Avian spatial and temporal cognition

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Declaration

The work contained within this thesis is my own and has not been done in collaboration, except where otherwise stated. The text does not exceed 70000 words. No part of this thesis has been submitted to any other university in application for a higher degree.
Abstract

I investigated the spatial memory abilities of male rufous hummingbirds (Selasphorus rufus) on their breeding grounds, where birds can be readily trained to feed from artificial flowers presented in two- and three-dimensional arrays. I found that birds use height as a cue when encoding flower locations, preferring to visit taller flowers. Performance in three-dimensional arrays is not however consistently better than in 2-D arrays. I also examined the possibility that hummingbirds follow a fixed flight path when returning to an array of flowers, as has been seen in the Hymenoptera. By manipulating the number of doors by which a bird could enter an arena to feed on an array of flowers, I found some evidence that birds do use systematic movements when relocating rewarding sites.

Rufous hummingbirds feed on floral nectar, a resource that varies in time as well as space. To determine whether these birds use time as a cue when foraging, I provided them with an array in which flowers were refilled after intervals of either ten or twenty minutes. The birds were able to learn these intervals, as they returned to flowers on or shortly after their expected refill time. I used an analogous experiment to investigate timing abilities in coal tits (Parus ater), a food-storing species, and great tits (P. major), a non food-storing species, in the laboratory. Storers and non-storers might differ in their ability to time intervals due to the demands, on storers, of reliable cache recovery. Coal tits were able to track the availability of rewards in three different locations associated with reward schedules in the 30-120s range. I found no consistent differences in the abilities of coal tits and great tits to time intervals in the 40-70s range.

In conclusion, rufous hummingbirds were shown to use both spatial and temporal cues whilst foraging at flowers. Coal tits and great tits were able to time short intervals in the laboratory, but I found no overall differences in timing behaviour between the two species.
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Chapter 1: Learning and memory in birds: the roles of space and time

1.1 Introduction

Most animals are faced with similar problems every day – finding their home, locating food, avoiding predators, and so forth (Bell, 1991; Cheng & Spetch, 1998). While some species inhabit environments diverse in form, others have to navigate through spaces that, to human eyes at least, appear featureless. Despite this, species as diverse as willow tits *Parus montanus* foraging in woodlands and desert ants *Cataglyphis fortis* moving across sand possess nervous systems and mental capacities permitting them to move around in an efficient manner (Brodin, 1994; Wolf & Wehner, 2000). One way in which resources can be exploited in an efficient manner is to learn and remember them (see e.g. Gallistel, 1990; Healy, 1998; Shettleworth, 1998; Pepperberg, 2001; Wynne, 2001 for reviews). In this thesis I will address questions on avian spatial and temporal cognition using hummingbirds and tits as model systems. The role of learning and memory will be explored, with specific reference to the kinds of foraging tasks these animals face in the “real” world.

1.2 Cognitive abilities in animals

Learning and memory research was, until around 30 years ago, largely the domain of experimental psychologists. In the tradition of Thorndike and Pavlov, active in the early 1900s, much of the work on learning was, and continues to be, conducted on a narrow range of species using laboratory operant tasks (Gonzalez et al., 1967; Fantino, 1977). Thorndike and Pavlov worked on cats and dogs respectively, more recently rats and pigeons have been favoured as experimental subjects. The findings of Thorndike and others, showing that different species had qualitatively similar learning abilities, led to the suggestion that throughout the animal kingdom
(including man) there were general principles of learning applicable to all animals. This belief led to a decline in comparative studies and an accompanying concerted effort to describe, in depth, the putative laws of learning. This was done in species chosen arbitrarily and probably largely for convenience (Bitterman, 1975).

Alternative ideas to a so-called general process learning theory only began to take hold in the 1970s (for review see Wasserman, 1993). This was helped by earlier surprising results, such as the phenomenon of long delay taste avoidance learning in rats (Garcia et al., 1955). The discovery that rats were able to make an association between the ingestion of food and subsequent illness when the relevant stimuli were not presented at the same time caused difficulty for generalist accounts of learning.

In 1973, Tinbergen, Lorenz and von Frisch shared a Nobel prize in recognition of their founding of a new science – ethology – which emphasised the importance, or survival value, of different behaviours to the species performing them. Ethologists viewed differences in behaviour patterns as reflecting varying selective pressures. Instead of general processes applying to learning and memory – assumed as a consequence of common evolutionary descent – ethologists proposed that animals’ behaviours, including their mental capacities, could be explained in terms of natural selection. This latter notion paved the way for a new approach to investigating animal learning and memory where great emphasis was placed on examining behaviours under natural conditions.

Tinbergen (1963) had argued earlier that for a complete understanding of any behaviour, it was essential to address both proximate and ultimate questions. His four “whys” related to the proximate cause of any behaviour, its development, its function and its evolutionary history. Psychologists had traditionally concentrated on mechanistic approaches to study of learning, for example when addressing the question of how animals detect certain events (e.g. Blough & Blough, 1977). Ethologists, on the other hand, favoured the study of animals in natural environments and sought to understand the functions of behaviours they observed in this context (Kamil & Yoerg, 1982). A lack of synthesis between the behaviourist and ethological schools of thought led to a lack of mutual understanding regarding the
role played by learning in behaviour. In the mid-70s and 80s, however, an "ecological" approach to learning emerged as an alternative to the traditional general process view.

Around this time behavioural ecology also emerged. This approach emphasised the current function and evolution of behaviours. A central tenet of behavioural ecology was that animals had evolved optimal solutions to the problems they encountered (for discussion see Maynard Smith, 1978). Optimal foraging theory, for example, examined whether animals moved between and fed on patches in an efficient manner (e.g. Stephens & Krebs, 1986; see also Perry & Pianka, 1997). An assumption was that those individuals able to forage efficiently would ultimately be more successful than less capable conspecifics. It was proposed that natural selection had also acted on cognitive abilities (see e.g. Shettleworth, 1984). The rationale for this latter proposal stemmed in large part from observations of animals, under natural conditions, performing behaviours with adaptive value (e.g. foraging in an efficient manner). This idea led to a more ethological view of learning and memory, which has gained in popularity (Yoerg, 1991; Dukas, 1998a; Healy & Jones, 2002).

The early 1990s saw the advent of "cognitive ecology" (Real, 1993; see also Dukas, 1998b). A hallmark of this approach has been to attempt to integrate psychological and neural mechanisms of behaviour with the functional approach of behavioural ecologists (Shettleworth, 2001). A further hope is to bridge findings from experiments on animal cognition to observations made in naturalistic contexts (for an appraisal, see Shettleworth, 2001; see also Healy & Braithwaite, 2000).

Perhaps the most successful demonstration of the cognitive ecology approach comes from work on food-storing birds. A number of studies have shown that storers (e.g. some parids) make greater use of spatial cues when compared to closely related non-storers (Shettleworth, 1990; Hilton & Krebs, 1990; Brodbeck, 1994; McGregor & Healy, 1999). This finding is consistent with the hypothesis that the differences may be because storers, reliant on retrieving cached items of food, have enhanced spatial memory abilities. However, such a correlation is insufficiently compelling to ascribe differences in spatial memory between a storing and a non-storing species to
requirements for successful food retrieval (e.g. remembering where an item was cached previously). Alternative possibilities (e.g. species differences in home range size) must be excluded (Krebs, 1990). Even then, correlational data can never be convincing alone and should be complemented by experiments.

An example of a combination of approaches leading to detailed insights into behaviour comes from investigations of differences between storing and nonstoring birds. The hippocampal complex, the brain region responsible for processing spatial information, occupies a greater proportion of the brain in food-storers than in nonstorers (Krebs et al., 1989; Sherry & Vaccarino, 1989; see also Bingman et al., 1995). Additional findings, such as food-storing coal tits *Parus ater* remembering spatial information for longer periods than non-storing great tits *P. major* (Biegler et al., 2001) support the suggestion that a larger hippocampus plays a role in birds' use of spatial information when foraging. Black-capped chickadees (*P. atricapilla*) from Alaska (where winters are harsh) have relatively larger hippocampi, store more food and perform better in spatial tasks than those from milder Colorado (Pravosudov & Clayton, 2002). In addition to opening up the possibility of further within-species comparisons of putative cognitive specialisations, further supporting is gained for the hypothesis that the conditions animals face in nature select for successful foraging.

The findings of these and similar studies suggest that animals may learn and remember information specific to the problems they face in nature. Despite this, some workers have questioned the validity of a cognitive ecology approach.

Recently, it has been argued that considerations of function say nothing about mechanism (Macphail & Bolhuis, 2001; Bolhuis & Macphail, 2001). According to this argument, there is no reason why the mechanisms of learning in a food-storing bird (for example) ought to differ from those in a non-storing bird. The storer naturally stores and the nonstorer does not. Consequently there is no reason to invoke different cognitive mechanisms in the two species; as food-storing is a specialisation unique to the storer and does not inform us about any proximate causes of behaviour. Some workers further argue that methodological differences in previous storer/non-storer comparisons have produced spurious significant
correlations between the size of the hippocampus and the extent of storing behaviour (Brodin & Lundborg, 2003). Instead, then, it is more parsimonious to assume that the mechanisms are the same. Despite the arguments put forth by Bolhuis and Macphail, their interpretation is not universally accepted (see e.g. Flombaum et al., 2002; Hampton et al., 2002; MacDougall-Shackleton & Ball, 2002). Animals’ performance in learning and memory tasks may differ as a consequence of different cognitive abilities per se, or in differences in, for example, perceptual processes or motivation. Where adaptive specialisations appear to exist, examining the processes of learning and memory and the use of various cues seems appropriate. It makes sense, then, to at least test various hypotheses on animals’ cognitive abilities (Dwyer & Clayton, 2002).

Perceptual processes can act as a window onto higher level processes of learning and memory (Desimone, 1996). Although in this review and throughout this thesis I will concentrate on learning and memory, it is worth emphasising that adaptive behaviour can also emerge from specialisations in how animals process the sensory information they receive. Attention, for example, refers to the preferential use of certain stimuli over others by an animal’s nervous system, which is constantly bombarded with more sensory inputs than can be dealt with at once. As a process that can be inferred only from an individual’s behaviour, attention can nonetheless reveal interesting differences in animals’ information processing abilities (Bushnell, 1998).

The investigation of cognitive abilities in animals has, for many reasons, been dominated by laboratory studies (see e.g. Dukas & Kamil, 2000 for a study on attention in blue jays pecking at touch screens). In the laboratory, a wide range of physical variables, including temperature and light regime, is readily controlled. The possible effects of conspecifics on an animal’s performance can be removed by excluding them or minimising contact, whether visual and/or acoustic. Various cues including olfactory traces and landmarks can be eliminated. Thus, a multitude of factors that might affect an animal’s behaviour can be controlled for (or at least quantified) in many laboratory paradigms (Timberlake, 1990).
Field experiments, on the other hand, offer less control over potentially confounding variables. For example, free-living experimental subjects can interact with other animals and their wider environment in ways over which control simply cannot be exerted, due to problems of scale and logistics. Despite these factors, studies in the field do offer some advantages (Cuthill, 1991; Balda et al., 1998). By making observations of individuals performing a normal behavioural repertoire in the field, insight is gained into what animals actually do, as opposed to what they can do (Altmann & Altmann, 2003). It is also under field conditions where selection ultimately acts.

Where possible, it would seem sensible to attempt corroborating results from both the laboratory and the field (Krause et al., 2000). Homing pigeons *Columba livia* allowed to locate a food goal alone in the laboratory were better at finding the same goal at a later time than conspecifics who had previously been allowed to follow an experienced conspecific to the goal (Burt de Perera & Guilford, 1999). In a complementary field experiment, however, pigeons that had been “tutored” by a conspecific homed at the same speed as untutored birds (Banks & Guilford, 2000). Despite sometimes conflicting evidence, the role of visual landmarks in pigeon orientation remains amenable to both laboratory and field investigations (Holland, 2003).

In summary, the study of animal cognition has benefited from workers adopting a variety of approaches. In this thesis, I will describe tests of learning and memory carried out both in the field and in the laboratory using two systems. The first involves learning and memory in hummingbirds. The second involves aspects of timing behaviour in parids.

### 1.3 Why hummingbirds?

Hummingbirds offer a number of advantages for investigating the potential role of learning and memory in the field (for reviews see Healy & Hurly, 2001; Healy & Hurly, 2003). These can be subdivided into biological and logistical factors.
1.3.1 Biological factors

Energetics

Hummingbirds are nectarivores with small body sizes and an energetically expensive mode of flight (Pearson, 1954; Cotton, 1996; Chai & Dudley, 1996). Nectarivores in general, and hummingbirds in particular, have often been assumed to be under stronger pressure than many animals to forage “efficiently” (Gill & Wolf, 1977; Kamil, 1978; Hainsworth, 1981; Wolf & Hainsworth, 1983). Early studies on hummingbirds were however largely descriptive (e.g. anatomy, community of plants visited), making it difficult to assess the consequences of individuals’ foraging routines (see e.g. Snow & Snow, 1972). The emergence of behavioural ecology in the 1970s caused a greater emphasis to be placed on testing specific predictions. For example, Pyke (1981) found that neither rufous nor broad-tailed hummingbirds S. rufus and S. platycercus minimised the distance travelled between flowers. This would appear to contradict earlier findings on for example Hymenopteran pollinators, many of whom appeared to forage efficiently by following simple decision rules (Heinrich, 1975).

