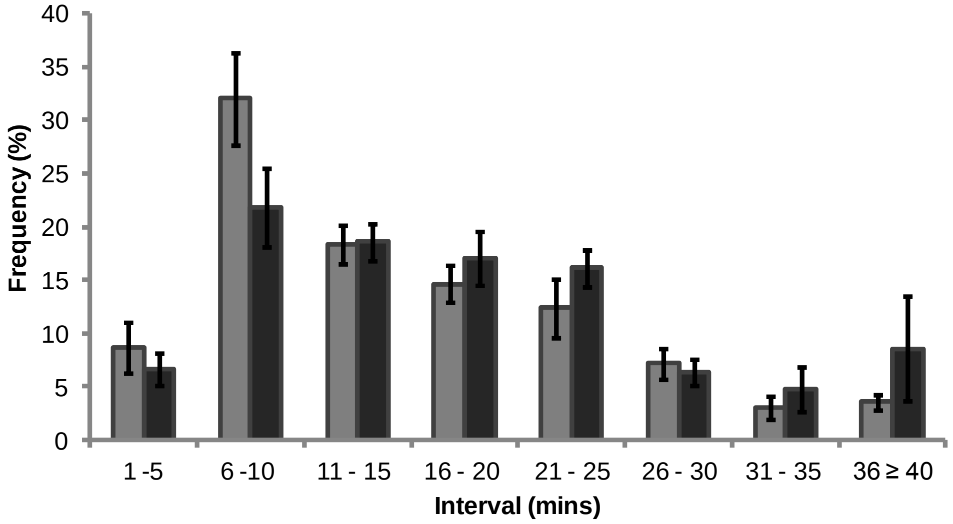

Figure 5.2. The frequency of time intervals at which birds visited the array, regardless of the patch (%, mean ± S.E., n =8). The light grey bars represent the first 50 bouts of the experiment while dark grey bars represent the last 50 bouts.

If birds visited patches in sequence one at a time so that each patch was visited only after the other two patches had been visited, then the inter-visit interval for each patch would approximate 30 minutes. In all cases the inter-visit intervals for each patch were significantly shorter than 30 minutes (tested using a two-tailed one-sample Wilcoxon signed rank test: Table 5.1; Figure 5.3). Rather than waiting 30 minutes to revisit a patch, the birds took on average 17:03 ± 00: 07 (mean ± S.E., n = 8) minutes to revisit a particular patch (Figure 5.3). But in fact, the hummingbirds visited different patches at different intervals. Six of the eight birds visited at least one patch every 20 minutes (Birds 1, 2, 3, 4, 5 and 8) and the inter-visit interval did not differ significantly from 20 minutes (Table 5.1). While Bird 1 visited not only one but two of the patches every 20 minutes, Birds 6 and 7 visited all three patches significantly sooner than 20 minutes (Figure 5.3; Table 5.1).

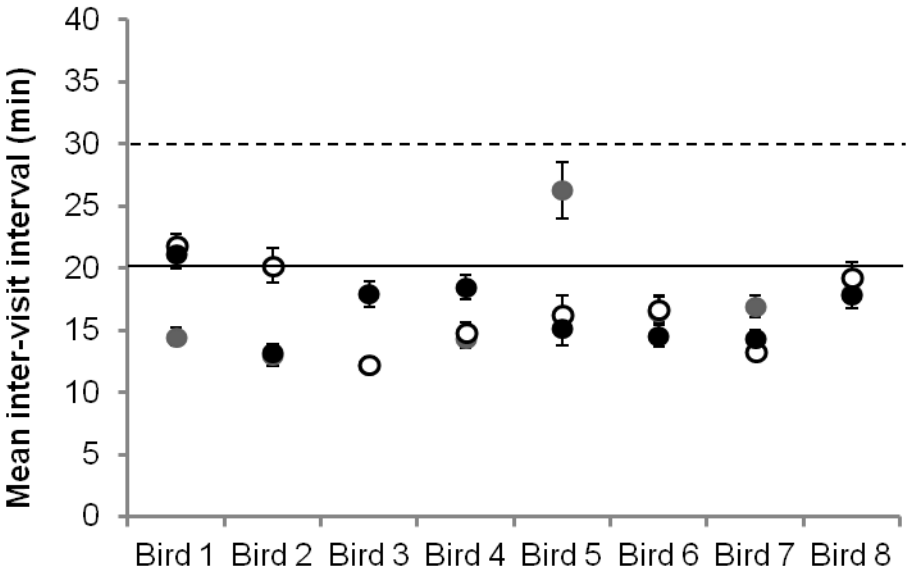

Figure 5.3. Mean (± SE, n = 8) of the inter-visit interval time in minutes each bird took to revisit each patch. White, black and gray circles represent different patches. The dotted line at 30 minutes represents the interval that would be expected if hummingbirds visited the patches in the same order at the appropriate time. The solid line at 20 minutes represents the interval at which patches were replenished.

Table 5.1. Summary statistics of the two-tailed one-sample Wilcoxon signed rank tests comparing the time the birds (n = 8) took to return to each patch (3 patches per bird). The distribution of the birds' inter-visit interval was compared to a 30 min interval and a 20 minutes interval. Birds and the patch identity are coded as B 1-8 for the bird and B for blue, G for green, M for morado (purple), O for orange and P for pink to identified different patches.

		30 minutes		20 minutes	
Bird/ Patch	*N*	*W*	*P*	*W*	*P*
B1/G	104	24	< 0.001	752	< 0.001
B1/P	67	125	< 0.001	1419	**= 0.07**
B1/M	70	228	< 0.001	1217	**= 0.628**
B2/B	108	156	< 0.001	651.5	< 0.001
B2/G	69	239.5	< 0.001	1030.5	**= 0.6339**
B2/P	99	104	< 0.001	495.5	< 0.001
B3/B	119	1.5	< 0.001	281	< 0.001
B3/P	119	0	< 0.001	379.5	< 0.001
B3/M	80	164	< 0.001	1026	**= 0.0352**
B4/G	111	52	< 0.001	826.5	< 0.001
B4/O	107	45.5	< 0.001	1010	< 0.001
B4/M	88	139	< 0.001	1348	**= 0.0514**
B5/B	70	773.5	< 0.05	1561	**= 0.0626**
B5/O	108	645	< 0.001	1584.5	< 0.001
B5/P	125	703.5	< 0.001	1546	< 0.001
B6/B	109	300.5	< 0.001	1355	< 0.001
B6/G	105	308	< 0.001	1371.5	< 0.001
B6/O	126	289	< 0.001	1136.5	< 0.001
B7/B	90	77	< 0.001	1047	< 0.001
B7/P	112	295	< 0.001	573	< 0.001
B7/M	105	18	< 0.001	701	< 0.001
B8/B	110	603.5	< 0.001	1958.5	< 0.001
B8/O	104	486.5	< 0.001	2146.5	**= 0.1092**
B8/P	112	378	< 0.001	1976	< 0.001

These data suggest that most birds visited two of the patches twice as often as they visited the third patch. To determine whether this was the case, I compared the number of visits birds made to each of the three patches. For half of the hummingbirds the number of visits they made to each of the three patches differ significantly (Bird 1: $X^2_2 = 9.9$, $P = 0.007$; Bird 2: $X^2_2 = 8.66.$, $P = 0.013$; Bird 3: $X^2_2 = 9.3$, $P = 0.009$ and Bird 5: $X^2_2 = 15.31$, $P = 0.0004$). This result confirms that these birds visited some patches more than the others. For the other four birds, however, the number of visits to each patch did not differ significantly (Bird 4: $X^2_2 = 2.87$, $P = 0.23$; Bird 6: $X^2_2 = 1.83$, $P = 0.4$ and Bird 7: $X^2_2 = 2.27$, $P = 0.32$ Bird 8: $X^2_2 = 0.4$, $P = 0.81$). This is not surprising for Birds 6 and 7 because the inter-visit interval also suggested that those birds often visited all three patches on a single bout. For Birds 4 and 8 the number of visits to the three patches was not significantly different because these two birds too, often visited three patches per bout.

Did the hummingbirds visit the patches in sequence?

Overall, hummingbirds visited one patch during 50.9 % of the bouts, two patches 35.5 % and all three patches during 13.4 % of the bouts. The hummingbirds revisited the same patch during a single bout on only eight occasions. For more than half of the bouts (54.34 ± 2.84 % mean ± S.E., n = 8) the hummingbirds revisited a patch that they had visited on the previous bout.

If birds had moved sequentially between patches and had visited only one patch at a time, then the number of times a bird visited a patch as his first choice in a bout should have been equal across the patches. The hummingbirds did not do this. Rather, the birds visited one of the patches more often and tended to visit that patch before visiting the other patches. The distribution of the number of first visits to each patch was compared to a random distribution with a Chi-squared goodness of fit test as a proxy of whether hummingbirds were following a repeated order of visitation to the patches. For six of birds the distribution of first visits to each patch was significantly different than expected by chance (Bird 1: $X^2_2 = 37.30$, $P < 0.001$; Bird 2: $X^2_2 = 30.65$, $P < 0.001$; Bird 3: $X^2_2 = 38.23$, $P < 0.001$; Bird 5: $X^2_2 = 74.95$, $P < 0.001$; Bird 7: $X^2_2 = 19.33$, $P < 0.001$ and Bird 8: $X^2_2 = 9.91$, $P < 0.01$). These birds visited one of the patches first in most of the bouts. Two birds however, made the same number of first visits to each of the three patches (Bird 4: $X^2_2 = 0.97$, $P < 0.6142$ and Bird 6: $X^2_2 = 5.47$, $P < 0.06$; Appendix 5.1).

The number of times a bird visited two patches in succession in a bout (transition between patches) was significantly different from that expected by chance for all hummingbirds (Markovian chain Likelihood ratio test comparing observed and expected matrices $P < 0.001$, Appendix 5.2). Furthermore, of the nine possible patch transitions (transition between the three patches as well as within a patch were included), hummingbirds went from one patch to another in a sequence of transitions that occurred significantly more than the other possible ones (transitions that had a Z-score > 1.96 were statistically different at a significant level of 0.05, Appendix 5.2; Figure 5.4): after visiting one of the patches, birds were more likely to visit one patch rather than the other one.