Distribution of resources and territoriality

Hummingbirds’ floral resources vary both in time and space (Wolf & Hainsworth, 1977; Gill, 1988; Healy & Hurly, 1995). Individuals able to keep track of which flowers they have visited and emptied, and when, would presumably benefit from avoiding flowers until enough time had elapsed for them to refill (a few hours: Armstrong et al., 1987). This applies particularly to territorial species (e.g. rufous hummingbirds Selasphorus rufus) as territory owners must deal with the problem of finding and feeding on flowers that can be distributed widely as well as defending these flowers from theft by other pollinators.

One possibility is to employ some kind of systematic foraging (e.g. searching from the edge of the territory in to the middle as the day proceeds). Pied wagtails Motacilla alba appear to do this when searching for insect prey along predictable routes which appear to match patterns of prey renewal (Davies & Houston, 1981).
Similarly in some hymenopteran species, a systematic "traplining" occurs, where the pattern of flower visits can be highly stereotyped (bumblebees: Thomson, 1996; Thomson et al., 1997; wasps: Corbet et al., 1981).

Plants generally offer their pollinators limited nectar rewards, forcing animals to visit multiple flowers each day (Kadmon, 1992; Sutherland & Vickery, 1993). In nectarivorous birds including amakihis *Loxops virens* (Kamil, 1978) and sunbirds *Nectariniafamosa* (Wolf et al., 1975) and *N. reichenowi* (Gill & Wolf, 1977) a correlation exists between systematic foraging and the defence of a territory. In general, feeding territories provide enough energy to sustain the owner, and flowers outside the territory contain lower than average amounts of nectar. For example, long-tailed hermit hummingbirds *Phaethornis superciliosus*, a traplining species, tend to use predictable routes to visit dispersed sources of nectar (flowers tens to hundreds of meters apart) (Gill, 1988). Nectarivorous birds thus appear to track the availability of nectar resources.

1.3.2 Logistical factors

In contrast to subjects tested in the majority of learning and memory studies performed in the laboratory, hummingbirds require little in the way of training to approach and feed from flowers and feeders. Whereas pigeons require many hundreds of training trials (see e.g. Randall & Zentall, 1997), rufous hummingbirds learn to feed from artificial feeders containing sucrose after a few visits, normally taking no more than a couple of hours. Once trained, they will feed from artificial feeders regularly (around every 10 minutes) (Healy & Hurly, 1995; Waser & McRobert, 1998). The amount, volume and concentration of sucrose in feeders can all be readily manipulated (Broom, 1976; Stromberg & Johnsen, 1990; Biernaskie et al., 2002) as can the number and locations of the feeders. Furthermore, the birds readily habituate to human presence and their behaviour can be monitored from a distance of a few metres (Stiles & Wolf, 1973; Hurly & Healy, 1996).
1.4 Testing for learning and memory abilities in hummingbirds

1.4.1 Learning and memory in hummingbirds: colour

In many North American gardens, people put out red hummingbird feeders containing “sugar-water”. Observations of hummingbirds in situations such as this produced many anecdotes of interesting behaviours such as the vigorous defence of “nectar” resources, an apparent preference for red, and a tendency to go to feeder locations even when the feeder had been removed (see e.g. Storer, 1930; Johnsgard, 1997). In the context of hummingbirds’ learning and memory, early experiments addressed the role that colour might play.

The willingness of subjects to approach and feed from artificial feeders, both in the field and in the laboratory, was exploited in studies addressing hummingbirds’ use of colour. This work failed to detect consistent colour preferences (black-chinned hummingbirds *Archilochus alexandri* Bené, 1941; Anna’s hummingbirds *Archilochus (Calypte) anna* Collias & Collias, 1968; Stiles, 1976; ruby-throated hummingbirds *A. colubris* Miller & Miller, 1971). Instead, hummingbirds learn to differentially associate colours with rewards. Black-chinned hummingbirds *A. alexandri*, for example, learn to feed from feeders in the laboratory lit by green light as rapidly as from feeders lit by red. Having learned one colour-reward association, they also readily learned the reversed association (Goldsmith & Goldsmith, 1979).

Hummingbirds will sometimes display preferences when given different choices. In Goldsmith and Goldsmith’s (1979) experiment, birds presented with the option of feeding from red and blue versus green and yellow feeders preferred the former. In laboratory experiments, red has been used as a colour to assist in birds making a rapid association between a novel food source and reward (see e.g. Altshuler & Nunn, 2001 for work on ruby-throated, rufous and broad-tailed hummingbirds). Innate colour preferences exist in some animals (e.g. the hawkmoth *Macroglossum stellatarum*), yet these are labile and readily change as result of experience (Kelber, 1996) as appears to be the case for rufous hummingbirds.
In tests using artificial flowers at least, hummingbirds place a much greater emphasis on spatial cues than on colour cues. This suggests that although colour may be a useful cue in the initial identification of flowers, this may not be the case in determining which flowers are visited next. In a field experiment, Hurly and Healy (2002) presented rufous hummingbirds with arrays containing either one or three rewarded flowers (out of a total of ten). The flowers within the array were all different colours/patterns, and the birds received consecutive trials of each array configuration. Once the birds learned the location of the rewarded flower(s), the array was shifted 2m. The shifted array was the same as before or differed in either/both the positions/colours of flowers. When the colour and position of the rewarded flowers remained the same as before, or the positions of the rewarded flowers remained the same, the birds made fewer mistakes learning the new array than in learning the original array configuration. In a further experiment, the possibility of the birds using spatial information derived from the shape of the array was removed by presenting arrays of different shapes in the two phases of a trial. The birds made fewer errors post-shifting when all possibilities of using spatial information learned in the first phase were removed (Hurly & Healy, 2002). Colour therefore can be used but is apparently subsumed by the use of spatial cues.

1.4.2 Learning and memory in hummingbirds: space

Hummingbirds readily learn an association between the location - and contents - of flowers and/or feeders (Bené, 1941; Miller & Miller, 1971; Gass, 1979; Miller et al., 1985). Spatial learning and memory has various components. For example, animals may differ in memory capacity (how many things are remembered), memory persistence (time scale of memory) and/or spatial resolution (the detail of the memory) (Biegler et al., 2001). These can be tested in different types of experiment, including those where visual and spatial cues are manipulated and/or placed in conflict. Experiments on hummingbirds, both in the laboratory and in the field, have involved these kinds of approaches being used.

An early investigation of spatial learning examined whether hummingbirds avoid recently depleted flowers (Cole et al., 1982). To test this, Cole and her colleagues
presented three hummingbird species in the laboratory with a spatial task in which birds were rewarded either for returning to the same feeder (win-stay) or for visiting an alternative (win-shift). Both males and females of the species tested (including black-chinned *Archilochus alexandri* and blue-throated *Lampornis clemenciae* hummingbirds) learned much more readily to select a new feeder rather than the one previously visited. The birds trained to the win-stay paradigm learned the task, but made more errors before reaching criterion.

Rufous hummingbirds *S. rufus* are able to make associations between cues and rewards even when the cues are spatially separated from the rewards (Brown & Gass, 1993). In a laboratory study, birds were presented with six feeders, one of which was rewarded, and a constant visible colour cue situated 2.5cm distant. The subjects learned more rapidly with a cue present than when it was absent and when in the middle of the experiment the cue was moved, the birds visited the wrong feeder. The birds appeared to use the cue to determine which was the rewarded feeder.

In a further experiment, rufous hummingbirds were presented with a coloured cue spatially separated from, or contiguous with, a rewarded feeder (Brown & Gass, 1993: Experiment 2). The positions of cue and feeder in the room were different in each trial. Feeder choice was more accurate the closer the cue was to the feeder.

Animals are often able, after a single exposure, to learn and remember information that they can then use later. This requires that individuals recall, for each site, information relating to spatial location and perhaps other characteristics, for example the presence of a tree nearby. One-trial associative learning tasks have been used both in the field and the laboratory to examine this kind of learning (see e.g. Bingman et al., 1995; Hurly, 1996).

There are two components to one-trial associative learning, both relevant to a foraging hummingbird. The initial phase involves the animal locating a food item. On its return the animal is usually offered a choice of the original and an alternative. Using this paradigm, Hurly (1996) found that hummingbirds returned to flowers they
had left with food remaining in them rather than visit alternative, water-filled flowers.

Whether hummingbirds prefer to use location or visual cues when returning to rewarded flowers was investigated in another field study (Hurly & Healy, 1996). In their first experiment, birds were given four unique flowers in a square configuration. The rewarded flower was randomly selected, and contained more sucrose than the bird could empty on one visit. After the bird's first visit the rewarded flower was emptied and switched with one of the other flowers. When the birds returned to the array, they returned most often to the flower correct with respect to spatial cues rather than to the flower correct with respect to colour/pattern cues. Hummingbirds, like food-storing birds (Brodbeck, 1994, Clayton and Krebs, 1994) thus appear to place greater emphasis on spatial information than on cues such as colour and pattern.

A second experiment was then carried out to examine whether the spacing between flowers might affect birds' choices. In the first phase, the birds visited a sucrose-filled focal flower and partially depleted it. Upon returning, a choice of two flowers was available: one a new sucrose-filled flower identical to the one from which the bird had originally fed, and another water-filled distracter flower. The distracter flower was either the same or different in colour to the focal flower, and was placed at a distance of 3, 40 or 80cm from it. When the distracter flower was different in colour to the focal flower, the birds favoured visiting the focal flower. This preference was greater as the distance between the flowers increased. When both flowers were the same colour, however, the number of visits to the focal flower decreased as the distance between the flowers increased. The authors suggested that this result may not have been due to the birds making spatial mistakes but rather to the birds sampling flowers that looked like the original, rewarded flower (Hurly & Healy, 1996).

Whether or not the birds would avoid flowers they had recently emptied was tested using an analogue of the radial maze (Healy & Hurly, 1995). The radial arm maze (RAM) is a piece of laboratory apparatus that has been used extensively in tests of
animals' (especially rodents) spatial working memory abilities since its invention in the 1970s (Olton & Samuelson, 1976; Wilkie et al., 1983; Foreman & Ermakova, 1998; Lipp et al., 2001). Species other than rats have also been tested, using an analogue of the RAM. Ring doves *Streptopelia risoria*, for example, are able to remember up to fourteen locations, as assessed by their avoidance of previously-entered arms (Wilkie et al., 1983). In a food-storing species, the Clark’s nutcracker *Nucifraga columbiana*, subjects performing a RAM task remembered (and subsequently avoided) previously visited locations for up to three hours (Balda & Kamil, 1988).

To perform a RAM task using a spatial strategy, the animal must firstly identify a number of spatial locations (the maze “arms”). In mazes commonly eight or twelve arms radiate horizontally from a central point, although some have more arms (Wilkie & Slobin, 1983) and can be three-dimensional (Grobéty & Schenk, 1992). “Open-field” analogues of the RAM (after Spetch & Edwards, 1986; see also Spetch & Honig, 1988) have also been used. In the “open-field” RAM, arms with walls accessed via a central point are replaced with locations accessible by direct movements between sites. An animal in a RAM must then visit all or most of the arms during an acquisition phase, learning as it goes which sites are rewarded. In the choice phase, after a retention interval, the animal is rewarded through avoiding returns to previously visited arms.

Healy and Hurly (1995) used an open-field analogue of the eight-arm RAM to investigate the spatial working memory abilities of adult male and female rufous hummingbirds. Birds were tested using arrays involving the presentation of artificial flowers mounted on stakes. The arrays used were two-dimensional, with flowers mounted horizontally on the stakes above the ground. Each flower acted as an “arm” of the maze and birds could fly, uninhibited, between any of the available flowers. Two trial types were used: in “forced” trials birds were presented with eight stakes. Rewarded flowers were mounted on four of the stakes, and in each trial the bird had to visit all of them. In “free” trials, the bird was presented with eight flowers and had to visit four of them in its first visit to the array. In both trial types a retention
interval separated the first visit (phase one) from the return visit to the array (phase two). In the second phase, all eight flowers were available in the same array, situated in the same spatial location. Each trial was conducted in a new spatial location to ensure that the birds did not simply learn always to return to the same places to gain reward.

In both trial types the birds performed significantly above chance levels, preferring to visit new flowers over previously emptied flowers. Control trials eliminated the possibility that this performance was due to the birds using cues intrinsic to the flowers (e.g. odours). The birds were able to perform above chance in the task over retention intervals up to 42 minutes, showing that they remembered something about the flowers for at least this long. The hummingbirds' performance may seem poor compared to other species: nutcrackers *N. columbiana* tested in a laboratory open-field RAM, for example, were able to perform above chance over retention intervals of 6 hours (Balda & Kamil, 1988). Unlike the nutcrackers, held under restricted conditions during retention intervals, the hummingbirds were however free to engage in other activities between visits.

An open field maze has also been used to assess whether hummingbirds treat previously seen and previously unseen flowers differently, a situation that could arise in nature with flowers opening and closing throughout the day (Henderson et al., 2001). The birds were presented with trials of three types: in “mixed” trials, the bird could choose four from six available flowers on its first visit, in “forced” trials the bird could only visit four flowers, and in “free” trials it could visit four from eight. Mixed trials therefore provided the bird with two options upon its return: two flowers it had seen before and two new flowers. In free and mixed trials birds performed better than chance. In the second phase of mixed trials new flowers were more than twice as likely to be visited than those previously visited.