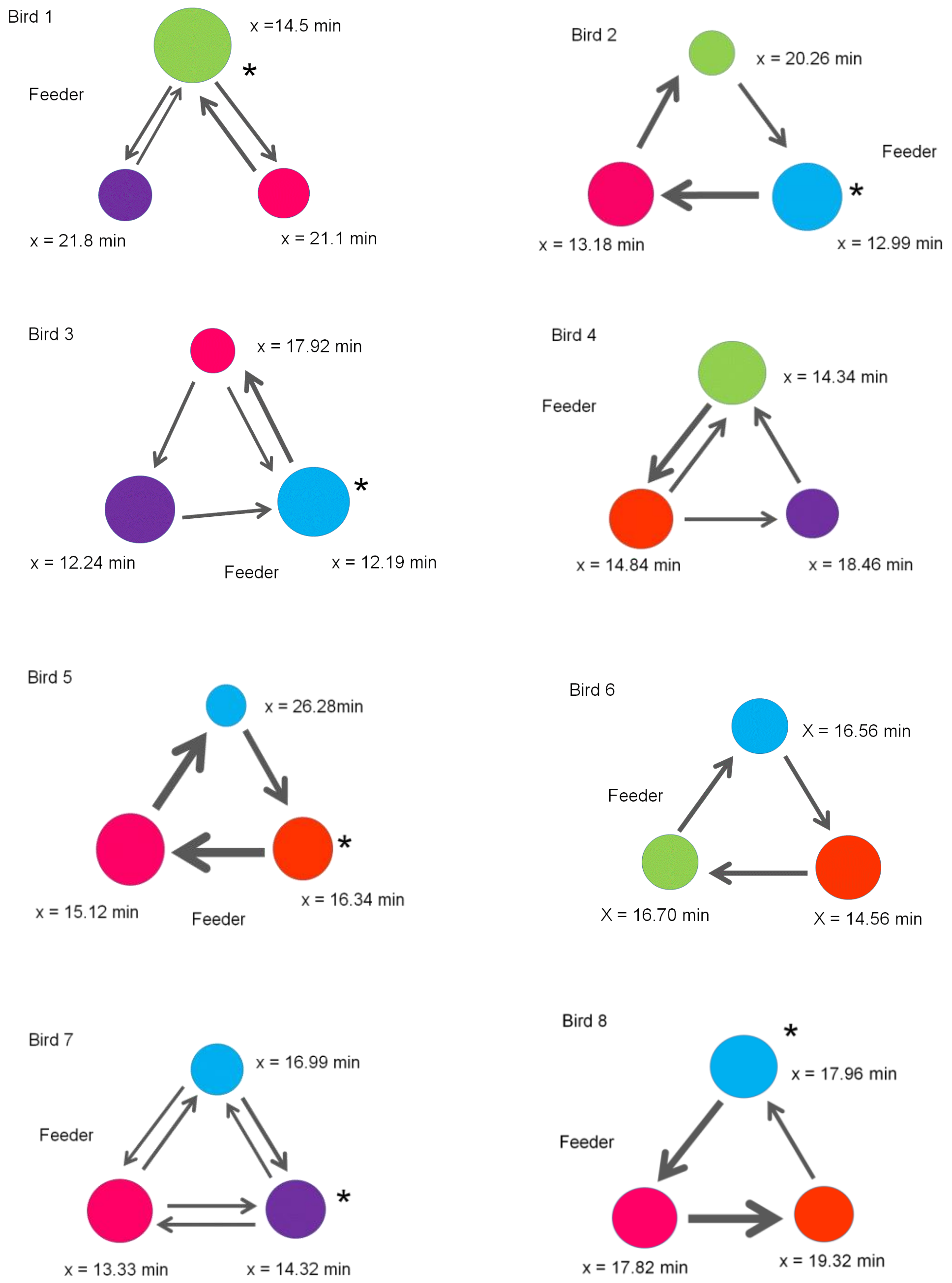

Figure 5.4. Diagrams of the transitions between patches that occurred significantly more often than expected by chance for each of the eight birds. The circles represent the three different patches each bird could visit. The circle size is proportional to the frequency of visits to that patch. Arrow size is proportional to the conditional probability of the transition. Diagrams only include the transitions with significant Z-scores (Z > 1.96). The asterisk denotes the patch that was visited first in a bout most frequently. X denotes the mean inter-visit interval for that patch.

Did the birds avoid revisiting empty flowers?

The majority of birds visited one of the three patches more frequently than the other two patches and they were more likely to visit that patch first. This meant that during half of the bouts birds first visited an unrewarded flower (because birds revisited the one patch before the 20 minute refilling interval) and then moved to the other patches that contained rewarded flowers. The number of possible rewarded flowers changed depending on the interval between visits and on the number of flowers the bird had emptied on the previous visit. Therefore, in order to estimate whether hummingbirds visited first a rewarded flower more often than predicted by chance, the chance level for each bout was set at the number of flowers that were rewarded at each bout divided by the total number of flowers (12). For example, if all the flowers in one patch were empty and all the flowers of the other two patches were rewarded the probability of visiting a rewarded flower was 0.66 (8/12). Overall the mean chance level across birds and all bouts was 48% ± 0.5 (mean ± S.E. n= 1560 bouts. This means that on average almost half of the flowers were rewarded at a time.

On average the hummingbirds visited a rewarded flower first more often than the mean chance level of 48% (Figure 5.5). Since the probability of visiting a rewarded flower varied across birds, instead of using the average probability to determine whether individual birds visited a rewarded flower more often than chance, I used a bird’s own average proportion of rewarded flowers as the probability of visiting a rewarded flower for that bird. Using a bird’s own chance probability of visiting a rewarded flower, I was able to estimate whether birds learned to avoid empty flowers and visit the rewarded flowers first. The percentage of first visits to a rewarded flower did not change with experience (Wilcoxon matched-pairs test, $W = 17$, $P = 0.94$) but it differed across birds (Appendix 5.3). For example, even during the first 50 bouts, Bird 4 first visited a rewarded flower more often than chance and continued doing so through all the experiment (Appendix 5.3), while the percentage of first correct visits for Bird 8, however never differed significantly from chance.

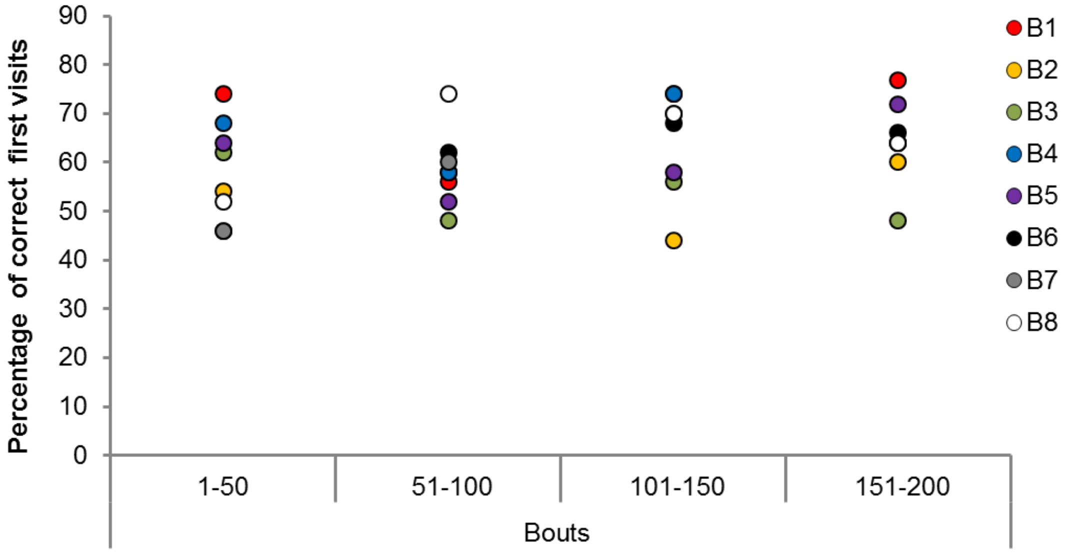

Figure 5.5. Percentage of correct first visits (mean ± SE, n = 8) made during the 200 bouts in bins of 50 bouts.

DISCUSSION

Hummingbirds free to visit three patches of flowers that were refilled after a 20 minute interval developed a particular sequence of visits to those flowers: birds were likely to move from one of the patches to another and to the third in a repeatable sequence. Most of the birds (6 out of 8 birds) avoided visiting one of the three patches before the 20 minutes it took to refill. Hummingbirds visited one or two of the patches more frequently and first than the other one. This suggests that the hummingbirds could learn the time interval the patches took to refill. However, rather than alternating visits between the patches, the birds preferably visited one or two of the patches during most bouts and visited the third patch only after they found a patch to be unrewarded.

This is the first study to look at how hummingbirds develop a trapline around several locations that have a temporal component to the availability of reward. Although the hummingbirds did not visit one patch per bout, they repeated the order in which they visited the patches. This foraging behaviour matches the description of foraging by traplining whereby animals are expected to repeat the order, or sequence of visits, in which they visit rewarding locations.

Hummingbirds visited a patch that they had visited during the previous bout en route to the next rewarded patch, which suggests that they were following a route and that the proximity to a commonly rewarded location (the feeder) influenced where the hummingbirds started their route. It might be the case that even if the hummingbirds had learned that the flowers took 20 minutes to refill when returning to feed, because those patches were “on the path” to a patch currently containing food, then hummingbirds might still visit them. In nature, hummingbirds may avoid revisiting recently depleted flowers because those flowers take at least four hours to refill (Armstrong, Gass, and Sutherland 1987). In the present experiment, flowers could be rewarded during every other bout and therefore hummingbirds did not need to avoid empty flowers for more than one bout. In order to learn to avoid visiting a patch or a flower, hummingbirds might need to find that patch empty on more than one occasion or for longer periods of time.

Hummingbirds can learn to visit different patches at different times of day, as shown in Chapter Four, and they can also learn to visit different flowers at different intervals as shown in previous experiments. For example, when a patch of two different colour flowers that were separated by 60 cm from each refilled at either 10 or 20 minutes intervals, birds learnt to visit the flowers at different intervals after fewer trials compared to birds that were presented with flowers of all the same colour (Samuels, Hurly, and Healy 2014). Those results suggested that hummingbirds can associate the interval a flower takes to refill with a colour cue. Associating colour cues to refill intervals will be advantageous to hummingbirds that move within patches of different species flowers with different refill intervals. The results of the present experiments suggest that when moving across patches of flowers that have the same refill interval, hummingbirds pay more attention to the spatial distribution of those patches rather than the refill interval of individual flowers. Additionally, in some bouts hummingbirds did not visit all the flowers in a patch and therefore not all of the flowers in a patch would have been empty when the bird returned to feed in that same patch. This would have required the birds to keep track of the refill intervals of all 12 individual flowers. While it is possible that they can do this, perhaps the spatial proximity of the patches meant that the costs of checking patches at regular intervals was not great.

Traplining is thought to help animals outcompete intruders by allowing the animals to deplete the resource before the intruders do. If the hummingbirds in this experiment had visited only one patch per bout, there would have been two patches with sucrose available at all times. Instead, the hummingbirds visited the patches in such order and frequency that the number of patches containing a reward was one of the three patches. This means that the standing crop available to intruders was lower than it would have been if the birds' traplining was 'perfect'. In nature, a traplining hummingbird has the option of delaying revisits to a flower and therefore increasing the sucrose accumulated, but by revisiting the same patches frequently, a traplining hummingbird could reduce losses to competitors through defense by exploitation (Paton and Carpenter 1984; Gill 1988). This was shown to be the case when territorial purple-throated carib hummingbirds (*Eulampis jugularis*) feeding from Heliconia patches decreased the duration of their inter-visit intervals in response to simulated competition (Temeles et al. 2006).

It is then possible that the sequences that the hummingbirds followed in my experiment could have helped them to exclude intruders by exploiting the patches in a way that decreased the total amount of sucrose available between bouts. On average a territorial male rufous hummingbird takes 100 µl of sucrose per bout (Karasov et al. 1986). When visiting two patches, however, hummingbirds were taking as much as 200 µl. This is surprising, because even though the crop size of these hummingbirds is about 600 µl (Hainsworth and Wolf 1972), territorial males tend to drink small amounts of sucrose to carry less weight when making flying displays to females and chasing off intruders from their territory (Carpenter et al. 1991). In my experiment, however, hummingbirds might have prioritized depleting as much of the resource as possible to prevent loss to intruders. Instead of visiting the array more often, hummingbirds continued to visit the array about every 10 minutes, but ingested more sucrose whenever it was available. Whether the frequency of intruders affected how much sucrose hummingbirds took or how often birds revisit a patch was not measured.

These birds are central place foragers that go back and forward between feeding locations and a couple of perches in a location that allows them to “monitor” for intruders and females (Hixon, Carpenter, and Paton 1983). In nature hummingbirds might revisit flower patches between days so that in the morning they will first visit the patches that are peripheral to their central perches and later on the day they will visit the patches closest to the perch (Paton and Carpenter 1984), only revisiting a location the next day. The territorial male rufous hummingbirds that I tested for this and all other experiments have made a territory around an artificial feeder. Although these birds will feed from natural flowers from time to time almost all of their sucrose intake comes from the feeder or the artificial arrays provided four or five meters away from where the feeder normally hangs. In this scenario the hummingbirds feed so many times from the same location that even when the feeder is moved only half a meter the hummingbirds will still visit the empty space of the feeder and sometimes take minutes to find the new location of the feeder. It is therefore not surprising that the hummingbirds started to feed from the patches that were located closest to where they normally feed. Starting by the same patch during must bouts will only leave two other patches to be visited next and so the closest to the first one will be the next option, leaving the more distant patch to be visited only after the previous two had been visited.