1.4.3 Spatial learning in hummingbirds: points in space or patterns?

Rufous hummingbirds in the field possess spatial memory for at least four locations, allowing them to avoid revisiting empty flowers for periods of up to 45 minutes
(Healy & Hurly, 1995). It is not clear, however, whether hummingbirds encode the specific locations of individual flowers, or whether general spatial patterns, such as the arrangement of flowers within an inflorescence, are what the birds learn and remember.

In an attempt to assess whether hummingbirds remember spatial patterns as opposed to point locations, Sutherland and Gass (1995) tested rufous hummingbirds on a laboratory task in which four different patterns of reward were used. A 2-D array containing 64 feeders (small wells containing sucrose) was arranged in an 8 x 8 configuration. Four spatial arrangements of reward were used: a) halves, where all the feeders on one half of the board were rewarded; b) quarters, where all feeders in two diagonally opposite sides of the board were rewarded; c) checkerboard, where 2 x 2 squares were rewarded; and d) random, where rewarded sites were arranged haphazardly (see Figure 1.1). Learning occurred most rapidly on the halves and quarters arrays.

In a further experiment the birds were trained first on a quarters configuration and then the pattern of reward was changed to a corresponding mirror image configuration (Sutherland & Gass, 1995). Performance decreased as soon as the shift was made, indicating that the birds were not following simple rules of thumb, or movement rules, to relocate rewarded sites (Pyke, 1981).
A 2-D array with an 8 x 8 configuration was used. Four spatial arrangements were used – halves (top left), quarters (top right), checkerboard (lower left) and random (lower right). In Sutherland and Gass' experiment, the birds learned the halves and quarters arrays most rapidly. Half of the flowers (filled circles) were rewarded.
The possibility that birds feeding from multiple rewarded sites might learn actual spatial locations rather than relative spatial relationships was tested in a field experiment by Healy and Hurly (1998). They presented rufous hummingbirds with a regular array comprising 5 equidistant flowers. These were arranged in a cross configuration with four “corner” flowers and a central flower. The birds were required to learn that the flower in the middle of the cross was rewarded. Once the birds had learned this, probe trials were conducted where the array was randomly shifted in the direction of one of the four main compass orientations. In the probe trials, the central flower occupied the same spatial location as a previous “corner” flower (Figure 1.2).

Figure 1.2 Experimental set-up used by Healy and Hurly (1998).

The circles show the positions of the flowers during training (only the middle flower was rewarded). The horizontal spacings between the flowers ranged from 5 cm to 320 cm. Filled squares denote the positions of the flowers during the test trial, when all flowers were empty. In this example, the flowers were shifted one unit to the east.

Seven different nearest-neighbour flower spacings were used within-trials, ranging from 5 to 320 cm. At spacings of up to 40 cm, birds were found to return to the relative centre of the array. When flowers were further apart, birds returned instead
to the absolute spatial location of the (former) reward (Healy & Hurly, 1998). As in the Sutherland and Gass (1995) study, it was not possible to exclude the possibility that the birds were remembering routes between flowers. In chapter 3, I investigate the possibility that wild rufous hummingbirds use a route-based strategy to locate rewarded flowers.

1.4.4 The role of other cues in hummingbird foraging

Flower Height

Birds may use a variety of cues to encode locations in memory. One of these is flower height.

Lima (1991) observed that Anna’s hummingbirds *Calypte anna* often opt to avoid feeding close to the ground. When feeding at locations with an obstructed view of the rest of the environment the birds in Lima’s study fed more erratically, a finding consistent with the notion that birds were responding to a perceived or actual predation risk (see also Dukas, 2001).

In order to examine the potential role of flower height in foraging, Blem and coworkers presented rufous hummingbirds in the field with sucrose at a variety of heights, ranging from 0.25 to 3m above the ground (Blem et al., 1997). The birds preferred the highest feeders (small vials) and this was interpreted in terms of predator avoidance. Broad-tailed hummingbirds *Selasphorus platycercus* and rufous hummingbirds *S. rufus* foraging on natural inflorescences of scarlet gilia *Ipomopsis aggregata* also prefer visiting tall flowers (Wolf & Hainsworth, 1990). Although hummingbirds are generally regarded as not suffering extensive predation, a few accounts do exist in the literature (Spofford, 1976; Graves, 1978; see also Lima, 1991; Johnsgard, 1997). There may however be reasons other than predation contributing to hummingbirds’ preference for high flowers.

Many hummingbirds (including those in Blem et al.’s study) often prefer perching in high positions (Storer, 1930; Healy & Hurly, 1995; Healy & Hurly, 1998). Although this may aid in vigilance behaviour, birds may also prefer feeding from high flowers
as a result of them being more conspicuous than flowers lower down or among other stands of vegetation. There is some support that hummingbirds do use height as a cue under field conditions (Henderson et al., 2001). Males were presented with an open-field analogue of the RAM, with eight flowers presented at two different heights of 40 and 110 cm. The birds were given two trial types—“forced” and “free.” In forced trials, four flowers (two randomly chosen flowers at the high and low positions of the array) were available in phase one. In free trials, the birds were free to visit any four of the eight rewarded positions. Eight flowers were available for the bird to choose from in phase two in both trial types. The birds performed at levels greater than chance in both free and forced trials, returning more often in the second phase to previously unvisited flowers. The observed performances were better than in the earlier two-dimensional array experiment (Henderson et al., 2001: Experiment 1), suggesting that the presence of flowers at different heights aided the birds in encoding their location.

Finding flowers in three dimensions

The finding that rufous hummingbirds’ performance in a 3-D spatial task was better than in an analogous 2-D task leads to the possibility that the addition of an extra dimension aids birds in remembering flower locations. Animals can attend to spatial information presented in three dimensions (e.g. rats *Rattus norvegicus* Grobêty & Schenk, 1992; fox squirrels *Sciurus nager* Jacobs & Shifflet, 1999). The rats were tested in mazes that could be oriented horizontally, vertically, or tilted at an angle. Subjects trained in vertical and tilted mazes found rewards more rapidly than those trained in horizontal mazes, suggesting that the addition of a vertical component to the task enhanced their performance. Furthermore, fewer errors were made in the vertical dimension.

There are two implications in testing animals on 3-D tasks (Grobêty & Schenk, 1992). The first is an increase in spatial complexity. To successfully complete a 3-D task an animal may need to form a representation in which three spatial coordinates (*X*, *Y* and *Z*) are integrated instead of two, as in 2-D tasks. Secondly, the addition of
the vertical component (the Z component) is likely to affect an animal's perception of its position in space. The Z component lies parallel to the force exerted by gravity, and thus moving in the vertical plane entails higher energetic costs than moving in the horizontal plane. Although this was not measured, by being able to learn quickly where rewards were located in the 3-D mazes the rats probably reduced the energetic cost of exploration.

A hummingbird foraging in the wild is faced with a 3-D foraging task every time it feeds at flowers, in contrast to the largely two-dimensional environment navigated by rats. Furthermore, hovering at flowers is a mode of flight that is costly, and involves movements of the body in the vertical plane (Chai et al., 1998; see also Hainsworth, 1991). We might expect, then, that hummingbirds would make use of information from the vertical plane; perhaps by minimising the amount of energy expended in hovering at flowers. In chapter 4, I explore hummingbirds' use of relational information and the use of absolute versus relative spatial cues with flowers positioned at different heights in the vertical plane. The potential interactions between flower height and foraging in 2-D and 3-D mazes are investigated in chapter 5.

1.4.5 Do birds also use temporal cues?

Learning and remembering when events of biological significance occur, as well as where they occur, is thought to be a fundamentally important process in animal cognition (Gallistel, 1990). By tracking temporal regularities that exist in the world animals might be able to exploit their environment more efficiently. A large literature exists on animals' timing abilities, typically from the laboratory (see e.g. Gallistel, 1990; Church, 1997; Gibbon et al., 1997), and rather little on the use of temporal cues in natural situations (although see Gill, 1988; Wilkie et al., 1996). In this respect, hummingbirds offer a potentially useful system for investigating timing in a biologically realistic context. For a hummingbird, making return visits to flowers relies not only on remembering the locations of rewarding flowers, but when they were last visited. This is akin to the needs of food-storing birds when retrieving cached items (e.g. Clayton & Dickinson, 1998).
Spatial and temporal cognition in birds

Three timing systems, each of which has been shown to exist in birds, are thought to have evolved in animals. These are ordinal, phase and interval timers (Carr & Wilkie, 1997).

**Ordinal timing**

Ordinal timing refers to a timing system that allows animals to anticipate an event (e.g. the arrival of food) that occurs reliably within a temporal sequence. Garden warblers *Sylvia borin*, for example, anticipate the unsignalled arrival of food on a daily basis. In one experiment, in which the spatial location of food varied according to time of day, garden warbles *S. borin* were able to learn appropriate time-place associations, resulting in them receiving food rewards by visiting different experimental chambers at certain times of the day (Biebach et al., 1989). This behaviour persisted when all chambers could be visited. That the birds persisted in visiting the chambers in the previous sequence provides strong evidence that they had made an association between where and when food could be found (see also Wilkie et al., 1996).

**Phase timing**

Phase timing permits animals to successfully anticipate events occurring within a cycle. Oystercatchers *Haematopus ostralegus*, for example, track the daily tidal cycle. This permits them to move to the coast from inland roosting sites to forage at the appropriate time of day, when invertebrates are available on the mudflats and beaches (Daan & Koene, 1981). Nectar-feeding bees can learn when to land on particular petals in order to gain reward according to the time of day (Gould, 1987). For an animal visiting foraging sites in a particular predictable sequence, the ability to time phases could provide a useful way of exploiting resources (see also chapter 6).

**Interval timing**

Animals capable of timing intervals are able to discriminate important events (e.g. the availability of food) that occur a fixed amount of time after another event (e.g. a previous visit to a flower). In contrast to phase timing, these events do not occur
within a cycle. Instead, interval timing has been likened to a stopwatch as an interval timer can be stopped, reset and restarted (Carr & Wilkie, 1997). In contrast to bees learning to associate rewards being available on certain petals at certain times of day (see e.g. Gould, 1987 for work on phase timing), hummingbirds visiting a number of different flowers throughout the day might be expected to use an interval timing sense to keep track of individual flower visits.

Interval timing has been studied extensively in the laboratory and has revealed some important generalisations. A common finding is that of the scalar property of timing (see e.g. Malapani & Fairhurst, 2002). This states that 1) the mean of an animal's estimate of a duration will increase in a linear fashion with the duration of the to-be-timed interval and 2) the standard deviation of an animal's estimate of a duration will increase linearly with the duration of the to-be-timed interval (Church, 1997).

Although ubiquitous in laboratory investigations of timing behaviour involving animals timing single intervals, the conditions under which these properties emerge are not readily applied in the real world where animals move freely and are exposed to a number of different biologically meaningful stimuli in addition to those associated with the arrival of food. Despite this, it is possible to assess animals' potential use of temporal cues in natural contexts by asking, for example, if shorter intervals are more readily learned than longer ones.

**Timing in natural contexts**

Nectar-feeding birds and territorial hummingbirds in particular offer a model system for investigating the putative use of temporal cues in foraging. Brood-parasitic cowbirds provide another example of how time might be used in a different context. In the same way that hummingbirds could usefully anticipate when a flower might replenish its supply of nectar after a previous visit, a brood-parasitic cowbird might similarly update information on the status and location of potential host nests by using temporal cues while patrolling its home range (Reboreda et al., 1996). However, little is known about animals' use of temporal cues in natural contexts.

The question of whether hummingbirds use temporal cues in foraging is addressed in chapter 6. The potential use of temporal cues in coal tits *Parus ater* and great tits *P.*
major performing analogous timing tasks forms the basis of chapter 7. As food-storers, coal tits may rely more heavily on temporal cues than non-storing great tits. In a similar way that hummingbirds might benefit from being able to remember where and when visited rewarded flowers were visited, food-storing birds may also benefit from the ability to integrate both spatial and temporal information (Olton, 1985).
Chapter 2: General Methods

2.1 Introduction

This work in this thesis describes experiments testing cognitive performance on two avian systems: free-living rufous hummingbirds *Selasphorus rufus* (chapters 3-6), and coal tits *Parus ater* and great tits *P. major* tested in the laboratory (chapter 7). Here I describe the background and general methods used in the field tests. More detailed methods are described in the individual chapters.

2.2 Hummingbirds in Canada

Rufous hummingbirds *S. rufus* have been termed North America's "extremist" hummingbirds (Calder, 1993). In the early spring months they leave their nonbreeding habitat in Mexico to fly to their breeding grounds, travelling as far as Alaska. All of the observations described in this thesis were of hummingbirds living in a valley in the Eastern Range of the Rocky Mountains in Alberta (49° 29' N; 114° 25' W; Figure 2.1).

In all three seasons the rufous hummingbirds arrived in the study valley in early May. Around this time feeders containing sucrose solution were placed in potential trees (on trees and bushes). Upon their arrival male rufous hummingbirds readily established (and subsequently defended) territories centred around these feeders, separated by approximately 100-200m.