The results of the experiment presented in this chapter provide confirmation that hummingbirds will follow a sequence when moving from one rewarded location to another. These data also suggest, however, that these birds do not necessarily avoid visiting depleted patches once they are a part of a sequence. Whether hummingbirds learn the sequence of a trapline by linking one patch at a time or if they learn the order in which they should visit each location needs further experimentation.

Chapter 6: Use of spatial cues by female hummingbirds

INTRODUCTION

Hummingbirds are a useful group in which to address sex differences in cue use because in most species the sexes differ in foraging behaviour and in their use of space. Males are typically territorial, defending and feeding from hundreds of flowers within their territories every day (Kodric-Brown and Brown 1978) and in most species they are considered to be polygynous. Females, on the other hand, are not usually territorial, are thought to forage by traplining (Temeles et al. 2006) and provide all parental care. There are no previous studies, however, in which the foraging behaviour of traplining females has been investigated. If, indeed, male and female hummingbirds do differ in their foraging behaviour, then it is possible to expect that, as in many other species, there are sex differences in the types of cues used when foraging. Although wild male rufous hummingbirds preferentially use spatial information to return to a previously rewarded artificial flower (Hurly and Healy, 1996) the types of information that females use to relocate a rewarding location are still unknown.

When trained to return to a rewarded location males typically outperform females. One consistent feature of the male performance is that they use distance, direction and feature cues while females tend to prefer to use feature cues to solve the task (Collins and Kimura 1997; Gaulin and Fitzgerald 1986; Jozet-Alves, Modéran and Dickel 2008; Kavaliers et al. 1996; Lacreuse et al. 1999; Schmidtke and Esser 2011; Seymoure, Dou and Juraska 1996). This effect appears to hold both across species and tasks. For example, when returning to a platform in a Morris water maze male rats outperform conspecific females when extra-maze landmark cues are absent but not when a landmark is made available (Roof and Stein 1999). Similarly, on a computer screen version of a delayed non-matching-to-sample task, women were more likely to use feature than location cues, whereas men used both types of information equally (Jones and Healy 2006). Even when describing the location of a place, men tend to provide Euclidean information (distance and direction) whereas women provide information on landmarks (Dabbs et al. 1998). Furthermore, for male rats spatial information overshadows landmark information but for female rats the opposite is true (Rodríguez, Chamizo, and Mackintosh 2011).

The sex difference in cue use appears not to be confined to mammals as male domestic chicks *Gallus gallus domesticus* used position cues to relocate a food reinforcement while female chicks used colour cues more readily (Vallortigara 1996) and female shiny cowbirds *Molothrus bonariensis* retrieved food rewards faster than did males only when the food was associated with a colour cue (Astié, Kacelnik, and Reboreda 1998; Bateson, Healy, and Hurly 2003). But the avian data are more mixed than are the data from the mammalian literature. For example, both male and female great tits *Parus major* used a position cue to relocate a reward hidden in one of three wells rather than the colour of a cloth on top of each well (Hodgson and Healy 2005). Furthermore, if cue preference is associated with selection pressure for better spatial ability, then in the cowbirds one would expect the females to be the sex that prefers/uses spatial rather than feature information as it is the females that appear to have the great spatial memory demand (as they alone search for nests to parasitize; Reboreda, Clayton, and Kacelnik 1996) and the better spatial memory: female brown-headed cowbirds (*Molothrus ater*) outperformed conspecific males in a spatial memory task (Guigueno et al. 2014). The lack of a compelling association between spatial demand/performance and preferential use of spatial information in birds led Hodgson and Healy to suggest that cue use/preference might be due to the relative value of those cues in the specific context in which the animals were tested i.e. that cue use might be context dependent rather than favoured by selection.

To determine whether female hummingbirds preferred to use feature rather than spatial cues I tested female hummingbirds of three different species using a similar task to that used by Hurly and Healy (1996). In this test birds visited a four-flower array in which all four artificial flowers were a different colour and only one of them contained reward. Prior to the birds' return, I emptied the flower and switched it with one of the other flowers. If the birds preferred to use feature cues to choose the flower it expected to contain reward (the original flower contained more sucrose than the bird could consume in a single visit), it should visit the flower of the 'correct' colour. If, however, it preferred to use spatial cues, it should visit the flower in the original location (the 'correct' spatial cue). I tested female rufous hummingbirds at the same site in Canada as described in the earlier chapters. Then I also tested females of the white-eared hummingbird *Hylocharis leucotis* and of the magnificent hummingbird *Eugenes fulgens*, which are also thought to be trapliners, at a site in Mexico. As I had the opportunity, I also tested male white-eared hummingbirds at that site.

My expectation was that if space use selects for a preference for using spatial cues, then like female mammals, the females hummingbirds should preferentially use feature information rather than spatial information when returning to a previously rewarded artificial flower. The male white-eared hummingbirds should, however, prefer to use spatial cues.

METHODS

Subjects and field site

I tested eleven female rufous hummingbirds in this experiment. Instead of marking females with ink, however, I identified individuals by their unique throat feather patterns. I used this method of identification, instead of marking females as I did the males in the previous chapters, because once I had marked two females, they abandoned their territory. In Mexico, at the National Park “La Malinche”, Tlaxcala, in Central Mexico (19º 14’ N,98º 58’ W with a 3000 m elevation), I tested eight males and three female white-eared hummingbirds and seven female magnificent hummingbirds. I identified individual birds in Mexico by a detailed record of their visit rates to an artificial nectar feeder and identification of perch sites. To avoid retesting individuals only one bird of each species or sex was tested at a particular site..

In Canada, by the first week of July male rufous hummingbirds start to abandon their territories around the artificial feeders and females start visiting them regularly and will defend the feeder from other females. At this point I watched female hummingbirds and recorded all of their visit and visible perches, as well as the visible patterns on their gorgets (Figure 6.1).

The hummingbirds tested in Mexico were identified in the same way as were the rufous female hummingbirds but as female white-eared and magnificent hummingbirds do not have distinctive gorgets, I made a more detailed account of their visits and perches. I first placed artificial feeders at locations within 1 km of La Malinche research station and waited for the hummingbirds to visit regularly. At La Malinche both the white-eared hummingbird and the magnificent hummingbird are year-round residents with both males and females migrating attitudinally to match the blooming schedule of different flower species (Lara, 2006).

Figure 6.1. Photograph of a female rufous hummingbird perching at the artificial feeder. In the centre of the throat she has a small patch of iridescent feathers in the form of a small gorget.

Initial training

I trained females that were defending feeders and had distinctive throat patterns to feed from artificial flowers. To do this I first lowered the feeder 20 cm at a time until it had reached a height of 60 cm above the ground and I then replaced the single yellow plastic flower on the feeder with another artificial ‘flower’. This flower comprised a plastic vial full of 25% sucrose solution, which was surrounded by a yellow cardboard circle (2 cm in diameter). After the bird had fed from the cardboard “flower” once, I replaced it with a larger one, also yellow (6 cm in diameter). Finally, after the bird fed from that flower, the feeder was removed and the experiment began. This training procedure ensured that the bird was exposed to both the spatial and colour cues the same number of times. This training procedure took four visits to the feeder by each bird.

Experimental trials

For the experiment I presented the bird with an array of four artificial flowers mounted on 60 cm height wooden stick in an 80 cm square where only one flower was filled with 600µl of 25% sucrose solution. The other three flowers were empty. The amount of sucrose solution in the rewarded flower was enough so that a single foraging bout was not sufficient for the birds to empty the flower. The rewarded flower was always the same colour as the feeder's plastic flower and was always placed in the same location as that where the bird had fed from the cardboard flower when it was still attached to the feeder. The three other flowers were each of one of three colours (possible colours were: yellow, red, pink and purple) and the location of these flowers within the array was assigned pseudorandomly (Figure 6.2).

Once the hummingbird had fed from the rewarded flower (Phase 1) and left the array, I switched the rewarded flower with one of the other flowers in the array so that its colour and its absolute location were in conflict (Figure 6.2; Phase 2). I emptied all of the flowers and ensured that the birds did not return to the array for at least five minutes. When the bird returned to the array I recorded the first visit made. Visits were defined as a probe into a flower. Each bird was tested only once.

Statistical analyses

I compared the distribution of observed first visits made to all flowers during Phase 2 to a distribution expected by chance with a Chi-squared of goodness-of-fit test. Since the expected frequencies of the distribution of the visits were smaller than 5, a randomization test with 10, 000 permutations was used to determine the probability of obtaining the observed and more extreme results (Sokal and Rohlf, 1995). Since the *P* values of both the Chi-squared of goodness of fit test and the randomization test were similar I present only the *P* values for the randomization test. If the birds visited flowers in the array randomly the chance of visiting any flower was assumed to be equal.

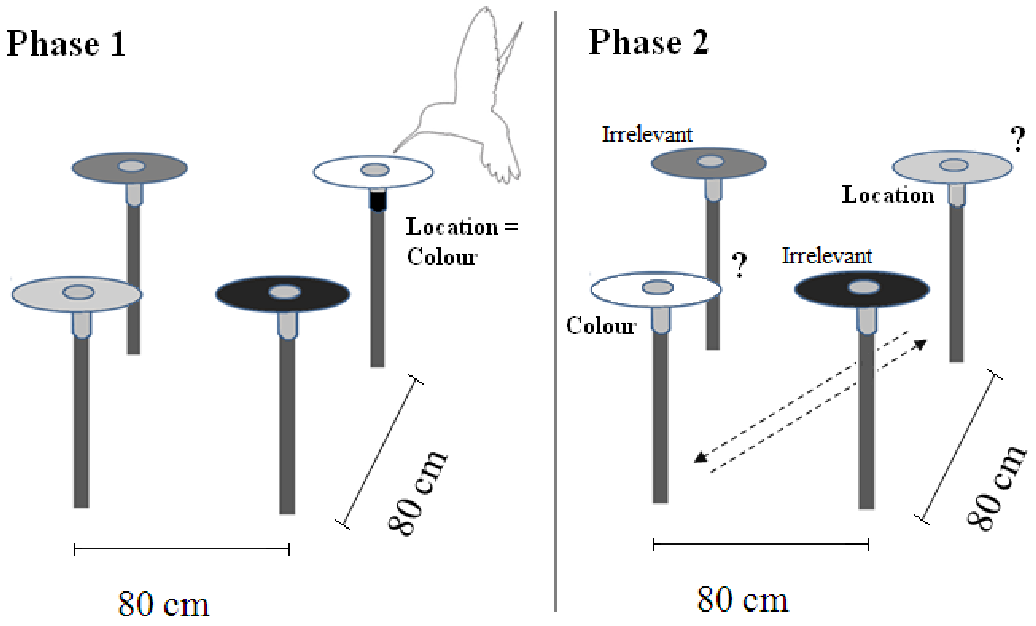

Figure 6.2. Diagram of the experimental set up. During Phase 1, a hummingbird had to search for one rewarded flower amongst four flowers presented in an 80 cm square array. Before the bird returned, I switched the rewarded flower with the flower in the diagonal corner. During phase 2 all flowers were empty. The dashed arrows indicate the flowers that had been switched in Phase 2.

Nevertheless, for the purpose of the analysis the probabilities of the two irrelevant flowers were pooled together so than the probability of visiting an irrelevant flower was 0.5 while the probability of visiting one of the relevant flowers was 0.25 each. I also analysed the bird's preference for the three types of flowers by comparing the number of birds that visited each type of flower (location, colour or irrelevant) against the proportion expected if birds were to visit the array at chance level by using a Binomial test. Again, the expected proportion for both the location and colour flower was 25% whereas the expected proportion for the irrelevant flowers was 50%.

RESULTS

Female rufous hummingbirds

The mean (±SE) inter-trial interval was 20.6 ± 6.2 min. Since all flowers were empty during Phase 2, the rufous females could have searched all four flowers but only two birds did. Most birds searched only two flowers and for two birds both visits were to the location flower. During Phase 2, six of the 11 birds first visited the correct location, one bird visited the flower of the correct colour first and four birds visited an irrelevant flower first (Figure 6.3). The overall distribution of choices was skewed towards location but not significantly different from the frequencies expected by chance (as tested by a Chi-square goodness of fit: $\chi^2_2 = 5.364$, with a randomization $P = 0.057$).