Once a male was observed to be consistently defending a feeder in any particular territory, he was marked (females defend much smaller feeding territories, are generally less visible, and were not used as subjects in any of the experiments). Males in contiguous territories were individually marked with a small daub of non-
Hummingbirds were observed in the Eastern Range of the Rocky Mountains south of Calgary in the province of Alberta, Canada (top; 1 cm = 250 km). The field site was a valley located to the west of Lethbridge in the vicinity of Pincher Creek (middle). The University of Lethbridge Westcastle field station (bottom) was the base for work.
toxic paint applied to the breast feathers while temporarily perched at a feeder. This did not require catching the bird, and different colours were used on individual males to allow residents to be distinguished from occasional intruders. The marking method has been used at this field site for nine years. Once marked, birds continue to approach and feed from feeders with no major changes in behaviour. None of the birds in this study lost their territory following marking. All fieldwork was carried out under license from the Alberta Fish and Wildlife authorities.

These birds readily learn to approach, and feed from, artificial flowers (see Figure 2.2). Details of how the birds were trained to feed from artificial flowers were slightly different for the four experiments described, and can be found in each chapter. In general, however, training a naïve hummingbird to feed from artificial flowers only took about 2 hours and once completed did not have to be repeated on subsequent days. Wherever possible experimentally naïve birds were used, although in one experiment (chapter 4) this was not possible due to time constraints. Around the middle of July the males leave the study site, so all experiments had to take place between late May and the end of July.
Figure 2.2  Artificial flowers presented to hummingbirds in field experiments.

"Flowers" were circular cardboard discs (diameter 60cm) of various colours mounted on wooden sticks. In the middle of the disc a modified syringe tip acted as a reservoir for sucrose "rewards". The top picture shows an example of flowers as used in chapter 5, with one flower mounted on each stick. The lower picture shows the kind of flower arrangement used in chapter 4, with two flowers on a stick.
Chapter 3: Do hummingbirds follow routes to remember the locations of flowers?

3.1 Introduction

Animal movements vary both spatially and temporally, from numerous short duration foraging trips made over the course of a day to long-distance migrations over weeks or months (Bell, 1991; Papi, 1992; Collett & Zeil, 1998; Weimerskirch & Wilson, 2000). Learning and remembering where and when important resources can be found enables some animals to exploit their environment efficiently. For example, some hummingbirds remember flower locations during foraging, and they use various cues – including flower colour and the distance between flowers – to do this (e.g. Miller et al., 1985; Healy & Hurly, 1998). Honeybees *Apis mellifera* also remember rewarding flowers (Burmeister et al., 1995; Brown et al., 1997) and use route-based information (e.g. following flight paths) to return to them when they are distant (see e.g. Srinivasan et al., 1996; Zhang et al., 1996). Ants *Leptothorax albipennis* follow distinct routes in proximity to features such as walls, and will follow edges in a consistent way to maintain their position while foraging (Pratt et al., 2001). It is possible that hummingbirds may also fly routes to and from rewarding flowers.

Route learning in insects often involves individuals finding their way around a familiar territory area. Insects navigate by learning information such as their position relative to landmarks found along the route home and the presence of landmarks at or near other sites of interest (e.g. food sites, for review see Collett & Collett, 2002; see also Barnes, 1993). In addition to this view-based navigation, insect movements can be guided by path integration. Path integration involves animals using idiothetic (self-generated) cues. They update their position relative to their starting point and return home by comparing a self-generated vector to a stored goal-to-nest vector (Etienne et al., 1998). This process can be done in the absence of learned references,
but extrinsic (e.g., celestial, visual landmark) cues can be used in combination with idiothetic path integration in order for animals to maintain position (Papi, 1992).

Path integration depends on the insect being able to measure both distance and direction and although orientation can be accurate in complete darkness (Etienne et al., 1998), bees moving to rewarded locations during the day use cues such as flower colour and shape (Ne'eman & Kevan, 2001; Spaethe et al., 2001). Insects use motion cues to determine how far they are from the goal (Lehrer, 1996; Lehrer et al., 1988). Honeybees *Apis mellifera* flying through tunnels measure distance through tracking the amount of optic flow (Srinivasan et al., 2000) and they commonly fly midway through experimental flight tunnels, thus balancing the optic flow on each side of the body (Srinivasan et al., 1996; Esch et al., 2001). However, bees can also learn to navigate through unmarked mazes, without the use of cues such as scent, colour marks and external landmarks from the wider surroundings. This indicates that sequences of movement between rewards may also be remembered (Zhang et al., 1996).

In view-based navigation, on the other hand, goals are specified by their location relative to local landmarks (Collett & Zeil, 1998). It has been proposed that insects “home-in” on a goal by a form of image-matching (Cartwright & Collett, 1983). In this model, the insect’s current retinal view is compared to a stored “snapshot” and by reducing the disparity between these two representations the goal is reached. Support for the snapshot model comes from the observation that insects often follow fixed routes whilst foraging (Chittka, 1998; Zhang et al., 2000). Bumblebees, for example, appear to remember the direction from which they arrived at flowers, and prefer to leave in the same direction (Pyke & Cartar, 1992). In so doing, they can maintain a consistency of scenes on approach to the goal. Bees and wasps also often perform highly structured orientation flights on their initial journeys from the nest. These suggest that by orienting in a specific way while flying towards the goal the animals form specific retinal snapshots that aid in relocating the goal later on (Collett & Lehrer, 1993; Zeil et al., 1996; see also Graham & Collett, 2002 on walking wood ants *Formica rufa*). Other nectarivorous animals may also do this.
Hummingbirds approach and feed from numerous flowers throughout the day, and will often revisit flowers visited earlier in the same day. The possibility that they may also follow routes between flowers arose in the context of “traplining” (Feinsinger & Colwell, 1978; Gill, 1988; Garrison & Gass, 1999). Trapliners (e.g. the long-tailed hermit hummingbird *Phaethornis longirostris*) revisit flowers in a regular and predictable pattern.

In this study, I tested whether hummingbirds use routes when returning to a patch containing rewarded flowers. Birds were trained to approach an arena containing flowers. Two treatments were used – the birds could either enter and leave the arena through one door, or were free to move through any combination of four doors. In tests, only one door was available (in one door trials always a different door to the one the bird had used during learning). If birds learned a flight path for returning to rewarded flowers when trained from a single direction, in tests they should not visit those flowers rewarded during the previous learning. When trained with four doors open the birds may either a) choose to use a flight path and come in and out of a single door or b) use all four doors and learn the locations of each flower relative to the landmarks surrounding the arena.

3.2 Materials and methods

3.2.1 Subjects and study site

The study area was the same as that described in chapter 2. Five experimentally naïve males were the subjects. The experiment took place from May-July 2001 between 0800 and 2000 hours (Mountain Standard Time).

3.2.2 Initial training

After marking, territorial birds were trained to feed from an artificial flower in the absence of the feeder. An artificial flower (a white cardboard disc, diameter 6cm) was mounted on a wooden stake (height 60cm) placed in the ground. Mounted vertically on top of the stake, above the cardboard disc, was a blue syringe tip forming a well capable of holding 120µl solution. A 120µl (20%) sucrose reward
was available to the bird on each visit. Birds learned to feed from this flower, which occupied a new spatial location (around 50-100cm away from the previous one) on each bout. Three more white flowers, mounted individually on new stakes, were then placed in the ground. Once the bird was consistently feeding from four flowers the initial training was complete. This usually took no more than two hours.

3.2.3 Training to the experimental arena

A square arena, with four walls and a ceiling, was constructed from thin grey plastic mesh. The array was 180cm long and 140cm tall (Figure 3.1) and was held upright by green sticks (height 150cm) situated at the corners. To add stability to the array, each stick was attached to string and held in the ground with a tent peg.

The next stage was to train birds to feed from flowers within the experimental arena. This began immediately after the initial training phase or, when training was completed late in the day, the next day. The bird's feeder was removed, and a flower was placed in the ground.

The rewarded training flower was moved closer to the arena after each visit by the bird. Over time, the bird fed from a flower placed inside the arena. Two more white training flowers were then placed inside the arena (120μl rewards). The bird fed from some or all of these flowers on each visit. One door was then placed over one of the entrance points. After two visits, another door was added to further restrict the number of open doors. The order in which doors were closed was chosen in no systematic way. The bird made at least two visits to the arena with 4, 3, 2 and then finally only 1 door open. Once all the combinations were complete, and the bird had become accustomed to flying in and out of the arena on different sides, training was complete. This usually took a few hours (range 2-6). The experiment began later the same day or, if training was completed in the evening, on the next day.

3.2.4 Experimental arrays and trial types

The arena was moved 2-5m from the location used during initial training. Sixteen flowers were laid out in the arena in a 4 x 4 configuration, with nearest-neighbour
distances between flowers of 40cm. Four flowers contained 30μl (20%) sucrose rewards and the remaining flowers contained water. A pseudo-random schedule was used to allocate the positions of rewarded flowers with the only restriction imposed that two of the rewarded flowers could not be positioned immediately adjacent to any open doors. Similarly, no more than two flowers in the array were nearest-neighbours.

3.2.5 Protocol

Two trial types were used. Common to both trial types was a learning period when, over a number of visits, the bird would learn where rewarded flowers could be found. Once criterion was reached, a test took place where all flowers were empty. A break then occurred before the next trial took place.

In “all doors” trials, all four arena doors were open during training. In “one door” trials, one door was chosen at random as the only one available for the bird to enter during the learning phase. Each trial was conducted in a new spatial location with trial-unique flowers. For recording purposes, doors were designated arbitrarily according to each of the four major compass orientations. These did not necessarily correspond to the actual orientation of the array in space.

After each visit each rewarded flower that had been visited was shaken out and refilled with 30μl (20%) sucrose. When on three consecutive visits the bird visited at least 3 of the 4 rewarded flowers (criterion of 75% or greater), a test was conducted.
Figure 3.1 Experimental set-up used in route-following experiment.

A schematic diagram of the arena used in the experiment. The four "walls" and "roof" were made of plastic chicken wire and were held still at each corner using tent pegs (not shown). The birds could either access the arena through one or four doors, depending on the trial type. An array of 16 flowers (4x4, not shown) was located within the arena. The picture shows the arena as used in the field.
Each subject first completed 3 all doors trials and 3 one door trials carried out in random order. The configuration of rewarded flowers differed in each of these 6 trials. In the 6 trials thereafter, the same configurations were used but the trial type was switched. For example, one bird's first trial was a four door trial in which flowers 2, 8, 9 and 11 were rewarded. The same bird's eighth trial was a one door trial in which flowers 2, 8, 9 and 11 were rewarded. Each bird completed twelve trials. In the event of early termination of a trial due to bad weather or failing light a new trial was conducted later in a new place using new, trial-unique flowers.

3.2.6 Tests

In tests the position of the arena and array remained the same as in the learning phase. All flowers were empty. In both types of trial, the test phase had only one door open. For one door trials, this was a randomly selected door that was not the one used during the learning phase. For all doors trials, which door was open was randomly chosen. As before, the time and sequence of flower visits made by the bird were recorded during the test.

After each test the bird's feeder was returned and the arena removed. The next trial started with the arena in a new site (at least 3 metres away from its previous location). There was an interval of at least one hour between trials.

3.3 Results

I assessed performance by looking at the proportion of birds' visits to correct (previously rewarded) flowers in the tests. Tests for normality were performed and the data normalised by applying the arcsine square root transformation prior to analysis using parametric tests.

3.3.1 Performance data

All doors trials

If birds remembered the absolute locations of the rewarded flowers, they would be expected to return to them first in the tests. I examined the birds' first choice, and
the first four choices, made in the test phases. The mean performance of the five subjects was compared to chance (0.25 for both comparisons). In the all doors trials, birds were no better than chance using the first choice measure (one-sample $t$-test: $t_5 = 2.11, p = 0.10$; Figure 3.2). Across the first four choices, however, birds were more likely to return to flowers in the correct absolute location (one-sample $t$-test: $t_5 = 6.2, p = 0.003$; Figure 3.2). None of the individual birds performed significantly better than chance (one-sample $t$-tests: first choice; all $n = 6$, Bird 1: $t = 0.75, p = 0.49$; Bird 2: $t = -1.00, p = 0.36$; Bird 3: $t = 0.01, p = 0.99$; Bird 4: $t = 1.58, p = 0.17$; Bird 5: $t = 1.58, p = 0.17$; across first four choices: all $n = 6$, Bird 1: $t = 0.81, p = 0.46$; Bird 2: $t = 0.01, p = 0.99$; Bird 3: $t = 1.58, p = 0.17$; Bird 4: $t = 2.24, p = 0.07$; Bird 5: $t = 0.35, p = 0.74$; Figure 3.3).

**Figure 3.2** Performance in all doors trial tests (absolute locations).

Overall performance (mean proportion correct ± s.e., $n = 5$ birds) in all doors trials, as assessed by visits made in the tests to those flowers in the same absolute locations as the rewarded flowers in the learning phase. The horizontal line denotes chance performance (0.25).
Figure 3.3  Individual performances in all doors trial tests (absolute locations).

The graph shows individual birds' performances (mean proportion correct in first four choices ± s.e., $n = 5$ birds) with respect to returning to the absolute locations of those flowers rewarded during the learning phase in all door trials. Chance performance (0.25) is denoted by the horizontal line.