Importantly for this experiment, where the birds were expected to visit the flower with the correct colour, in fact, more female rufous hummingbirds returned to the flower at the previous correct location than expected by chance (Binomial test with an expected proportion of 0.25 for the location and colour flowers: location: 6/11, $Z = 2.263$, $P = 0.034$; colour: 1/11 $Z = 1.218$, $P = 0.311$; irrelevant flowers with an expected proportion of 0.50: irrelevant: 4/11, $Z = 0.904$ $P = 0.548$).

Female white-eared and magnificent hummingbirds

Due to the small sample size of female white-eared hummingbirds I pooled the visits of both Mexican species of females together. The mean (±SE) inter-trial interval of the females was 26.8 ± 2.0 min. The majority of females (nine of eleven) returned first to the flower at the correct previous location. Only one female first visited the flower with the correct colour and no female went first to an irrelevant flower (Fig. 6.3). Together, the distribution of first visits to the three different types of flowers was significantly different to the distribution expected if the birds were revisiting the array at random (tested by a Chi-squared goodness of fit test; $\chi^2_2 = 22.8$, with a randomization $P < 0.001$).

This preference for the flower in the original location was significantly higher than expected by chance, while the visit to the flower of the correct colour did not differ from chance (binomial test with an expected proportion of 0.25, 0.25 and 0.50 for the location, colour and irrelevant flowers, respectively; location: 9/10, $Z = 4.746$, $P < 0.001$; colour: 1/10, $Z = 1.095$, $P = 0.468$ and irrelevant: 0/10, $Z = 3.162$, $P < 0.001$).

Male white-eared hummingbirds

The mean (±SE) inter-trial interval for the male white-eared hummingbirds was 13 ± 1.0 min. Like the females from all three species, the majority of white-eared males (six of eight birds) returned first to the flower at the correct previous location and not the flower of the correct colour. Only one male visited the flower with the correct colour first while another went first to an irrelevant flower (Fig. 6.3). The general distribution of first visits to the three different types of flowers was significantly different to the distribution expected if the birds were revisiting the array at random (as tested by a Chi-squared goodness of fit test, $\chi^2_2 = 10.75$, with a randomization $P = 0.007$).

The visits made to the flower at the original location were significantly greater than expected by chance while the visits made to the colour flower were no different from chance. The visits made to the two irrelevant flowers were significantly fewer than expected by chance (Binomial test with an expected proportion of 0.25, 0.25 and 0.50; location: 6/8, *Z* = 3.265, *P* = 0.004; colour: 1/8, *Z* = 0.816, *P* = 0.688 and irrelevant: 1/8, *Z* = 2.121, *P* = 0.035).

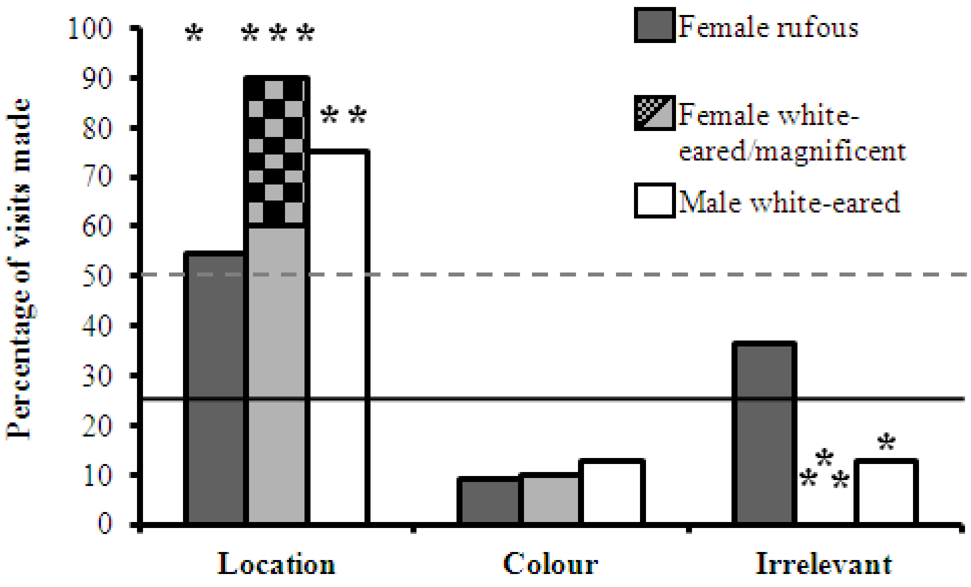

Figure 6.3. The proportion of first visits made to each flower type during Phase 2 (N = 11 female rufous hummingbirds, 3 female white-eared hummingbirds, 7 female magnificent hummingbirds, 8 male white-eared hummingbirds). The black horizontal line represents chance for both the correct location and correct colour whereas the grey dashed line represents chance for the two irrelevant flowers. Asterisks indicate the statistical significance of comparisons between observed and expected values (* *P* < 0.05, ** *P* < 0.005, *** *P* < 0.001).

DISCUSSION

Contrary to expectation, female hummingbirds of the three species I tested here did not prefer to use colour cues when returning to a flower array: only one of the 11 rufous females and one of the seven magnificent females returned first to the flower bearing the same colour as the flower rewarded in Phase 1. None of the three white-eared females tested returned first to the flower with the correct colour cue. Rather, like the rufous males tested by Hurly and Healy (1996) the majority of the females first visited the flower at the correct location (55% of the rufous females and 90% of the white-eared and magnificent females). Male white-eared hummingbirds also first visited the flower at the correct previous location (75%). Although the birds tested differed in their species, sex, territoriality, breeding stage (breeding or non-breeding season) or whether they are latitudinal migrants, most of them preferred to use spatial cues to relocate a reward.

Although my results are not consistent with the previously reported female preference for feature cues in other species (e.g. Dabbs et al. 1998; Astié et al. 1998; Jones et al. 2003) they do concur with a preference for spatial cues in both sexes of great tits (Hodgson and Healy 2005). As the birds in that study experienced the same location and colour 10 times in a row before they were tested, the authors speculated that it was the repeated nature of the training that lead to both sexes preferring spatial cues. That would not, however, explain the outcome I observed because my hummingbirds experienced the rewarded flower only once before they had to choose between colour and spatial cues.

Female hummingbirds, just like male hummingbirds forage amongst hundreds of flowers every day. Within a female’s foraging range or a male’s territory, the colours between flowers species might differ (e.g., red paintbrush *Castilleja* spp versus yellow columbines *Aquilegia flavescens*) but colour alone would not be an informative cue on whether a particular flower has been previously visited. Conversely, the location of a flower alone is sufficient information to relocate an exact specific flower. Therefore, perhaps it is not surprising that females also prefer to use spatial cues to relocate a rewarded flower.

There are, however, instances where feature cues might be more informative than spatial cues (for both sexes). While migrating, as hummingbirds arrive at a new stopover site, colour cues are likely to be helpful initially in locating flowers and at that time one might expect birds to prefer to use feature cues to locate profitable sites. Once the birds learn the locations of rewarding plants in their new territories (which they establish temporarily along their migration route), however, they should switch to using spatial cues. This possibility is supported by evidence that males can be flexible as to their preferred cue use when in their breeding territory: after only three trials in which a rewarded artificial flower was moved after each time the male fed, male rufous hummingbirds switched from using spatial cues to relocate the rewarding site to using colour cues (Flores-Abreu, Hurly, and Healy 2012). One would need to test hummingbirds' cue preferences along the migration route to confirm this suggestion.

Furthermore, hummingbirds (and probably other animals) use different types of cues at different scales. When male rufous hummingbirds had to return to a single rewarded flower in an experimental array, for example, they used the relative position of the flower within the array when flowers were spaced at 40 cm or less but when flowers were further apart (i.e., 80 cm or more) the birds used the absolute location of the flower (Healy and Hurly 1998).

In sum, these data seem to add weight to the suggestion made by Hodgson and Healy (2005) that sex differences in cue use are context dependent rather than ecological and that it is likely that selection has favoured flexibility in cue use rather than fixed strategies that differ between the sexes.

Chapter 7: General Discussion

As a result of the different experiments I carried out during my PhD I have shown that territorial male rufous hummingbirds will follow traplines when foraging, at least in an experimental context, and that, contrary to expectations, female hummingbirds will also use spatial cues to relocate a rewarded site.

As a first step to determining whether wild free-living hummingbirds might forage by traplining, I decomposed traplining foraging behaviour into some of its key components. Because traplining can be accomplished by learning the order of flower visitation, I first tested whether these birds would learn to visit a flower array following a predetermined order (or sequence). I found that the hummingbirds followed their own preferred routes incorporating first the flowers that were located closest to a usual feeding site. In a second experiment I confirmed that indeed these birds will repeat the order of flower visitation. Since the flowers from which hummingbirds feed will refill with time, I also tested and confirmed that these birds can learn when to visit one of four patches, an ability referred to as "time-place learning" (Gallistel 1990). Then, in a fourth experiment, I showed that hummingbirds flew traplines around several patches even when the flowers on those patches had a fixed refill interval and were rewarded only after a certain duration. Finally, although male and female hummingbirds have long been considered to differ in their foraging behaviour there were no empirical data to confirm (or not) this assumption. I therefore tested whether there were, indeed, differences in the types of cues used by males and females when foraging. I found that, just as for male hummingbirds, females of different species prefer to use the more reliable spatial cues rather than feature cues to relocate a reward.

Combined, these results may offer insight into the natural foraging behaviour of hummingbirds in the wild. Instead of assuming how hummingbirds visit multiple locations based on their visits to focal flowers I have recorded, for the first time, how they develop their own routes when visiting multiple locations. Consequently, the results from the different experiments I conducted also challenge the entire classification of foraging behaviours that has been accepted for hummingbirds. My data also confirm that even though hummingbirds can learn to use temporal information when visiting several patches of flowers, the spatial location of those patches has a larger influence when the birds return to visit previously rewarded flowers. Hummingbirds use the shortest routes to visit several locations and prioritize those locations closest to a central perch. These data are, therefore, relevant for both the study of the foraging ecology of hummingbirds as well as the cognitive abilities of foraging hummingbirds.

The traplining foraging behaviour of hummingbirds

Although traplining is thought to be taxonomically wide spread for most of the species that are considered to forage in this way, this behaviour is only now receiving much quantitative attention (Lihoreau et al. 2013). This is due to the difficulty of tracking animal movements through the environment without disturbing their natural foraging behaviour. Since individually marked hummingbirds can be trained to visit artificial flowers in their own territory I developed a method to record the traplining behaviour of hummingbirds to test how and when hummingbirds use traplines without the use of telemetry. When five flowers were added to a board one at a time, male territorial rufous hummingbirds repeated the order in which they visited the artificial flowers in the array. The order of flower visitation was not influenced by the order of flower presentation. Birds flew only a very small subset of routes despite of the large number of possible routes around the flower arrays and those routes were most often the shortest distances routes around the flowers. These data are the first quantitative evidence that hummingbirds may very well develop traplines when foraging and they are consistent with the hierarchy of cues hummingbirds use when foraging in the wild.

A plethora of studies have established that hummingbirds much prefer to use spatial cues rather than other cues (e.g. feature cues, temporal cues) when foraging (Sutherland and Gass 1995; Healy and Hurly 1998; González-Gómez and Vásquez 2006; Flores-Abreu, Hurly, and Healy 2012). For example, hummingbirds will continue to search for an artificial flower at the previous rewarded location even when the rewarded flower has been moved only three meters away (Flores-Abreu, Hurly, and Healy 2012). However, as hummingbirds need to feed from hundreds of flowers every day to maintain a positive energy budget (Hixon, Carpenter, and Paton 1983), it is possible that to forage efficiently these birds use an alternative mechanism to that of remembering each of the spatial locations of hundreds of individual flowers. Traplines might be that more effective foraging strategy. In my board experiment, the hummingbirds used the shortest routes around all flowers even though it was not the route predicted by the order of flower presentation. This ability is not a trivial one. In fact, the board problem is analogous to the Travelling Salesman Problem in which animals are expected to find the shortest route around several locations before returning to an origin, in this case a central perch. As the number of locations to visit increases the number of possible routes increases factorially. For example, in the board experiment, when there were five flowers to visit the hummingbirds could have followed 120 different possible routes (5!). Instead, hummingbirds used one or two routes more often than all other routes and those preferred routes were the shortest possible ones.

The Travelling Salesman problem is common to many central place foragers and animals are expected to solve it in order to forage with an optimal route even though no efficient mathematical solution is known for this problem (Applegate et al. 2006).There are no algorithms that can solve the problem efficiently because the processing time increases as the number of locations increase (Reynolds, Lihoreau, and Chittka 2013). The easiest way to solve the problem would be to try all permutations and then to select the shortest one but this is impractical for both the animals in the wild and for the computer algorithms because as the number of locations increases so does the number of possible permutations (Anderson 1983; Lawler et al. 1995). Nevertheless, nectarivores, including the hummingbirds I tested, can complete this task. For example, bumblebees *Bombus terrestris*, given a task similar to that presented in Chapter 2, also followed the shortest possible routes around five flower locations after only 60 bouts (Lihoreau, Chittka, and Raine 2011). As impressive as this behaviour might be, however, it need not be the result of a "complex cognitive process" but rather the result of a simple heuristic rule. When the flower visitation sequences of bumblebees were compared with a simple iterative improvement heuristic model of a bumblebee collecting nectar from a stable flower array, the model closely matched the observed behaviour of these animals (Lihoreau et al. 2012). The model included a bee comparing the length of the route just travelled with a previous one and keeping the shorter of the two, with just this positive feedback the route segments that resulted in a shorter route would be reinforced. This strategy would allow the bees to use an optimal trapline while also readjusting their routes in response to changes in environment. Whether hummingbirds use the same strategy to shorten their routes is still unknown but my data show that they can do so after as few as 60 bouts.

Since the environment is not static and the distribution, availability and competition for a resource changes constantly, an animal foraging efficiently should readjust its foraging routes accordingly. For example, central foraging animals might face a trade-off between using an optimal route that reduces the distance flown around profitable locations or a route that prioritises the locations that present a larger reward. This was shown to be the case for the bumble bees that, after stabilising a stable optimal route around five flower locations, readjusted their traplines in the presence a highly rewarded flower even though that meant that the overall distance flown would be greater (Lihoreau, Chittka, and Raine 2011). This flexibility in traplining behaviour suggests that at least some animals can weight different information depending on the context. Whether hummingbirds too would adjust their traplines in response to a volume change in one of the locations needs further experimentation.

Another factor that might influence how hummingbirds use their traplines is the time flowers take to refill their depleted nectar supply. To investigate how hummingbirds might incorporate a temporal component into their trapline, I tested whether hummingbirds could learn when to visit four patches that were rewarded for one hour only per day. Most of the hummingbirds learned when to visit each of the four patches and some learned the time-place association after only one day of training. After a test day in which the patches were only presented at the fourth hour, it appeared that both the time of day as well as the order of patch visitation were necessary for these birds to visit the correct patch at the correct time. These data are consistent with the data from a previous study in hummingbirds (Marshall et al. 2013) and several in rats (Carr, Tan, and Wilkie 1999; Pizzo and Crystal 2002; Thorpe et al. 2012). In the laboratory however, when tested with different time manipulations animals seem to time events by using either an ordinal, time of day or interval timing mechanism. For example, rats learned to get a reward from a T-maze by going to one arm in the morning and the other in the afternoon. In one of the tests the light cycle was inverted and yet the rats continued to visit the temporally correct arm, suggesting that these animals were not using the light transmission to time their visit to the different arms of the maze. Then, in another test, the morning session or the afternoon session was skipped but again the rats were able to visit the correct arm, ruling out an ordinal timing mechanism. It was concluded that rats used a circadian mechanism (or the time of day) to visit the two arms correctly (Mistlberger et al. 1996). In the field, however, the possibility of manipulating time experimentally is limited to skipping sessions. It is possible that in a controlled environment where external cues are minimized by the testing protocols, animals can learn a time-place association by either learning the time of day or the order of events. For animals in the wild however, both types of cues are necessary and neither is sufficient. Regardless of which timing mechanism the hummingbirds might have used, the results from my time-place experiment suggested that indeed hummingbirds could include temporal information when visiting several locations and that they would visit a patch only when it was likely to be rewarded.

Having found that hummingbirds will not only repeat the order of flower visitation in traplines but that they could also learn to go to different flower patches at different times, I then investigated whether hummingbirds could integrate the temporal information of flower refilling intervals and the spatial information needed to form a trapline. The birds were presented with three patches of flowers that would refill only after 20 minutes had passed from the last visit. Birds repeated the order in which they visited the patches by first visiting one or two of the patches while not visiting the third patch until more than 20 minutes had passed. Hummingbirds did not develop a route to maximize their sucrose intake during the first flower visit of a bout; rather the birds used the routes that lead to them visiting first those flowers nearest to the location usually occupied by their feeder. Hummingbirds followed a direction around the patches so that one patch would follow another, but the start of the trapline was always the flowers closest to the location of the feeder (removed for the duration of testing). This suggests that the hummingbirds prioritize visiting flowers in a sequence from the closest to the furthest rather than waiting 20 minutes to revisit those patches that were in route to the rewarded one.

Although these data bear some resemblance to my first experiment, this result was a little surprising since there is plenty of evidence that these birds can readily use the temporal information of the specific refill schedules of individual flowers adjusting their visit rates to flowers depending on when those flowers were last visited (Gill 1988; Henderson et al. 2006; González-Gómez, Bozinovic, and Vásquez 2011; Marshall, Hurly, and Healy 2012). Furthermore, when flowers with different refill intervals were of different colours, hummingbirds learnt the refill interval significantly sooner than when flowers were of the same colour (Samuels, Hurly, and Healy 2014). Therefore, even though the refill time of the artificial flowers used in those experiments was shorter than is the natural refill interval of flowers, those data show that hummingbirds can use temporal information to modify their foraging behaviour. A difference between the experiment I described in Chapter Five and those in the previous published studies is that in the latter, the flowers with different refill intervals were all presented 60 cm from each other, forming what might be considered a single patch. In the experiment described in Chapter Five, flowers within a patch were 40 cm apart and the patches of flowers were separated by 3m. When flowers are spaced more than 80 cm apart, hummingbirds remember the exact spatial location of individual rewarded flowers and not the relative position in relation to other flowers (Healy and Hurly 1998). This is relevant because the hummingbirds that were feeding in the patches separated by 3 m may have remembered the flowers in different patches as patches and not as individual flowers. The birds may, then, not have used the information regarding the refill rate of individual flowers when moving across the patches.

When in 1978 Pyke applied optimal foraging theory to the movement patterns of foraging bees he assumed that nectar was a non-renewable resource and therefore there was no energetic gain to revisiting a flower. This is true, however, only when the time between revisits does not allow flowers to refill (Pyke 1978). As more time passes between revisits, however, flowers do replenish and pollinators would benefit from revisiting flowers before they are visited by a competitor. Therefore, the time scale at which pollinators revisit a flower will determine whether is an advantageous or disadvantageous behaviour (Gill 1988; Possingham 1989). Depending on how rapidly flowers refill, revisiting flowers at regular intervals may reduce the variance of the reward encounter (Possingham 1989). Hummingbirds that continued to visit the flowers before the 20 minutes refill interval (Chapter Five) depleted the reward in the patches to a greater extent than if they had waited until the 20 minutes had passed. Their behaviour meant than only a third of the reward was available at any one time. This could be a useful strategy to outcompete intruders. Allowing hummingbirds to feed freely amongst several rewarded patches that will only refill after a longer, more realistic interval (around three to four hours) might clarify how hummingbirds actually incorporate temporal information into their foraging.

In any case, instead of remembering individual flowers, perhaps hummingbirds should remember the patches that they have visited in the morning, avoiding them in the afternoon by moving to a new area later in the day. Remembering the sequence of patches visited and not the refill intervals of individual flowers might be a more plausible reflection of how hummingbirds actually forage. In my first experiment I tested if hummingbirds could indeed learn to visit flowers in a predetermined order. The experiment I used to test this hypothesis was similar to protocols that are aimed at testing the ability of animals to learn a sequence. I therefore presented six hummingbirds with two different artificial flower arrays where birds had to learn the ordinal sequence in which flowers were rewarded. One flower array had three flowers while the other one had five. Each flower within the array was rewarded in an ordinal sequence so that only one flower in the array was rewarded at a time. In both arrays, flowers were separated by 72 cm or 1 m. In both arrays birds did not visited the array at random, but rather started to visit the array by the flower closest to the location of the feeder. In the array of three flowers the hummingbirds showed a primacy and recency effect at the beginning of the training trials. In the array of five flowers birds also showed a recency effect, which meant that flower 5 was correctly visited more frequently than would be expected by chance. As in the patches of flowers in the experiment in Chapter Five, the proximity to location of the feeder was important and hummingbirds flew their own sequences when visiting the flowers, rather than the sequence I attempted to impose. These data led to me to all of the experiments that followed.

Taken together, all of these results suggest that even though hummingbirds can remember and use temporal information in a traplining context, the time of day, the order of flower reward or the interval between rewards may not be especially relevant to these birds when foraging between multiple locations.

Since tracking animals as they forage naturally is still methodologically complicated, in the past indirect measures have been use to assess how animals forage in the wild. In the case of hummingbirds, training them to visit artificial flowers has allowed us to determine what animals can do but not what they actually do when foraging naturally. For example, we know that they can learn to time their visits to match a 20-minute refill interval, showing that these animals have the ability to time intervals that are shorter than the time it takes natural flowers to refill. When foraging naturally, however, this ability might not be used because flowers do not refill after 20 minutes, instead this ability might be use to time how long a female sits at the nest or how long a male stays on one perch before moving to another. The truth is that even though much is known about the foraging and cognitive abilities of hummingbirds as a result of experiments like mine, how these birds really use their cognitive abilities in their daily lives warrants more research.

In recent years, Passive Integrated Transponder (PIT) tags have been used to record the flight paths hummingbirds use to return to specific locations (Rousseu, Charette, and Bélisle 2014). Furthermore, radio tags have also been successfully fitted to ruby-throated hummingbirds while allowing the birds to fly freely inside an aviary (Zenzal, Diehl, and Moore 2014). This methodological advance, combined with in-depth knowledge of hummingbird behaviour, could mean that in the future we will be able to follow hummingbirds as they forage naturally. When tracking hummingbirds as they forage freely becomes a feasible field technology we will be able to answer all sorts of unknown questions. For example, do hummingbirds optimize the routes they use to visit all flowers in their territory? Or, do hummingbirds time their visits to match the natural refill rate of different plant species? Do male and female hummingbirds differ in their foraging behaviour? When do hummingbirds switch from using spatial cues about the location of rewarded flowers to using feature cues to find new flowers? Combining the methodology so far used to study hummingbirds with tracking technology will allow us to determine what information the animals learn, remember and use and whether any differences are the result of different ecologies.

Hummingbirds use different information to solve different tasks. For territorial birds spatial cues are reliable when relocating flowers that do not move in space and therefore these birds tend to rely more on spatial than feature information when solving many foraging tasks. When migrating however, hummingbirds might use visual information to first locate an area with red flowers. After finding a site with several flower patches rufous hummingbirds will establish a temporary territory and then may switch to remembering where they have found rewarding flowers rather the colour(s) of the flowers. A rufous hummingbird foraging efficiently, then, will be able to acquire and use information of one type if other types of information are not available or are unreliable.

Individual differences in the types of cues used may be the result of which cues an individual has used more often. Only when one type of information is always available and reliable, can we expect natural selection to favour the use of one cue type above all others and to find a preference for using a specific type of information across all individuals of a species and across contexts. Rufous hummingbirds seem to be equipped with the ability to use one or the kind of information depending on different situations, however given their ecology, it is also possible that the strong preference for spatial information has been favoured by natural selection.