Instead of returning in the tests to those flowers in the same absolute location as those rewarded during the learning phase, the birds may have returned to flowers which were correct relative to the entry door (Figure 3.4). In all doors trials, this opportunity would arise if the birds favoured a certain door in the learning phase. However, there were no instances in all door trials of birds exclusively using certain doors throughout the learning phase. There were seven occasions (23%) in the last three visits prior to the test (those visits determining criterion performance) when birds flew in and out of the arena through the same door. In these trials, the same door (e.g. North) was used to enter the arena before the test was conducted. The test performed in each of these seven trials, by chance, never involved the door randomly
Figure 3.4 Measurements of performance using absolute and relative flower position

A schematic diagram to illustrate the measurement of performance using absolute and relative flower location. In this hypothetical one door trial four flowers (black-filled circles) were rewarded in the learning phase. The entry door was S (South). In the test, birds used the W (West) door. If the bird returned to all correct flowers in the test trial using absolute position (corresponding to the actual position of the previously rewarded locations), the same four flowers would be chosen. If instead the bird used relative position, different flowers would be chosen (see bottom right). Similar logic applies to all doors open trials where one door is favoured.
chosen to be open matching the previously favoured door. I checked whether in these trials the birds went to locations in the array that were correct with respect to relative rather than absolute position. There was no difference in performance between these trials and all the others (comparing returns to flowers correct with respect to relative location against returns correct with respect to absolute location; Mann-Whitney tests: first choice $W = 246, p = 0.38$, first four choices $W = 98, p = 0.59$).

**One door trials**

I tested first whether, in one door tests, the birds preferred returning to those flowers occupying the same absolute position as the (previously) rewarded flowers. Chance performance was 0.25 for the first, and the first four, flowers visited in the tests. On neither measure were birds better than chance (one-sample $t$-tests; first choice: $t_5 = 1.34, p = 0.25$, first four choices: $t_5 = 2.28, p = 0.08$; Figure 3.5).

**Figure 3.5** Performance in one door trial tests (absolute locations).

Overall performance (mean proportion correct ± s.e., $n = 5$ birds) in one door trials, as assessed by visits made in the tests to those flowers in the same absolute locations as the rewarded flowers in the learning phase. The horizontal line denotes chance performance (0.25).
None of the individual birds performed significantly better than chance, as measured across the first choice in the test (one sample t-tests, all \( n = 6 \): Bird 1: \( t = 0.40, p = 0.71 \); Bird 2: \( t = 0.40, p = 0.71 \); Bird 3: \( t = 1.98, p = 0.11 \); Bird 4: \( t = -0.50, p = 0.64 \); Bird 5: \( t = 1.98, p = 0.11 \)). Across the first four choices, only one subject (Bird 1) performed significantly better than chance (one-sample t-test: \( t_6 = 2.71, p = 0.042 \); Bird 2: \( t = -0.42, p = 0.70 \); Bird 3: \( t = -0.01, p = 0.99 \); Bird 4: \( t = -0.28, p = 0.79 \); Bird 5: \( t = 2.00, p = 0.10 \); Figure 3.6; all \( n = 6 \)).

**Figure 3.6** Individual performances in one door trial tests (absolute locations).

The graph shows the birds' performances (mean proportion correct ± s.e., \( n = 5 \) birds) with respect to returning to the absolute locations of those flowers rewarded during the learning phase in one door trials. Bird 1 performed significantly above chance (0.25). Chance performance is denoted by the horizontal line.

I also examined the possibility that in tests the birds returned to those flowers correct with respect to relative location (Figure 3.4). Overall, the effect as measured across the first choice in the test was very close to significance (one-sample t-test against chance (0.25): \( t_5 = -2.74, p = 0.052 \); Figure 3.7) with birds performing worse than chance. There was no significant effect across the first four choices (one-sample t-
test against chance (0.25): \( t_5 = -1.17, p = 0.31 \); Figure 3.7). In terms of the first choice in tests, the birds were poor at relocating the flowers correct with respect to relative cues. Using this measure, two birds (Birds 1 and 5) never visited such a flower. The remaining three subjects did so only once each (all \( p > 0.6 \)). Across the first four choices, none of the birds performed significantly differently from chance (one-sample t-tests: all \( n = 6; \) Bird 1: \( t = -1.54, p = 0.19 \); Bird 2: \( t = -1.58, p = 0.17 \); Bird 3: \( t = 0.99, p = 0.36 \); Bird 4: \( t = 0.99, p = 0.99 \); Bird 5: \( t = -1.58, p = 0.17 \); Figure 3.8).

**Figure 3.7** Performance in one door trial tests (relative locations).

Overall performance (mean proportion correct ± s.e., \( n = 5 \) birds) in one doors trials, as assessed by visits made in the tests to those flowers in those positions correct relative to the locations of the flowers rewarded in the learning phase. The horizontal line denotes chance performance (0.25).
The graph shows the birds’ performances (mean proportion correct ± s.e., n = 5 birds) with respect to returning to flowers in positions correct relative to those rewarded during the learning phase. None of the birds performed significantly above chance (see text). Chance performance (0.25) is denoted by the horizontal line.

3.3.2 Patterns of visits to flowers

I looked at the sequences of flower visits in the three visits preceding tests for evidence that the birds were using stereotyped routes to move among flowers. In each of the three visits made before a test, a minimum of three and a maximum of four flowers could be probed to fulfil the criterion of 75% (3 visits to rewarded flowers (100%) or 4 visits with one error (75%)). When four were visited either all flowers were rewarded or one might be unrewarded. I analysed data on the choices made using a method similar to that used by Brown and Terrinoni (1996). They analysed the movements rats made in a regular 5 x 5 pole maze where rewards were positioned in a linear fashion (five in a row) or as a square of four. In trials the subjects could move from baited poles to adjacent poles (which could be baited or
not). The rats chose the rewarded poles in the maze according to their spatial configuration more often than would be expected by chance (Brown & Terrinoni, 1996). In this study, I examined hummingbirds’ sequence of visits to all the flowers in the array (and not just to adjacent flowers to the one currently being visited). An index of similarity allowed me to describe the choices made: a maximum score of 12 resulted from a bird visiting the same four flowers in the same order in the three bouts before the test. Conversely, a score of zero indicated that no sequence regularity was present. In this case, the required number of flowers was visited but in an irregular sequence. I performed an analysis on all trials (all doors versus one door). The total score for each trial type was computed (the sum of scores for the three visits before the test) and compared. There was no significant difference in the scores between the two trial types (Mann-Whitney test: $W = 794.5, p = 0.065$; median score of 6 in one door trials and 4 in all door trials) although the birds in one door trials tended to visit flowers in similar sequences more often than they did in four door trials.

3.3.3 Comparison between trial types

The reward configurations in each trial type were matched such that each bird was given the same configuration in both trial types. I compared performance between the two trial types according to the configuration used. There were no differences between the trial types using either the first choice ($t_6 = 0.56, p = 0.60$) or the first four choices ($t_6 = 0.19, p = 0.86$).

3.3.4 Differences in learning between the trial types

It may have been more difficult for birds to learn which were the rewarded flowers when all four doors were open during learning than when only door was open and the flowers could only be reached from a single direction. For both trial types I looked at the number of visits to the arena before criterion was reached. There were significantly more visits to the arena before tests in all door trials than in one door trials ($t_7 = 3.28, p = 0.013$; Figure 3.9). There was no difference between the trial
types, however, in the number of visits made to unrewarded flowers during learning (two-sample t-test: \( t_5 = 0.03, p = 0.98 \)).

**Figure 3.9** Measure of task acquisition – number of visits made to the arena before tests.

The bars show the mean number of visits (± s.e.) to the arena before tests were conducted for both trial types.

3.4 Discussion

In this experiment, I examined whether rufous hummingbirds use stereotyped routes to return to rewarded flowers. Using an arena containing an array of flowers, birds were trained to access the flowers via either one or four doors. They were tested with only one door open. When trained with all the arena doors open, the hummingbirds successfully relocated previously rewarded flowers in the test. On the relatively rare occasions when use of a single door was favoured before the test, there
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was no evidence that the birds returned to the flowers that were in the correct positions relative to the open door. When trained with a single door open, the birds also tended to return to flowers in the correct absolute locations, but this effect did not seem to be as strong as in the all door trials. In contrast, performance in one door trial tests was worse than chance with respect to returning to flowers correct according to relative position. The hummingbirds in both trial types seem to have remembered which were the rewarded flowers independently of their position relative to the open door. If the birds develop a stereotyped route to visit flowers, the one door training trials should have shown the strongest effect. In fact, the effect was in the opposite direction. These data, then, do not support the proposal that individual birds repeatedly use the same flight path into the array.

However, with respect to possible route following among flowers within the array, there was a tendency in one door trials for birds to visit flowers in similar sequences. Rufous hummingbirds feeding from an open array of ten artificial flowers readily remembered the locations of up to three rewarded flowers; however, the possibility that birds might use routes was not investigated (Hurly & Healy, 2002).

Although in this experiment the hummingbirds appeared not to be using routes to return to the array, they did appear to use them to some extent between flowers. The use of routes on a small scale such as this was also seen in a visual discrimination task in which hens were trained to recognise objects placed in front of two walls. After training, the birds displayed marked preferences for going around one side of objects to find food rewards. They also moved their heads in repeatable ways when moving towards them, although the birds’ eyes did not follow fixed trajectories across trials, as do bees and wasps (Dawkins & Woodington, 2003; Collett, 1992).

The hummingbirds in this study were not constrained in any way as they flew from their territory vantage points to the arena and flowers. It would perhaps be worthwhile to investigate birds’ body movements near flowers in greater detail. Through techniques such as high speed filming, it might be possible to establish if hummingbirds “home-in” on flowers with their body positioned at a certain angle. The route-following mechanism proposed for bees depends on the animal acquiring a
representation, or snapshot, from a fixed angle (Cartwright & Collett, 1983). It would seem from the current experiment that the hummingbirds are not doing this. Homing bumble bees *Bombus impatiens* appear to be guided to the nest by distal cues and, closer in, by more proximal cues (Plowright et al., 1995). It is possible that the hummingbirds in this experiment also used a combination of cues derived in part from seeing the arena and surrounding landmarks (e.g. trees, bushes) from some distance away and then, closer up, the flowers within it. Bumblebees *Bombus terrestris*, for example, rapidly form associations between the size of flowers and rewards inside them (Blarer et al., 2002). This requires that they perceive the size of flowers accurately, and following a particular route is a way that this could be achieved.

Data from an experiment on humans provides some clues on why birds might be affected by viewing flowers from one (one door trials) or many (all doors trials) perspectives. In a task-set switching experiment, where the spatial relationships between simulated items (e.g. between an office and another room in a building) was manipulated, subjects took longer to respond in situations where multiple perspectives were incorporated in consecutive trials (Brockmole & Wang, 2003). It appears, then, that memories involving numerous perspectives and hence more complicated spatial reasoning require more time for animals to process. The human data are consistent with the finding in this study that the birds took longer to learn the rewarded flower locations in the all doors trials than in the one door trials.

Investigations of route-following under natural conditions have largely been concerned with how animals such as bees and wasps reach goals using landmarks (allocentric orientation). There is increasing evidence that many animals orientate effectively using a combination of an egocentric frame of reference and mechanisms such as view-based navigation and compass cues. For example, the ability of honey bees to locate food sites is not disrupted when they have no external directional reference point. Yet, if present, landmarks do provide useful cues for finding rewards (Fry & Wehner, 2002). Similarly, wood mice *Apodemus sylvaticus* distribute small items such as sticks on their way to places of interest and through
this "way-marking" probably move around more efficiently by reducing unnecessary
diversions (Stopka & Macdonald, 2003). It seems likely that animals often solve
orientation problems according to the solutions available to them, which vary
according to context (see e.g. Skinner et al., 2003). Further investigations of the role
of directional information in hummingbird foraging will be logistically challenging
due to the problem of working over a realistic spatial scale. Despite this difficulty,
this experiment (and others, see e.g. Tiebout III, 1992; Baum & Grant, 2001) have
allowed insight to be gained into hummingbirds' use of route-based information.
Chapter 4: Relational learning in hummingbirds

4.1 Introduction

When faced with making a decision among a range of items varying in quality, such as food or potential mates, the choice(s) that the animal make(s) may be based on what it has learned previously (Lewis, 1986; Craig, 1994; Miller & Todd, 1998). When two or more choices are present there is a possibility that an animal might use the relationship(s) between those stimuli to choose between them.

Animals learn spatial relationships between objects in their environment. For example, Clark’s nutcrackers *Nucifraga columbiana* were able to learn to find rewards located halfway between two landmarks even with landmarks at varying distances apart (Kamil & Jones, 1997). Invertebrates, too, for example the fiddler crab *Uca vomeris*, can perform similar feats (Hemmi & Zeil, 2003). A number of species, including rats and human children, may make mistakes in foraging tasks that demonstrate that they are using geometric information to make their choices: in rectangular arenas, for example, rats often search in the corner diagonally opposite to the correct one (Cheng, 1986). Although few other species have been tested, bees (Cheng, 1999), pigeons *Columba livia* (Kelly & Spetch, 2001), fish *Xenotoca eiseni* (Sovrano et al., 2002), and chicks (Tommasi & Polli, in press) can all orientate using geometric cues.