The results from my experiments suggest that traplining behaviour, as “complex” as might seem at first glance is not a hardwired behaviour and that there is a role for experience, depending on circumstances. All through my experiments I found individual variation in the sequences that were preferred by the different hummingbirds. This variation in foraging behaviour may have been caused, in part, by a predisposition to certain distances and turning angles of successive flights (Pyke 1978) but also by spatial learning (Thomson 1996). A difference in the amount of experience of a particular arrangement of flowers would also result in a variation in the traplines followed between individuals. For example, individuals that are territorial and have visited rewarded locations several times could potentially also use the renewal schedule, the competition level of that area, the age and state of some of the flowers to change their traplines as these factors change. On the other hand, individuals that are not territorial (there were many rufous males that did not defend territories at my site) and have only limited access to rewarding flowers might benefit from using other types of information. Instead of learning the location of a rewarded flower that they might never visit again, these foragers might use the feature cues of rewarded flowers to visit only those flowers that "look" like they might contain nectar. For example, the flowers of *Malvaviscus arboreus* var. *mexicanus* (Malvaceae) change colour and are more likely to be robbed as time progresses. Flowers pollinated by hummingbirds are often marked by holes or ruptures made by insects or other bird taxa that have tried to access a flower's nectar (Irwin and Maloof 2002). These flowers are referred to as "robbed" because those nectarivorous species consume the flower's nectar without pollinating the flower (Inouye 1980). Therefore, hummingbirds might use the change in colour or the hole left by the robbers as a cue denoting a possible lack of a reward and consequently avoid it (Gass and Montgomerie 1981).

Since most female hummingbirds are not thought to be territorial, if the types of cues used when foraging depend on gender or on territoriality, then the sexes should differ in the types of cues they use when foraging.

Sex differences in cue use

Hummingbird foraging behaviour has been classified as territorial or as traplining with females of most of the species considered to be trapliners. As I have discussed already, this classification is incorrect for two reasons. First, no previous studies had described the use of traplines in hummingbirds. Second, territoriality and traplining behaviour are not mutually exclusive. Indeed, hummingbirds that defend a territory may be more likely to develop traplines than are non territorial birds as the former will benefit most from learning the location of profitable resources that will be revisit in the future. Perhaps a more accurate description of what females, or non-territorial males for that matter, do is to search for food until they find a rewarded location, deplete the resource and then move on to the next available profitable patch (Ohashi and Thomson 2009). This foraging behaviour will leave searchers with a great deal of uncertainty as to future feeding opportunities. This description, however, seems a little unlikely since a female that has to feed herself and two chicks during the breeding season will remain in one location centred around the nest for at least a month (Baltosser 1996). In this sense female hummingbirds could also be classified as territorial because they too forage around a certain restricted area. Furthermore, when given the opportunity, the females that were observed and tested for the cue preference test described in Chapter 6 also defended feeders aggressively and would even “slap” another female if she came to feed from the artificial feeder provided. When the male rufous hummingbirds start to migrate back to Mexico, female rufous will compete with each other for access to feeders and just like males they will spend most of their time perching near the artificial feeder.

Rather than classifying species or individual of a species as a forager that uses one or other strategy when feeding, then, a more accurate description would acknowledge that animals are likely to change behaviour depending on the context. Female hummingbirds have been understudied because when they approach a feeder (which is used to then train territorial birds to feed from the artificial flowers) they are either chased off or harassed by male territorial hummingbirds (Hurly and Healy 1996; Temeles and Kress 2010; Pérez et al. 2011). For this reason, female foraging behaviour had not been described with the same degree of detail as has male foraging behaviour. But as males and females were thought to forage using different spatial strategies, I had reason to believe that females would use different types of cues to relocate a rewarded flower.

The range size hypothesis (Gray and Buffery 1971) suggests that polygynous males will benefit from enhanced spatial abilities because the males will cover a bigger home range compared to the females. Since males of many species do outperform females in spatial tasks, including humans (Kavaliers et al. 1996; Seymoure, Dou, and Juraska 1996; Collins and Kimura 1997; Lacreuse et al. 1999; Jozet-Alves, Modéran, and Dickel 2008; Kandori et al. 2009; Schmidtke and Esser 2011a) the cause of the difference in performance has been much investigated. In laboratory experiments males appear to use both spatial (e.g. distance and direction, location) and feature cues (e.g. colour), while females frequently use only feature cues (Gaulin and Fitzgerald 1986; Kavaliers et al. 1996; Seymoure, Dou, and Juraska 1996; Collins and Kimura 1997; Lacreuse et al. 1999; Jozet-Alves, Modéran, and Dickel 2008; Schmidtke and Esser 2011).

As a first approach to comparing the foraging behaviour of female hummingbirds I first recorded the preference for cue use (spatial or feature) of the females of three hummingbird species and the males of one Mexican species. Birds were allowed to feed once from a four-flower array in which only one flower was rewarded. When the birds returned, the colour and the spatial cue designating the rewarded flower had been dissociated. Although I had expected females to visit the flower of the correct colour (feature cue) first, during the test phase most of the birds (males and females) went to the correct spatial location (spatial cue). It appears, then, that in hummingbirds the preference for spatial cues is not specific to males and it seems more likely to depend on the relevance or value of a cue to the solution of the task.

To the best of my knowledge all female hummingbirds exclusively provide the parental care, which includes constructing the nest and feeding the chicks until they fledge while also feeding themselves. Therefore, the energetic demands of a reproductive female are expected to be the same if not greater than are the energetic requirements of territorial males. Moreover, due to their smaller body size females have higher thermoregulatory cost. However, when the territory size and density of flowers of males and a female purple-throated carib *Eulampis jugularis* were compared, males had smaller but more dense and energetically rich territories than the territory of the single female observed (Temeles, Goldman, and Kudla 2005). This suggests that females have a different foraging strategy (other than territoriality) to obtain all the nectar that they need to feed themselves and their offspring. Females of the green-backed firecrown *Sephanoides sephaniodes* are described as opportunistic, exploiting flowers scattered throughout several patches within male territories (González-Gómez et al. 2014). Females of this species have larger wings than males allowing them to travel longer distances saving energy and females also have a larger bill and higher intestinal enzymatic activity than males, which allows them to feed more frequently, whenever the possibility presents.

All these morphological adaptations suggest that the foraging behaviour of females might differ from that of males. Still, because the foraging behaviour of females is studied in detail (i.e., whether females trapline, when and how they establish territories) only when females are themselves territorial, it may not be surprising that no differences are found. I was only able to test female rufous hummingbirds once the males had set off on their southerly migration and left their territories, leaving only two weeks before females also began their return migration. In that time, females established a territory and would visit the artificial feeder frequently and will defend it from other females. When I tried to test whether females too would develop traplines, I discovered that females are less tolerant to changes in the flower array and would abandon a site if she found a flower empty. Studying the natural foraging behaviour of non-territorial females, then, can be difficult.

As pointed out earlier, the use of tracking technology to study the foraging behaviour of hummingbirds might also help us determine if indeed female hummingbirds forage by following traplines or if and when their foraging behaviour differs to that of male hummingbirds. It is possible that when females are breeding and need to stay close to the nest they follow traplines around the flower patches near the nest. During migration females might forage by locating flowers visually using the colour of morphological characteristics of rewarded flowers. Until the tracking technology becomes available, it appears that the easiest way to access how hummingbirds forage is by allowing them to use artificial flowers.

The ecology and cognition of hummingbirds

For well over 60 years hummingbirds have been a model for optimal foraging behaviour and, more recently, for the cognitive abilities that allow them to optimize different elements of their foraging. As it is often the case, however, many questions remain unanswered and some of the descriptions of the foraging behaviour of hummingbirds have turned out to be too simplistic. In this thesis I have shown that territorial hummingbirds follow routes around several rewarded locations, that they can use temporal information to avoid visiting empty flowers but that the spatial proximity to a previous rewarded location is more relevant for these birds. I have also shown that females of different species will also prefer to use spatial cues when foraging.

With this thesis I hope I have shown that any behaviour, as simple as might look at first glance, can be fascinating if studied in depth. Hummingbirds that need to make hundreds of foraging decisions in a day, can use many different types of information, however given the choice spatial information and the optimization of flight distances will be prioritize.

The traplining behaviour and the type of information use by the hummingbirds described in this thesis are very similar to that of the Hymenoptera even though these two groups are not related. This similarity is probably due to the common ecological problems faced by these two pollinating groups. Therefore one might expect that other distantly-related groups faced with foraging problems similar to these two groups may also resolve them by traplining.

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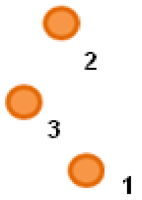
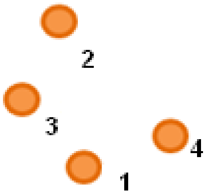
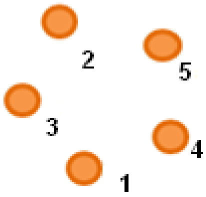
Appendix 3.1. Visitation sequences of each bird to the flowers on the board. Sequences are presented in chronological order from bout 1 to bout 60. In the case where a bird visited the board with the same sequence for 9 out 10 bouts, then that stage ended and a new flower was added to the board. Numbers 1 to 5 in each cell represent the flower on the board. Above each panel there is a schematic of the position of each flower at that stage of the experiment. Colored cells indicate the sequences repeated by that bird more often than expected by chance during that stage of the experiment (Binomial test with a random probability as the total of sequences observed for that bird at that stage: $p < 0.05$). Extended sequences where birds revisited one or more flowers in the same bout are indicated using red font and were excluded from all analysis * represent sequences that resulted in a longer distance flown.



2 Flowers on the board						
	Bird 1	**Bird 2**	**Bird 3**	**Bird 4**	**Bird 5**	**Bird 6**
Bout 1	2121	21	1	2	1	1
Bout 2	21	2	2	2	2	2
Bout 3	21	2	2	1	21	1
Bout 4	21	12	12	12	1	1
Bout 5	12	21	21	2	121	21
Bout 6	21	12	12	2	2	12
Bout 7	12	2	2	2	21	12
Bout 8	21	12	2	1	12	12
Bout 9	2	21	21	2	1	12
Bout 10	21	21	21	2	12	21
Bout 11	12	1	21	21	21	21
Bout 12	12	21	2	2	21	12
Bout 13	2	12	12	12	12	2
Bout 14	21	2	21	2	21	2
Bout 15	2	12	2	12	21	21
Bout 16	12	2	12	1	12	21
Bout 17	12	21	2	21	21	12
Bout 18	12	12	21	21	12	12
Bout 19	12	2	2	2	21	12
Bout 20	12	21	21	21	1	21
Bout 21	12	1	2	21	2	12
Bout 22	12	12	2	12	12	12
Bout 23	21	1	2	12	21	1
Bout 24	12	21	12	12	21	21
Bout 25	12	1	2	2	21	21
Bout 26	12	21	2	1	1	2
Bout 27		12	21	2	21	21
Bout 28		21	21	12	2	2
Bout 29		12	12	1	21	12
Bout 30		1	21	1	212	12
Bout 31		12	12	21	12	12
Bout 32		12	12	2	21	2
Bout 33		12	21	12	21	2
Bout 34		1	21	2	21	2
Bout 35		21	12	1	21	12
Bout 36		2	21	2	21	21
Bout 37		2	2	12	21	12
Bout 38		12	21	12	21	12
Bout 39		21	21	21	21	12
Bout 40		12	21	1	212	12
Bout 41		1	21	12	21	21
Bout 42		12	21	21		21
Bout 43		12	12	2		2
Bout 44		12	2	12		2
Bout 45		2	12	12		12
Bout 46		2	21	2		21
Bout 47		12	12	12		12
Bout 48		1	21	12		2
Bout 49		2	12	1		12
Bout 50		1	21	2		21
Bout 51		2	12	2		21
Bout 52		12	21	12		21
Bout 53		12	212	12		1
Bout 54		12	21	2		21
Bout 55		12	21	12		21
Bout 56		12	2	12		21
Bout 57		12	12	2		21
Bout 58		12	12	12		21
Bout 59		12	12	2		2
Bout 60		2		2		21

3 Flowers on the board					
Bird 1	**Bird 2**	**Bird 3**	**Bird 4**	**Bird 5**	**Bird 6**
13	132	132	132	312*	231
231	2	231	132	231	132
132	32	312*	13	312*	321*
31	32	321*	132	321*	231
132	13	32	312*	321*	231
132	23	312*	132	312*	231
132	132	32	312*	321*	32
132	13	321*	312*	132	23
231	132	32	312*	312*	312*
132	13	32	32	132	32
32	132	321*	312*	132	32
13	1	32	32	312*	231
132	132	32	32	132	132
132	32	321*	32	32	312*
132	32	321*	32	231	312*
31	32	32	3132	231	132
132	231	132	312*	31	231
132	312*	321*	321*	32	231
132	312*	321*	32	123*	132
132	132	321*	132	32	132
31	132	321*	132	32	312*
132	231	321*	23	312*	231
132	32	321*	132	321*	312*
132	32	312*	132	231	312*
132	3231	32	21	312*	13
132	32	321*	132	32	23
	321*	23	13	32	31
	13	231	23	32	321*
	3231	3231	31	321*	231
	32	312*	132	31	231
	23	213*	23	231	231
	23	312*	31	321*	132
	32	31	132	231	13
	132	321*	132	231	132
	32	32	132	231	23
	23	321*	132	321*	231
	32	321*	32	132	32321
	13	3132	132	32	13
	32	321*	132	231	132
	3	132	321*	132	132
	32	312*	231	231	132
	13	321*	312*	31	31
	132	321*	132	32	132
	3	321*	13	231	312*
	32	312*	32	3232	32
	132	231	132	231	321*
	32	231	123*	231	312*
	321*	321*	321*	132	132
	312*	312*	231	231	231
	32	312*	31	1321	132
	32	312*	132	123*	132
	32	312*	132	312*	132
	132	321*	23	1321	312*
	132	321*	32	31	31
	32	32	13	132	132
	132	321*	132	321*	132
	321*	231	32	231	312*
	321*	312*	32	32	312*
	231	312*	13	3231	132
	32	31	132	321*	32





	4 Flowers on the board					
	Bird 1	Bird 2	Bird 3	Bird 4	Bird 5	Bird 6
Bout 1	314	142	3142*	32	23412	324
Bout 2	31432	231413	3142*	314	3214*	3241*
Bout 3	3142*	3241*	314	32	3214*	132
Bout 4	41321	32	3241*	314	3214*	1413
Bout 5	41231	3214*	324	13	3142*	32
Bout 6	4132	3	2314	143	3214*	14
Bout 7	31432	232	324	314	32314	132
Bout 8	1432*	132	231	324	3214*	1423*
Bout 9	3241*	32	24	142	13241	132
Bout 10	4132	13241	314	32	231	3241*
Bout 11	4132	32413	3142*	32	32314	324
Bout 12	1324*	23141	324	142	2314	2314
Bout 13	4132	3241*	314	132	2314	32
Bout 14	4132	3241*	41432	3241*	2314	4132
Bout 15	4132	3241*	2314	324	32413	2314
Bout 16	4132	1432*	31	314	3214*	413
Bout 17	132	3241*	14	132	32314	41
Bout 18	3214*	231	324	314	3241*	143
Bout 19	132	3241*	324	143	3214*	314
Bout 20	314	1432*	241	32	2314	132
Bout 21	4132	3241*	324	3142*	32314	414
Bout 22	4132	324	314	3142*	2314	324
Bout 23	4132	1432*	324	3142*	3142*	4132
Bout 24	41324	4132	14	3142*	3241*	3142*
Bout 25	1324*	3241*	3241*	3142*	3241*	31
Bout 26	41324	13241	3142*	3142*	2314	4132
Bout 27	41324	3241*	14	142	3241*	314
Bout 28	4132	2314	3142*	1432*	4132	3142*
Bout 29	1324*	2341*	4132	324	3142*	3142*
Bout 30	14	14132	314	13142	3241*	3142*
Bout 31	412	23	314	314	3241*	314
Bout 32	4132	1423*	34	231	32314	231
Bout 33	4132	3241*	34	31	3142*	143
Bout 34	4132	4132	314	14	1342*	14132
Bout 35	4132	1324*	312	23	3214*	2314
Bout 36	4132	1432*	4132	324	2314	3142*
Bout 37	4132	2314	314	314	32314	3142*
Bout 38	4132	1432*	3412*	23	3142*	3142*
Bout 39	1324*	2314	3142*	32	32314	3241*
Bout 40	4132	23	3142*	314	4132	1432*
Bout 41	4132	32	324	324	2314	3142*
Bout 42		3	3142*	3142*	4132	2314
Bout 43		32	3214*	3142*	2314	3241*
Bout 44		32	3241*	324	2314	14132
Bout 45		324	314	314	4132	42
Bout 46		3214*	3241*	32412	4132	2314
Bout 47		1423*	3124*	3142*	4132	2314
Bout 48		3214*	132	32	4132	31432
Bout 49		32314	3142*	3142*	4132	314
Bout 50		3241*	31412	3142*	4132	143
Bout 51		3142*	31432	3241*	2314	1413
Bout 52		3241*	3142*	3241*	2314	3142*
Bout 53		3241*	4123*	3241*	14321	314
Bout 54		3241*	3142*	3241*	4132	413
Bout 55		32314	4132	3241*	2314	314
Bout 56		3214*	3142*	3142*	1432*	314
Bout 57		32	3241*	3241*	4132	1324*
Bout 58		2341*	3142*	324	231	314
Bout 59		3142*	4132	3142*	4132	314
Bout 60		3241*	3142*	32	2314	132

5 Flowers on the board					
Bird 1	Bird 2	Bird 3	Bird 4	Bird 5	Bird 6
414523	32541	231452	3145	45231	32541
452314	354123	32541	3251	32541	31452
413254	23145	45231	32541	145231	1452
52314	31452	54123*	1452	23452*	31452
254132	2314	54132	3251	32541	32541
413	32	54132	31452	32541	31452
31452	145231	54132	3254	14532*	31452
132	23541*	31452	32541	32541	32541
314523	14	5231	32541	23145	541
314523	14523	2541	32541	52314	32541
31452	325	54132	32541	14523	13254
25413	23	5413	31452	23145	23145
14532*	32541	153124	3254	2345	32541
314523	325	541	325	45231	13254
4132	541325	31452	325214	23145	13254
4135	32514*	54132	325	23145	3254
3152	32541	31452	325	32541	4132
413254	13254	54132	1452	32541	14523
413254	25	4152	31452	23145	31452
31451	32	4132	14523	325	2541
4152	145231	23145	32514*	3251	254132
32541	254132	54132	32541	32541	313254
41452	14523	45312*	325	32541	31452
3254132	32	4152	31452	13254	13145
325413	3	4531	32541	3245	13254
231452	145231	4152	1325	32541	325
254131	23145	5231	32541	32541	314
31452	1452	3145	1452	3145	4132
3134523	453	14523	31452	32541	41325
41452	45231	13254	31452	31452	32541
25413	1452	4132	31452	13524*	41325
31452	1325	4532	31452	32541	4132
254132	14532*	4132	31452	31452	45231
25413	1452	23145	14532*	3254	145231
3145	32	45321*	31452	23145	41325
41325	132	23145	41325	45231	31452
4152	1325	14523	31452	25413	41325
31452	14	45231	325	254132	23145
45231	14523	45231	41325	32541	31452
314523	14523	4531	31452	32541	32541
32541	4132	54132	31452	231452	32541
132541	14523	54132	52431*	13254	231
145	14523	54132	31452	1452	141325
4132	14523	254132	32541	452314	32541
13254	325	5234	315	31254*	31452
132541	1452	4132	325	32541	31452
31452	4532	4532	325	523145	231454
41352*	1452	45321*	25	54132	14523
41542	123	4532	32541	25314*	3145
413354	32541	54132	25413	452314	32541
231452	1452	5432	31452	23145	1452
4132	3254	4531	54132	23145	31452
31452	14523	452314	32541	45	31452
31452	32541	452314	3452	32541	314523
4513	32541	45132*	1452	32541	23145
251	32541	54132	32541	23145	31452
54132	32541	4532	31452	32541	454132
4152	14523	45132*	32541	32541	3145
41325	3251	4513	325	23145	31452
1452	32541	413	325	23145	254132

Apendix 5.1. Order in which each bird visited the patches during each bout. Each letter represents a different patch. B: Blue, G: Green, M: Morado (purple in Spanish), O: Orange and P: Pink.

Bout	Bird 1	Bird 2	Bird 3	Bird 4	Bird 5	Bird 6	Bird 7	Bird 8
1	G	G	B	MO	O	O	PM	P
2	GP	G	B	MGO	O	O	B	PO
3	M	G	BP	O	P	G	M	P
4	G	BP	M	MGO	B	O	P	B
5	GP	G	P	G	PB	O	MB	PO
6	G	G	P	MGO	O	OB	P	B
7	GM	G	B	G	P	G	M	PO
8	GP	B	P	MO	OPB	GB	PB	B
9	G	G	B	MO	PO	O	P	P
10	GPG	G	BM	G	P	OB	PB	BO
11	M	G	BP	MO	PB	G	M	OPB
12	P	B	P	MG	O	GO	P	P
13	G	P	M	OM	OP	B	PB	BO
14	M	B	B	G	B	GO	BM	PO
15	P	BG	BM	GO	OP	GBO	P	B
16	G	P	BP	M	O	BG	PB	O
17	M	B	PM	MG	OP	O	M	PO
18	GP	G	B	O	PB	GB	BP	B
19	G	P	M	G	O	OB	PB	BO
20	M	PB	BP	M	O	GOB	BM	BPO
21	MP	G	B	MG	P	GO	MP	PB
22	G	BG	BM	O	OB	O	P	PO
23	GP	B	P	OG	OP	G	M	PBO
24	M	BP	B	GO	O	O	PB	BPO
25	G	G	P	M	P	GBO	MB	BP
26	P	B	B	G	POB	G	P	PO
27	GP	PB	M	O	OP	BO	PMP	P
28	GM	G	BP	GM	O	GOB	B	OB
29	G	PB	B	MOG	P	GO	P	O
30	M	B	P	O	O	G	PBM	POB
31	G	BP	M	G	POB	O	PB	POB
32	GP	BPG	BP	M	P	OBG	MPB	BO
33	M	B	BP	O	O	BOG	BM	OP
34	G	B	PB	G	P	GBO	PB	OP
35	MP	BP	M	M	PO	GOB	PM	B
36	G	BG	B	O	PB	BOG	BM	BOP
37	MP	B	BM	OG	POB	BGO	PBM	POB
38	G	BP	P	GO	PO	B	BP	PB
39	G	B	B	MO	PB	GB	M	O
40	GP	BPG	BPM	OG	OPB	BO	B	PB