There is a possibility that the natural history of a species could have an effect on its performance in tasks involving the use of geometric information. Food-storing birds, who in nature are faced with the problem of relocating hidden caches using landmarks, are an example of animals that might benefit from the use of geometric cues (Jones et al., 2002). Rufous hummingbirds *Selasphorus rufus* might also be expected to learn spatial relationships. Throughout the course of a day they visit numerous different flowers, the nectar in which varies in availability both in space and time (see e.g. Gill, 1988). Hummingbirds will learn about patches of flowers
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and the positions of rewarded flowers within patches as well as the locations of individual flowers (Miller et al., 1985; Sutherland & Gass, 1995).

Healy and Hurly (1998) showed that, dependent on the distance between flowers, rufous hummingbirds remembered them either relative to the other flowers in the array (up to 40cm apart) or as individual locations. In another experiment, hummingbirds also appeared to learn the spatial configuration of rewarded flowers such that when tested on a similar array to that on which they were trained, birds were faster to learn which flowers were rewarded if they occupied the same array positions. This occurred even when the flowers in the second array were of different colour patterns to those in the training array (Hurly & Healy, 2002).

The current experiment was designed to test explicitly whether or not the birds learn which are the rewarded flowers based on the spatial relationship between the flowers. Whether or not animals learn a relationship between stimuli can be tested using a transposition paradigm (human children: Zeiler & Salten, 1967; cichlid fish: Mark & Maxwell, 1969; rhesus monkeys: Harmon et al., 1982; Pasnak & Kurtz, 1987; goldfish: Zerbolio, 1985; Californian sea lion: Schusterman & Krieger, 1986). Subjects are trained to discriminate between two stimuli which differ in a single physical dimension (e.g. size, shape, brightness or colour). In the test situation the animal is presented with the previously reinforced stimulus and a novel stimulus which varies in the same dimension as the training stimuli (e.g. small and medium (rewarded) object presented during training, followed by a medium and large object in the test). If the animal has learned the physical relationship between the stimuli it should choose the novel stimulus in the test (i.e. the large object). This is transposition. If choice is determined by experience of past reinforcement, however, the original object (i.e. the medium sized object) will be chosen. In this way, animals’ use of absolute and relative cues can be investigated.

Bumble bees *Bombus impatiens* can be trained to transpose flower height (Wiegmann et al., 2000). In one group, bees were trained to visit a single rewarded flower at a height of 6cm. Another group was trained with two flowers: one rewarded flower at 6cm and another, empty, at 10cm. Both groups were then given a
test in which two water-filled flowers were presented - one at 6cm and one at a lower height of 2cm. Those bees familiar with feeding at two flowers chose the lower flower significantly more often than the 6cm flower. Subjects that had only fed from the 6cm flower preferentially returned to the 6cm flower. Foraging bumble bees thus appear to be influenced to at least some degree by the absolute and relational properties of flowers, and the context in which decisions on which flowers to visit are made.

In this study, I used a similar design to that used by Wiegmann et al. to determine whether hummingbirds also transpose spatial relationships. I trained birds to discriminate between flowers at two differing heights and I predicted that if the birds learned relational properties they would visit the flower at the appropriate relative height in the test. If, however, they learned the rewarded flower's absolute height, in the test they would visit the flower at the training height.

4.2 Materials and Methods

4.2.1 Subjects and study site

The experiment took place in the same locale as described in Chapter 2. Six male rufous hummingbirds were tested, of which three were experimentally naïve. Three birds had previously taken part in an experiment that involved them feeding from an array containing flowers similar to those used in this experiment and hence did not require training. The remaining birds required training (see below). All training and experimental trials were conducted in open, flower-filled meadows within each male's feeding territory. Subjects were individually identifiable by means of small coloured ink marks on their breast feathers. The marking technique, involving no capture or restraint of birds, has been used successfully for 9 years. No loss of territory or disappearance from the study locale has ever been observed following marking. The experiment took place from June-July 2001, and observations were made between 0800 and 2000 hours (Mountain Standard Time).
Before training birds to feed from flowers commenced (3 of the 6 birds in this study), individuals were used to feeding from and defending artificial feeders filled with 14% sucrose solution. After marking the territorial male, training could begin.

4.2.2 Initial training

Training began with a progressive lowering of the bird’s feeder, followed by its temporary removal as artificial flowers were introduced. An artificial flower (a white cardboard disc, diameter 6cm), mounted on a wooden stake (height 60cm), was placed in the ground. Birds rapidly learned to feed from a small well containing a 20% sucrose reward (a blue syringe tip, capable of holding 120μl solution) mounted vertically in the middle of the flower. Once the bird had approached and fed from this single rewarded flower on a few occasions (around 10 visits), an additional three white flowers were added onto new stakes. Throughout training the stakes were moved 50-100cm between visits. Training ended once the bird was consistently feeding from the four flowers. This usually took 1-2 hours. When a bird was not being trained or tested, its feeder was returned.

4.2.3 Experimental trials

Two different trial types were used in the experiment: tall/medium and medium/short (henceforth T-M and M-S). The learning phase of all trials involved the presentation of one wooden stake placed vertically in the ground (see Figure 4.1 for trial set-up). Two identically coloured flowers were mounted on the stake, one low flower and one higher flower. A short length (5cm) of clear plastic tubing was used to attach the cork and flower to the central column of the stake. The flowers were trial-unique and were the same shape and size as those used in training. Flowers on the stake were mounted horizontally, one directly above the other. The flowers varied in colour and the geometric pattern (e.g. dots, squares) painted on them. A variety of colours (yellow, green, orange, blue, white, pink, red and purple) were used as background colours. The same flowers were used for all subjects but those used in any one trial type, at any stage of the experiment, were different across birds.
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**Figure 4.1** Arrangement of flowers in relational learning experiment.

Diagrammatic representation of the flower arrangements used. T = tall, M = medium, S = short. Not to scale.

In both trial types one flower contained a sucrose reward (120μl, 30% sucrose) and the other was filled with water, which the birds prefer to avoid. The pattern of reward differed according to trial type. Flowers were mounted at heights of 90cm and 70cm in T-M trials, and at heights of 70cm and 50cm in M-S trials (Figure 4.1).
The 70cm flower was rewarded in both trial types: the lower flower in T-M trials and the higher flower in M-S trials.

At the start of a day's testing the bird's feeder was taken down and a stake presented. After each visit to the flowers the stake was moved about 25cm and the emptied sucrose well was refilled. The criterion level for testing was three consecutive visits in which the first visit was to the 70cm flower. An additional requirement was that the bird must have visited the water-filled flower at least once during the trial. In tests, birds were presented with a stake with empty flowers at heights of 70cm and 50cm (T-M trials) or 90cm and 70cm (M-S trials). The height of the stake in tests was 70cm and 90cm for T-M and M-S trials respectively.

Twenty trials (10 x T-M and 10 x M-S) were conducted on five of the birds, with the order of presentation of T-M and M-S trials drawn from a random schedule. The sixth bird completed only two trials of each type as he lost his territory. The same flowers were used for all subjects but those used in any one trial type, at any stage of the experiment, were different across birds. In the event of a trial being terminated due to bad weather a further trial was carried out later in a new spatial location and with a new set of unique flowers.

Observations were taken from a position about 10m away from flowers. The time and sequence of visits to flowers in each foraging bout were recorded. Birds completed a mean of $4.7 \pm 0.36$ trials/day.

### 4.3 Results

Data were collected on the flowers visited in the learning phase, and the tests. Proportions (number of visits to each flower type) were calculated (this also allowed the incomplete data from one bird to be included), and were arcsine square-root transformed where appropriate for use in parametric tests. Non-parametric tests were used when data transformation failed to produce normal distributions. All tests reported are two-tailed.
4.3.1 Transposition

In T-M trials, birds were presented with flowers at 90cm and 70cm during the learning phase. In the tests, flowers were presented at 70cm and 50cm. If the birds were transposing the trained spatial relationship, they would be expected to visit the lower flower in the tests. In M-S trials, where subjects were given flowers at 70cm and 50cm in the learning phase, birds would favour the taller flower (90cm) in the tests if they were transposing. If however the birds learned the rewarded flower’s absolute height, they would choose the taller (70cm) flower in T-M trials and the lower flower in M-S trials.

The proportion of 70cm flowers visited in T-M trials (across all subjects and trials) did not differ from chance performance of 0.5 (one-sample t-test on arcsine square-root transformed proportions: $t_6 = -0.48, p = 0.65$). In M-S trials birds chose the 90cm flower significantly more often than the shorter flower (one-sample t-test on arcsine square-root transformed proportions: $t_6 = -2.84, p = 0.03$; see Figure 4.2).
Figure 4.2  Performance (visits to both flowers in tests).

Mean number of visits (% ± s.e.) to the two flowers available in the tests. Data are averaged across all subjects and trials. Medium (M, 70cm) and small (S, 50cm) flowers were available in T-M trial tests; medium (M, 70cm) and tall (T, 90cm) flowers in M-S trial tests. The line at 50% denotes chance levels. * denotes a significant difference at $p < 0.05$.

A paired $t$-test was used to test whether the proportion of 70cm flowers chosen in T-M trial tests differed from that in M-S trials. Performance on the two trials differed significantly ($t_6 = 3.08, p = 0.027$). The birds made more visits to the 70cm flower in T-M trials than in M-S trials. The following analyses, then, will consider both trial types separately.

4.3.2 Effect of experience

Although there was a criterion to be met before tests occurred, the transposition shown in M-S trials and not in T-M trials may have been due to differences in ease of learning during the training phase. To reach criterion there had to be three consecutive bouts in which the first choice was the 70cm flower immediately before the test and he had to have visited the unrewarded flower at least once.

Significantly more visits were made to the unrewarded flower during the learning phase in T-M trials than in M-S trials (Mann-Whitney test: $W = 3326.5, p = 0.0001$;
see Figure 4.3). Birds made more mistakes before learning which was the rewarded flower in T-M trials than in M-S trials. In T-M training the unrewarded flower was the taller of the two.

A further possibility is that the birds may have made more visits to the array in one trial type and not the other. As another measure of acquisition, I looked at data relating to individual foraging bouts. “Bouts” were defined as each visit made to the array during the learning phase. Across all T-M trials, the mean number of bouts before a test was conducted was $6.02 \pm 0.35$. In M-S trials, there were $5.27 \pm 0.19$ bouts before tests. The difference in the median number of bouts before tests in the two trial types was not significant (Mann-Whitney test: $W = 2897.5, p = 0.27$).

**Figure 4.3** Visits to unrewarded flower in learning phase.

Mean number of visits (± s.e.) to the unrewarded flower (90cm in T-M trials, 50cm in M-S trials) in the learning phase for both trial types. * denotes a significant difference.
4.3.3 Effect of return interval

The period between a bird's last visit during the learning phase and return to the new pairing of flowers in the test (the "return interval") was variable, and determined by the birds. Across the experiment return intervals ranged from 5-138 minutes, and return intervals did not differ between T-M and M-S trials (all return intervals pooled, subdivided by trial type; Mann-Whitney test: \( W = 2790.5, p = 0.69 \)).

In T-M trials, the mean return interval was 19.42 ± 2.5 minutes (range 6-138 minutes). In T-M trial tests where the birds chose the 70cm flower, the mean return interval was 22.25 ± 5.25 minutes (range 7-138 minutes). In those tests where the shorter flower was chosen, the mean return interval was 17 ± 1.57 minutes (range 6-52 minutes; see Figure 4.4). There was no difference in the median return intervals in T-M trial tests according to which flower (70cm or 50cm) was chosen in the tests (Mann-Whitney test: \( W = 651.5, p = 0.78 \)).

In M-S trials, the mean return interval was 16.55 ± 0.96 minutes (range 5-39 minutes). When the 70cm flower was chosen in tests, the mean return interval was 15.33 ± 1.81 minutes (range 5-32 minutes). In tests where the taller flower was chosen, the mean return interval was 17.05 ± 1.15 minutes (range 7-39 minutes; see Figure 4.4). There was no difference in the median return intervals for the tall (90cm) and medium (70cm) flowers chosen in the tests (Mann-Whitney test: \( W = 1020.5, p = 0.42 \)).

The time taken to return did not appear to affect performance in any of the trial types.
4.4 Discussion

The hummingbirds in this study were presented with a transposition problem in which two training stimuli varied in height and the birds were rewarded for visiting the flower at a height of 70 cm. In tests, the training flower was coupled with a flower either 40 cm lower or 40 cm higher than the unrewarded training flower. In M-S trial tests, prior to which birds had been trained to the higher flower (70 cm vs. 50 cm), the birds preferred going to the higher flower (now at 90 cm). The birds appeared to have learned the relationship between the two flowers instead of their absolute properties. In T-M tests, prior to which the birds were trained to visit the lower flower (70 cm vs. 90 cm) the birds showed no preference between the 70 cm flower and a lower flower.

One possible explanation for this result is that the birds simply preferred visiting taller flowers. This would fit with the findings of Blem et al. (1997) who found that rufous hummingbirds preferred to feed from feeders at the tallest positions (Blem et al., 1997). Although not consistent with the outcome of the T-M tests, the training
data support the idea that the birds preferred taller flowers. In T-M trials, the birds were trained to visit the shorter of the two flowers, which they appeared to find more difficult than learning which was the rewarded flower in M-S trials. During T-M training, the birds visited the unrewarded flower significantly more often than in M-S trials before reaching the criterion required for the test. The lack of preference for the taller flower in the test phase might suggest that training had induced a conflict in the birds when facing the new choice. In M-S trials, on the other hand, birds were trained to visit the taller flower, a training situation that was in accord with their natural preference.

In this study, one pair of flowers was presented during training and another pair during testing, and in each phase the stimuli were presented simultaneously. Different arrangements have been used in laboratory tests on relational learning and transposition. In a study on transposition in pigeons *Columba livia* two approaches were used (Wills & Mackintosh, 1999). These were a) presenting stimuli simultaneously within each trial and b) presenting the stimuli in separate trials. The birds sometimes showed enhanced discrimination, and more transposition in the tests, when stimuli were presented simultaneously. This suggests that pigeons can use relational learning and, at least sometimes, allowing subjects to compare stimuli in the same dimension facilitates the learning of the discrimination. In another test on transposition in pigeons, both relational and absolute responding occurred (O.F. Lazareva and E.A. Wasserman, personal communication). In this second study, relational responding increased as the number of training pairs was increased from one to four. As in the current study, with one training pair available at a time, birds' responses can change according to the context in which decisions are being made.

There is increasing evidence that individuals evaluate and choose between alternatives (from items of food to potential mates) using comparative mechanisms such as that demonstrated by the use of transposition (see Houston, 1997; Shafir et al., 2003). Although currencies such as energy intake rate are absolute, it appears that animals may not assign absolute values to options. In a laboratory experiment, honey bees *A. mellifera* were presented with artificial flowers in the laboratory
differing in corolla length and nectar volume. The cost of feeding varied between the two options. The majority of bees foraged in a manner consistent with both absolute and comparative evaluation of choices, which may have arisen from individuals weighting options differently, for example through differences in experience (Shafir, 1994). Similarly, foraging gray jays *Perisoreus canadensis* (Waite, 2001) and rufous hummingbirds *S. rufus* (Bateson et al., 2002; see also Hurly & Oseen, 1999) frequently choose between options in a context-dependent manner.

Similar arguments apply to the discrimination of absolute and relative properties of stimuli. In a transposition study, bumble bees *B. impatiens* familiar only with feeding from one flower at medium height preferred returning to the medium height in later tests with a binary choice between a medium and a short flower. In contrast, bees trained on two flowers and with experience of visiting both flowers transposed height and preferred the shorter flower in tests (Wiegmann et al., 2000). These results are consistent with bees making comparative evaluations dependent on context. An approach that could help to shed further light on transposition in hummingbirds would be to test subjects on an intermediate-stimulus problem. In this paradigm, three stimuli are available to choose during training. The rewarded stimulus is the one that lies in the middle of the stimulus continuum (e.g. the medium-sized stimulus of small/medium/large stimuli). In later test sets, the transposition response occurs when the animal chooses the intermediate stimulus. Choice of the test stimulus most similar (e.g. in size) to the rewarded stimulus in training is the absolute response. A third possibility is the “nontransposition-nonabsolute” response of choosing the third stimulus. Children presented with an intermediate-stimulus transposition problem responded variably, with each category of response occurring to some extent (Zeiler & Salten, 1967). Animals are commonly faced with more than two choices, and as this is known to impact on choice behaviour in other foraging contexts its role in relational learning in hummingbirds seems worthy of investigation (Bateson et al., 2003). Under natural conditions, for example, hummingbirds in different areas throughout the year not only feed from different flower types, but may also have to learn and relearn handling skills appropriate to the species they are feeding on (Woodward & Laverty,
It may be important, then, for birds to have the flexibility in learning spatial relationships according to the area(s) in which they are foraging.

In this study the flowers used in the tests were the same as those used during the learning phase. Reese (1968) states that in studies of transposition, duplicate stimuli should be used in tests to guard against the risk that subjects might respond to features intrinsic to the objects used previously. In my experiment, the same flowers were used. Once the bird reached criterion, I moved the two flowers from one stake to another for the test to take place. Although I did not then use new flowers, there was no systematic bias in terms of which of the two flowers used in the learning phase were placed in either the high or low position on the new stake. In another field experiment, where cues derived from flower colour and position were put in conflict, rufous hummingbirds attended much more to spatial cues than to colour cues when learning where rewards could be found (Hurly & Healy, 2002). In this experiment, transposition occurring in one trial type and not the other can not be explained by differences between the flowers used.

The information used by animals to learn has, historically, been the topic of extensive debate. Whereas psychologists such as Köhler believed that animals learn about stimuli depending on their relations to each other, and not their absolute properties; others, such as Spence, believed animals respond to absolute properties of stimuli (for review see Reese, 1968). The findings from this experiment support the suggestion that animals can learn both absolute and relative properties of stimuli and that choices are often made which are context-dependent. The challenge now is to attempt to predict when each type of learning might be expected to occur, and to relate this to the types of learning and memory problems faced by animals under natural conditions (Jones et al., 2002).
Chapter 5: The roles of flower height and number of dimensions in hummingbird foraging

5.1 Introduction

The radial arm maze (RAM) is a commonly used apparatus used to test animals' spatial working memory abilities (see e.g. Bond et al., 1981; Wilkie et al., 1983; Balda & Kamil, 1988; Kamil et al., 1994; Hughes & Blight, 1999; for review see Foreman & Ermakova, 1998). For an animal to perform well in this task it needs to visit all the available rewarded sites without repeating prior choices (Olton & Samuelson, 1976). In addition to the oft-used laboratory rat, species as diverse as gerbils Meriones unguiculatus, rabbits Oryctolagus cuniculus and hooded crows Corvus corone cornix have all demonstrated good spatial working memory abilities in the radial arm maze and its variants (Wilkie & Slobin, 1983; Lipp et al., 2001).

The traditional RAM typically involves animals moving from arm-to-arm in a horizontally placed 2-D maze and rapidly adopting a “win-shift” foraging strategy, whereby returns to empty arms are avoided. This behaviour has been likened to the situation a foraging animal faces in the real world, where decisions have to be taken on where to feed, how long to stay, and when to move on (Roberts, 1991; Timberlake & Hoffman, 2002). Although rodents live a largely 2-D life, lending credence to the suggestion that the fast learning of efficient foraging patterns in the RAM reflects what they may commonly do in nature, performances appear to be even better when more complex mazes are used.

Grobéty and Schenk (1992) presented rats in the laboratory with both 2-D and 3-D mazes. They found that in mazes with a vertical (or z) component, which involved the animals moving in the vertical plane (e.g. by climbing from one level to another), fewer errors were made than in those mazes where movement could only take place
Spatial and temporal cognition in birds in the horizontal plane. One potential explanation for this is that as vertical movement is more energetically costly than moving horizontally, fewer mistakes are made (Grobéty & Schenk, 1992). Other work has shown that animals select particular visual configurations including those in the vertical plane. In an experiment on golden hamsters *Mesocricetus auratus*, vertically positioned landmarks positioned against a continuous background pattern were more effective in allowing animals to home accurately than horizontal ones, as determined by animals' movement vectors between a nest and a goal (Etienne et al., 1995). It appears, then, that animals use information in the z dimension. As highly mobile foragers, accustomed to approaching, feeding and hovering from many flowers each day, hummingbirds too might be expected to use information from the z plane.

The possibility that the z dimension might play a role in hummingbirds' learning and memory capabilities has been investigated previously. Height as a flower cue was manipulated in an open field analogue of the radial arm maze (Henderson et al., 2001: Experiment 2). Instead of presenting an array with eight "arms" all at the same height, rufous hummingbirds were tested on an array with a cubic configuration. Four stakes were mounted vertically, each with two flowers. The flowers were mounted at heights of 40cm and 110cm, and on their first visit to the array (phase 1) birds had to visit four flowers. Subjects were given two trial types. In "free" trials, all eight flowers were available for the bird to choose from in phase 1, and any four could be visited. Four randomly positioned flowers (2 in high positions and 2 in low positions) were present in phase 1 of "forced" trials. The birds performed significantly better than chance in both trial types, and in free trials preferred visiting higher flowers in phase 1. Performance on this 3-D version was better than that on birds tested with a 2-D version maze (Henderson et al., 2001: Experiment 1). I aimed in this experiment to exclude an alternative hypothesis that may have led to better performance on the 3-D array than on a 2-D array.

A factor that may have caused performance in the 3-D array to be better than in the 2-D array was the arrangement of flowers. Within the 3-D array, there were more short inter-flower distances than long ones. The birds may have found the 3-D task
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easier, then, by being able to use relative spatial relationships to successfully locate new flowers. To disentangle the possible effects of both flower height and varying interflower distances on hummingbird learning and memory, I presented birds with an experimental set-up where these cues could be manipulated.

In the current experiment, I used a hexagonal array in which I manipulated the height and the horizontal distances between flowers. In the horizontal plane, rufous hummingbirds attend to relative cues when flowers are closer than 40cm (Healy & Hurly, 1998). When flowers are further apart, the birds instead favour using absolute spatial location cues. In order that the birds were using only one cue type, I chose interflower distances in this experiment that were not less than 80cm. Differences in performance on 2-D and 3-D mazes should not then be attributable to use of different spatial cues. I predicted that birds’ performances in 3-D arrays would be better than in 2-D arrays, and when flowers were placed closer together the birds would perform better than when they were further apart.

5.2 Methods

5.2.1 Subjects

The experiment took place in the locale described in chapter 2. Training and experimental trials were run between 0800 and 2000 hours Mountain Standard Time in June and July 2002. Ten male rufous hummingbirds were used in the experiment. One subject was experimentally naïve, the remaining birds had previously been used in an unrelated risk experiment in which they had fed from small wells on a perspex board. All the birds were naïve to the cardboard flowers used in this experiment.

5.2.2 Initial training

Before experiments could begin, birds underwent a short period of training (1-2 hours). Subjects were trained to feed from artificial “flowers” (small circular cardboard discs, diameter 6cm). These were white and were pierced in the middle by a blue syringe tip forming a well capable of holding 120μl of sucrose solution. After
feeding a few times from a full flower temporarily attached to its feeder, and gradual lowering of the feeder towards the ground, the feeder was removed. The bird learned to probe a single flower attached to a vertical wooden stake (height 123cm) in the ground. Once the bird had fed from this flower twice I placed three more stakes, each with one flower, in the ground nearby. These flowers were rewarded with 30μl (20%) sucrose solution. The birds learned to feed from these flowers, typically moving between them and emptying them on each bout. The sticks were moved a short distance (50-100cm) between bouts. When the bird had fed from these flowers on a few occasions, including at least two visits to a flower in low, middle and high positions (ca.10cm, 80cm and 120cm), training was complete. The experiment then began.

5.2.3 Experimental trials

Before beginning a trial, the bird’s feeder was removed. Seven flowers were presented in an array (one flower mounted on each 123cm tall wooden stick) in a hexagonal configuration (Figure 5.1). Arrays were constructed on flat and clear patches of ground where the birds were free to move between all the flowers without obstruction. The distance between the sticks was constant within trial types. Four main trial types were used: 2-D near and far and 3-D near and far (for details see Table 5.1).

Common to all trials was a regular hexagonal arrangement of the sticks in the ground. The hexagonal arrangement was chosen so as to equalise the nearest-neighbour distance between the flowers (Figure 5.1). Flowers in the array were presented in the horizontal plane (2-D near and far) or in both the horizontal and vertical planes (3-D near and far). In the 2-D near and far trials birds were tested with flowers at high, medium and low positions. In the 3-D arrays, birds were given the choice of visiting high and medium flowers (3-D near trials) or high and low flowers (3-D far trials). In the trials where flowers were at two different heights (3-D near and far trials) two regular hexagonal array configurations were possible – one with three flowers in the top position and four in the lower position and vice versa. Both configurations were used in the experiment.
Figure 5.1  The flower arrangements used in the field.

The pictures show the artificial flowers as used in the field. Flowers were positioned at three heights of 1) 122cm “high” 2) 89cm “medium” and 3) 9cm “low”. The seven flowers were either all at the same height (2-D near and far) or at two different heights (3-D near and far). In pictures 1) to 3) below, the horizontal distance between flowers was 160cm, representing 2-D far trials. In 4), the stakes were 113cm apart, and the configuration shown is thus a 3-D far trial.
The table shows the distances between the flowers used in the experiment, subdivided by trial type. In every array, the distance between the sticks in the ground was constant in a hexagonal configuration. When flowers were presented only in the horizontal plane (2-D near and far), the inter-flower distances (the actual distance between a flower and a nearest-neighbour flower) were constant. When flowers were at two different heights (3-D near and far), the inter-flower distances varied. High = 122cm, medium = 89cm and low = 9cm.