41	GP	B	BP	MO	P	OBG	PBM	BOP
42	G	B	B	G	PB	GBO	MBP	O
43	GP	BBP	PM	O	O	BGO	PB	BP
44	MG	BPG	P	M	OP	O	MP	BPO
45	MP	B	PM	GO	OPB	GB	MBP	P
46	MP	BP	M	O	P	G	PM	PB
47	G	B	B	MO	O	GBO	PBM	O
48	P	G	PM	G	O	OG	PB	B
49	G	P	BPM	O	PB	GB	M	O
50	PM	G	B	G	OBP	O	PM	OPB
51	G	B	BPM	GOM	O	G	B	B
52	GMP	G	P	O	OP	OBG	M	O
53	GP	P	B	OMG	P	O	M	O
54	GPM	P	BP	O	PB	GBO	P	O
55	G	B	M	G	PO	BO	PB	PBO
56	MP	BP	MB	O	P	O	M	BP
57	M	BPG	P	M	OP	OG	P	O
58	G	B	B	O	B	OB	PM	O
59	P	BP	PM	OG	O	GBO	B	B
60	GM	BPG	BMP	OGM	P	OBG	PM	P
61	PG	B	PB	GO	OPB	OB	BP	OPB
62	M	P	PM	M	O	OG	MBP	P
63	MG	BP	P	O	OP	B	BM	B
64	P	BPG	MB	G	PB	BG	BP	BPO
65	M	G	PBM	M	O	GB	BM	BP
66	G	B	BP	O	OP	OG	PMBP	PO
67	PM	P	PM	M	OPB	B	BMP	OP
68	G	G	BMP	GO	O	O	PBM	OPB
69	M	B	BMP	MG	P	G	P	B
70	GP	B	BP	MGO	PB	OB	M	B
71	G	P	BM	MG	O	GO	MP	PO
72	M	BPG	P	O	OBP	OG	MB	OPB
73	P	B	B	GOM	PB	OB	P	BPO
74	MG	P	B	G	O	BOG	M	B
75	G	G	P	OGM	OBP	O	MPB	OP
76	GP	B	M	OG	OBP	O	P	P
77	GM	PB	B	MG	OPB	OB	MB	OB
78	P	BP	BMP	OGM	O	G	PB	PO
79	G	G	PM	GO	P	OB	P	O
80	PM	B	PB	G	PB	G	M	B
81	GM	P	P	OM	O	GB	PMB	O
82	P	B	PM	GOM	OP	BO	B	B
83	G	PG	PB	MG	OB	G	P	P
84	M	BG	P	MOG	O	BO	M	B
85	MGP	P	PM	MGO	P	GBO	B	OP
86	G	B	PB	OGM	O	G	M	B
87	M	PG	BP	OG	OP	B	BP	P
88	GP	P	PM	GOM	P	GBO	P	O
89	G	PG	B	OGM	OP	G	BP	P
90	M	B	PBM	OG	B	OB	M	OB

91	MP	P	BP	MOG	O	GO	PM	OPB
92	M	BG	M	M	PB	OB	B	OP
93	MG	P	BP	G	OPB	G	MP	B
94	GP	GB	P	O	OB	B	PB	PO
95	PG	P	B	GM	OBP	GO	MPB	P
96	GM	P	M	GO	O	OBG	MP	O
97	G	B	BP	MG	PB	GO	PB	B
98	P	G	PM	O	OP	B	M	PO
99	M	PB	B	MG	OB	GO	P	BP
100	GP	PB	P	OG	P	GBOG	P	BOP
101	PM	BPG	M	M	PB	GB	B	BP
102	G	BP	PBM	GO	O	GO	M	OP
103	GM	PG	B	GM	PB	BO	P	B
104	P	B	P	O	OP	G	MPB	PO
105	G	BP	BM	G	PBO	B	PM	B
106	M	PG	B	GO	OPB	O	PB	OP
107	P	B	P	M	P	GB	MB	P
108	G	P	M	G	O	BO	P	B
109	PM	BG	PB	GO	PB	G	BM	O
110	MG	P	P	G	OPB	B	PM	POB
111	MP	B	BM	OM	OP	BO	MB	B
112	PG	BP	B	O	OB	G	MP	O
113	M	P	P	M	P	OB	MB	B
114	G	BG	BP	G	O	BO	P	O
115	P	P	M	GO	OPB	OG	M	P
116	GM	B	B	M	P	B	MB	B
117	MG	BP	P	G	O	OG	P	PO
118	GP	BG	M	GO	PB	BO	M	PBO
119	M	PB	BP	GM	OP	G	PMB	BPO
120	GP	P	PMBP	G	P	B	MP	BPO
121	G	B	MP	M	O	GOB	PB	POB
122	M	PG	BMP	GO	B	G	MP	POB
123	P	G	PB	MG	P	OG	B	BPO
124	G	BP	P	O	O	B	PM	B
125	M	PGB	BPM	G	OP	O	M	BPOPB
126	GP	P	B	M	PO	G	MB	BOP
127	MP	B	BP	O	P	GB	MB	B
128	G	B	PMB	M	PB	GBO	P	PO
129	GP	B	B	G	O	BOG	MP	PB
130	GMP	P	M	O	PB	BO	B	B
131	G	PG	BP	OG	PO	OG	MP	BP
132	GM	P	B	M	OPB	B	PMB	BPO
133	P	G	BMP	O	O	BOG	PM	P
134	M	B	P	G	P	B	M	BO
135	G	P	MB	OGM	PBO	GO	PB	POB
136	P	G	BMP	O	PO	OBG	PM	BPO
137	GM	P	BM	M	OBP	OGB	B	B
138	G	BP	P	G	OP	O	P	O
139	GM	PG	B	O	O	GB	M	PB
140	P	BG	PM	M	POB	O	B	BP

141	G	PG	P	G	PBO	GB	P	O
142	GM	BP	B	O	PB	BO	M	B
143	MP	G	PM	M	OP	OG	B	B
144	G	BPG	MP	G	OPB	B	P	P
145	M	PB	B	MO	P	OBG	M	O
146	G	G	P	OG	P	OG	B	PB
147	P	B	BPM	M	O	OBG	MP	OPB
148	G	P	B	G	P	OB	PBM	BP
149	GM	P	P	O	OPB	G	BB	O
150	GMP	BP	BM	GOM	P	BO	P	B
151	G	P	MPB	MOG	O	GB	M	POB
152	G	B	P	O	P	B	B	PO
153	P	G	BM	M	O	O	MB	B
154	PM	BP	B	G	PB	GB	P	O
155	G	G	BP	MO	OP	OBG	M	BPO
156	PM	B	PB	O	PBO	OBG	PBM	B
157	MG	GP	BM	G	P	BG	MB	BPO
158	GP	BP	B	GO	PB	GO	P	O
159	G	PG	P	M	O	OB	M	BP
160	PM	B	BM	G	P	BG	M	B
161	G	P	B	O	PBO	OBG	B	BPO
162	GP	PG	P	M	P	BOG	P	B
163	M	B	BM	G	PBO	OG	MP	P
164	P	BP	PB	M	P	O	B	B
165	G	PG	P	GO	O	G	P	O
166	P	B	PMB	G	OPB	B	PM	OP
167	P	BP	BM	M	P	O	PB	PB
168	G	P	P	O	PB	BG	BMP	BP
169	M	G	B	MG	O	O	P	OP
170	G	B	PM	M	P	OB	PB	B
171	GP	P	P	O	O	O	M	B
172	M	B	BM	G	B	G	PB	PO
173	GP	P	MPB	GOM	PB	OB	PB	BPO
174	G	BG	M	GOM	OP	O	PM	B
175	M	P	BMP	MG	PB	G	MP	BP
176	G	BPG	B	M	OBP	O	B	OPB
177		P	PM	GO	OP	B	PMB	BPO
178		G	P	GO	BO	OG	P	BP
179		G	PMB	G	PB	O	MPB	P
180		B	BPM	M	PB	B	M	O
181		P	P	OG	PBO	G	P	O
182		BG	B	G	PBO	O	MB	POB
183		PB	M	GO	O	OB	M	PBO
184		BP	P	OM	PB	BOG	MP	BPO
185		PG	PB	OG	POB	O	B	BPO
186		B	BM	GOM	PO	B	M	B
187		P	P	MOG	P	OG	P	POB
188		PG	PB	M	O	O	BM	PO
189		P	MP	O	PB	B	BM	BP
190		B	P	OG		O	P	BPO

191		PG	B	M		OG	MB	OP
192		PBP	BPM	O		B	P	B
193		B	P	MO		OG	M	POB
194		B	B	GO		OB	PB	O
195		P	PM	G		BOG	P	BP
196		G	BPM	MO		B	M	POB
197		B	PB	GO		OGB	PMB	OBP
198		P	P	MG		BO	PM	O
199		PG	B	M		B	B	BP
200		PG	B			OGB		BP

Appendix 5.2. Table with the G^2 statistic from Markovian chain Likelihood ratio test comparing observed and expected matrices. Transitions that had a Z-score > 1,96 were statistically different at a significant level of 0.05.

Bird	**G^2**	**P value**	**Z score for each transition**			
				Green (G)	**Morado (M)**	**Pink (P)**
1	48.23	< 0.001	**Green (G)**	-5.11	**2.66**	**2.93**
			Morado (M)	**2.09**	-3.69	1.43
			Pink (P)	**3.50**	0.81	-4.65
				Blue (B)	**Green (G)**	**Pink (P)**
2	51.13	< 0.001	**Blue (B)**	-4.04	-1.85	**5.74**
			Green (G)	**3.47**	-2.85	-0.94
			Pink (P)	0.95	**4.43**	-4.94
				Blue (B)	**Morado (M)**	**Pink (P)**
3	62.08	< 0.001	**Blue (B)**	-5.21	1.61	**3.75**
			Morado (M)	**2.92**	-4.92	1.52
			Pink (P)	**2.60**	**2.81**	-5.13
				Green (G)	**Morado (M)**	**Orange (O)**
4	90.44	< 0.001	**Green (G)**	-6.64	1.79	**5.00**
			Morado (M)	**3.43**	-5.15	1.40
			Orange (O)	**3.43**	**3.08**	-6.36
				Blue (B)	**Orange (O)**	**Pink (P)**
5	114.65	< 0.001	**Blue (B)**	-5.48	**5.04**	-0.17
			Orange (O)	-2.27	-5.16	**6.98**
			Pink (P)	**6.92**	0.71	-6.66
				Blue (B)	**Green (G)**	**Orange (O)**
6	70.94	< 0.001	**Blue (B)**	-5.15	1.52	**3.51**
			Green (G)	**3.88**	-5.81	1.86
			Orange (O)	1.22	**4.14**	-5.18
				Blue (B)	**Morado (M)**	**Pink (P)**
7	81.59	< 0.001	**Blue (B)**	-6.02	**3.21**	**2.56**
			Morado(M)	**2.78**	-5.83	**3.09**
			Pink (P)	**2.97**	**2.70**	-5.48
				Blue (B)	**Orange (O)**	**Pink (P)**
8	90.03	< 0.001	**Blue (B)**	-4.18	-1.40	**5.59**
			Orange (O)	**3.38**	-5.36	1.87
			Pink (P)	0.87	**6.64**	-7.41

Appendix 5.3. Tables showing the number of correct first visits over the number of 50 bouts, the mean probability of visiting a rewarded flower first, Z statistic as calculated from a Binomial test and the P value.

Bouts 1-50

	k/n	**mean p**	**Z**	**P**
B1	37/50	0.56	2.42	**0.0155**
B2	27/50	0.49	0.57	0.5687
B3	31/50	0.49	1.7	0.0891
B4	34/50	0.46	2.98	**0.0029**
B5	32/50	0.45	2.56	**0.0105**
B6	23/50	0.5	0.42	0.6745
B7	23/50	0.48	0.14	0.888
B8	26/50	0.52	0	0.99

Bouts 51-100

	k/n	**mean p**	**Z**	**P**
B1	28/50	0.48	0.99	0.1611
B2	29/50	0.45	1.7	0.0891
B3	24/50	0.41	0.86	0.3898
B4	29/50	0.42	2.15	**0.0316**
B5	26/50	0.45	0.85	0.3953
B6	31/50	0.49	1.7	0.0891
B7	30/50	0.44	2.14	**0.0324**
B8	37/50	0.63	1.47	0.1416

Bouts 101-150

	k/n	**mean p**	**Z**	**P**
B1	37/50	0.55	2.56	**0.0105**
B2	22/50	0.38	0.73	0.4654
B3	28/50	0.36	2.8	**0.0051**
B4	37/50	0.48	3.54	**0.0004**
B5	29/50	0.48	1.27	0.2041
B6	34/50	0.5	2.4	**0.0164**
B7	35/50	0.4	4.19	**0.0002**
B8	35/50	0.56	1.85	0.0643

Bouts 151-200				
	k/n	**mean p**	**Z**	**P**
B1	20/26	0.55	2.05	**0.0404**
B2	30/50	0.48	1.56	0.1188
B3	24/50	0.36	1.62	0.1052
B4	32/49	0.43	3.01	**0.0026**
B5	28/39	0.67	0.47	0.6384
B6	33/50	0.49	2.27	**0.0232**
B7	32/49	0.43	3.01	**0.0026**
B8	32/50	0.518	1.59	0.1118