<table>
<thead>
<tr>
<th>Trial type</th>
<th>Configuration</th>
<th>Flower arrangement</th>
<th>Inter-flower distances</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>2-D near</td>
<td>high</td>
<td>113cm horizontally</td>
</tr>
<tr>
<td>1b</td>
<td></td>
<td>medium</td>
<td></td>
</tr>
<tr>
<td>1c</td>
<td></td>
<td>low</td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>3-D far</td>
<td>3 high and 4 low</td>
<td>160cm diagonally between high and low, 113cm horizontally between adjacent lows</td>
</tr>
<tr>
<td>2b</td>
<td></td>
<td>4 high and 3 low</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>3-D near</td>
<td>3 medium and 4 low</td>
<td>113cm diagonally between high and medium, 80cm horizontally between adjacent mediums</td>
</tr>
<tr>
<td>3b</td>
<td></td>
<td>4 medium and 3 low</td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>2-D far</td>
<td>high</td>
<td>160cm horizontally</td>
</tr>
<tr>
<td>4b</td>
<td></td>
<td>medium</td>
<td></td>
</tr>
<tr>
<td>4c</td>
<td></td>
<td>low</td>
<td></td>
</tr>
</tbody>
</table>
Flower colour pattern was unique to each trial (colours used: purple, orange, green, pink, brown, red, yellow, white). The patterns used were typically lines and dots and within trials all the flowers were the same.

A one-trial learning paradigm was used and thus each trial had two phases. On his first visit to the array (phase 1) a bird was allowed to visit three of the seven available flowers. The bird either left after drinking from three flowers, or was chased away before he could feed from any more. The timing of phase 2 visits was primarily determined by the subjects, but I prevented the bird from returning to the array for a minimum of 5 minutes. On the rare occasions the bird returned before 5 minutes he was chased away. A trial was deemed valid if the bird fed from three different flowers in phase 1 and at least three flowers in phase 2. When these conditions were not met the trial was aborted. Aborted trials were repeated upon completion of all the other trials, using the same set of flowers but in a new spatial location.

Each trial was conducted in a new spatial location at least 3m away from the previous trial site. A minimum inter-trial interval (ITI) of 20 minutes separated consecutive trials, and a series of trials was conducted throughout the day (across all birds and trial types 8.42 ± 0.65 trials per day). During the ITI, and at all times when trials were not being run, the bird had unrestricted access to its feeder. Testing typically took 3-4 days on each bird; occasionally bad weather caused temporary delays. Data were collected on one subject at a time, and I sat approximately 5-10m from the array. The time and the flowers chosen by the bird were noted after each visit.

All birds completed six trials of each main trial type and hence 24 trials in total (for 2-D near and far trials two trials of each a, b and c permutation were completed; similarly, for 3-D near and far trials the bird completed three a and three b trials). The order in which trials were conducted was determined in a pseudo-random fashion. The 24 trials were divided into two blocks of 12 trials such that an equal
number of a and b trials occurred in each block. Within each block of 12, the order of trials was randomised. This process was repeated for each bird.

5.3 Results

Repeated visits to the same flower within phase 2 of trials were rare (4.9% of all phase 2 visits across all trial types), slightly less than those found in previous experiments on hummingbirds foraging in open-field analogues of the radial maze (6%: Healy & Hurly, 1995; 6.9%: Henderson et al., 2001). In instances of phase 2 revisits to a flower that had not been visited in phase 1, I did not count the repeat visit as "correct".

5.3.1 Comparison within trial types

In phase 2, birds could visit a combination of three empty flowers and four new (and full) "correct" flowers. I assessed performance by looking at both the first and the first three choices made in phase 2 to determine whether any differences in performance existed within trial types. A preference for feeding from tall flowers may, for example, have caused birds to perform better in the 2-D trials when the flowers were at the high positions than at the low positions.

Nonparametric statistics were used in the first four within-trial comparisons (2-D near - 1a vs. 1b vs. 1c; 3-D far - 2a vs. 2b; 3-D near - 3a vs. 3b; 2-D far - 4a vs. 4b vs. 4c) because the data (proportion correct flowers visited) were not normally distributed. The lack of normality was not corrected by applying the arcsine square root or any other standard transformation. Nonparametric Friedman tests were therefore used. There were no differences within each trial type in terms of birds' first visits in phase 2 (Friedman tests: 2-D near - 1a vs. 1b vs. 1c: $F_{r1} = 1.56$, $n = 10, p = 0.460$; 3-D far - 2a vs. 2b: $F_{r1} = 0.50$, $n = 10, p = 0.480$; 3-D near - 3a vs. 3b: $F_{r1} = 0.67$, $n = 10, p = 0.414$; 2-D far - 4a vs. 4b vs. 4c: $F_{r1} = 2.61$, $n = 10, p = 0.272$). Next, I performed a similar analysis using performance data from the first three phase 2 choices. In 3-D near trials birds tended to visit a correct flower more often in 3b trials than in 3a trials (Friedman test: 3-D near - 3a vs. 3b: $F_{r1} = 3.6$, $n = 10, p =$
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0.058); in all other trial types there were, however, no significant differences
(Friedman tests: 2-D near - 1a vs. 1b vs. 1c: Fr2 = 0.63, n =10, p = 0.729; 3-D far - 2a
vs. 2b: F1 = 2.0, n =10, p = 0.158; 2-D far - 4a vs. 4b vs. 4c: F1 = 0.5, n =10, p =
0.779). Overall, there was little evidence that the manipulations within the four
groups of trial types had any effect on performance. I therefore pooled the data for
each group of trial types for subsequent analyses.

5.3.2 Performance relative to chance

I compared birds’ performances to chance using both the first, and first three, phase 2
choices.

In phase 1, the birds always visited three of seven available flowers. The probability
of visiting a new flower in the first visit of phase 2 was therefore 4/7, or 0.57. I
computed the mean proportion of correct choices in phase 2 for each subject, pooled
across all trials (i.e. all 2-D near trials, all 3-D far trials, etc.).

In 2-D trials performance across the first choice in phase 2 did not differ significantly
from chance (2-D near: t9 = -1.21, p = 0.26; 2-D far: t9 = -0.99, p = 0.35). In 3-D far
trials there was a marginally non-significant difference (t9 = -2.13, p = 0.062). Birds
tended to perform worse than chance in 3-D far trials and significantly worse than
chance in the near trials (t9 = -4.41, p = 0.001; Figure 5.2).

I then compared birds’ performances across the first three choices in phase 2 with
chance. To calculate random expectation I used the formula –

\[ P = \left(\frac{n-1}{n}\right)^{c-1} \]

where \( n = 7 \) was the total number of sites and \( c \) was the order number of a given
choice (see also Bond et al., 1981; Prior & Güntürkün, 2001). On a seven arm maze,
with three flowers always visited in phase 1, random expectation across the first
three choices in phase 2 is thus equal to the sum of the probabilities across choices 4-
6. This is 1.63 out of three flowers, or 0.54. In 2-D near trials the birds performed
significantly better than chance (t9 = 2.26, p = 0.05); in all the other trial types there
were no significant effects (3-D far: \( t_9 = 0.05, p = 0.96 \); 3-D near: \( t_9 = -1.25, p = 0.24 \); 2-D far: \( t_9 = 1.07, p = 0.31 \); see Figure 5.2).

**Figure 5.2** Overall performance in 2-D/3-D array experiment.

Performance of hummingbirds in terms of the proportion of correct flowers visited in phase 2. The top graph shows the data across the first phase 2 choice and the lower graph shows the data across the first three phase 2 choices. Chance performance is denoted by the horizontal line.
5.3.3 Comparisons between two and three dimensional arrays

I used paired $t$ tests to make comparisons between the 2-D and 3-D trial types using the same performance measures as before (first and first three phase 2 choices). For each subject, I computed the mean proportion of visits to correct flowers and tested for differences between the 2-D and 3-D trials outlined in Table 5.2.

Table 5.2 Comparisons between 2-D and 3-D arrays.

The table shows the comparisons made between 2-D and 3-D trials. Flower heights: high = 122cm, medium = 89cm and low = 9cm.

<table>
<thead>
<tr>
<th>Trial types</th>
<th>Number of dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2-D vs. 3-D)</td>
<td></td>
</tr>
<tr>
<td>2-D near vs. 3-D near</td>
<td>flowers high</td>
</tr>
<tr>
<td>2-D near vs. 3-D near</td>
<td>flowers medium</td>
</tr>
<tr>
<td>2-D near vs. 3-D near</td>
<td>flowers low</td>
</tr>
<tr>
<td>2-D far vs. 3-D far</td>
<td>flowers high</td>
</tr>
<tr>
<td>2-D far vs. 3-D far</td>
<td>flowers medium</td>
</tr>
<tr>
<td>2-D far vs. 3-D far</td>
<td>flowers low</td>
</tr>
</tbody>
</table>

Performance tended to be better in the 2-D trials than in the 3-D trials in three comparisons. In 2-D near (medium) trials birds tended to perform better than in 3-D near trials ($t_9 = 2.19, p = 0.057$). The other two comparisons approached significance [2-D near (high) vs. 3-D near: $t_9 = 1.92, p = 0.087$; 2-D near (low) vs. 3-D near: $t_9 = 2.06, p = 0.070$; Figure 5.3]. The other comparisons were not statistically significant.
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[ 2-D far (high) vs. 3-D far: \( t_9 = 1.38, p = 0.20 \); 2-D far (medium) vs. 3-D far: \( t_9 = 0.10, p = 0.92 \); 2-D far (low) vs. 3-D far: \( t_9 = 0.72, p = 0.49 \); Figure 5.3].

5.3.4 Comparison within two dimensional arrays

There were no significant differences in performance between the 2-D near and far trials as measured across the first choice (\( t_9 = -0.81, p = 0.44 \)) and the first three choices in phase 2 (\( t_9 = -0.30, p = 0.77 \)).

5.3.5 Effect of retention interval

The interval between a bird's first visit to the array and its return visit (the retention interval, or RI) was a minimum of five minutes. Longer retention intervals were determined by the subjects, and ranged from 5–88 minutes (mean ± SE: 9.48 ± 0.45 min). To determine whether performance declined with increased RI, I divided the RI among the four trial types (ignoring possible non-independence within birds) and correlated performance (as measured by proportion of visits to correct flowers in the first three phase 2 choices) with RI. None of the correlations was significant (trial type 1: \( r_{60} = 0.191 \); trial type 2: \( r_{62} = 0.220 \); trial type 3: \( r_{62} = -0.146 \); trial type 4: \( 0.060 \); all \( p > 0.05 \)).
Figure 5.3  Comparisons between performance in 2-D and 3-D arrays.

Performance of hummingbirds in comparisons between 2-D and 3-D arrays. The top graph shows the data (proportion correct in phase 2) across the first choice and, in the bottom graph, across the first three choices. The horizontal lines denote chance performance.
5.4 Discussion

In this experiment in which birds were tested on mazes that were either 2 or 3 dimensional, the few effects that were found were contrary to predictions: birds’ performances on 3-D mazes were relatively poor, particularly when the flowers were close together, and birds tended to perform better on 2-D mazes.

It is not clear why performance in 2-D mazes was consistently better than in 3-D mazes. In Henderson et al.’s (2001) experiment in which flowers were positioned at different heights, birds appeared to do better than when flowers had all been at the same height (Henderson et al., 2001). However, in that study there was no direct comparison between performance on the 2-D and 3-D arrays, with order effects also possibly affecting the results. As the distances between flowers in this experiment were never less than 80cm, I aimed to eliminate the possibility that the results of Henderson et al. (2001) may have been due to birds using relative spatial relationships, as in Henderson et al.’s 3D array there were more short inter-flower distances than long ones. That the hypothesised differences between 2-D and 3-D arrays were not found could have arisen from the spacings used. The diagonal distances between nearest-neighbour higher and shorter flowers in 3-D near and far trials (113cm and 160cm respectively) may have simply been too far and, in 2-D arrays at least, rufous hummingbirds under some conditions are less likely to visit a focal flower when a distracter is placed up to 80cm away (Hurly & Healy, 1996).

One way to test this would be to perform a similar “scaled-down” experiment. This would perhaps reveal a switch in birds’ preferences for returning to relative versus absolute spatial locations at intermediate interflower distances less than those used in this study (see e.g. Eilam et al., 2003 for a similar approach).

Animals’ performances in maze tasks have traditionally been explained in terms of two main strategies. The first, the “cognitive map” theory, posits that animals orientate by developing a representation of the spatial relationships between objects in their environment (O'Keefe & Nadel, 1978; see also Dallal & Meck, 1990). Animals are thought to use a variety of intramaze cues (those from the actual locations being visited) and extramaze cues (those defining a location but not present...
at the location, for example distal cues in a testing room) to learn and remember food locations (Wilkie & Slobin, 1983; see Foreman & Ermakova, 1998 for a review). Others have instead characterised performances using a “sequential choice” model where animals make a series of go/no-go decisions, or microchoices, as they forage in a maze (Brown, 1992). Cognitive map theories assume that animals perform well in maze tasks by integrating a number of available spatial cues, and using this information to guide choices in a way analogous to a map. The sequential choice model, on the other hand, assumes that animals are unable to discriminate unvisited arms in a maze from those not yet visited on the basis of an internal map-like representation. Instead a “microchoice” has to be made which, in a typical laboratory RAM, involves a subject orienting towards an arm. Rats in the laboratory appear to spend time visually orienting towards maze arms before entering them (Brown & Cook, 1986), a finding not entirely consistent with the use of a stable map-like representation that ought to direct an animal to a food source without delay. By inspecting the cues at close range, then, the animal decides if it has experienced that arrangement of cues before and chooses accordingly. Although my data do not permit me to distinguish between theories of spatial mapping, it is possible that more detailed observations of birds’ choices at flowers within arrays – perhaps using filming techniques (see also chapter 3) - would allow a more thorough examination of how hummingbirds are guided by various cues. Similarly, if it were possible to perform noninvasive flight experiments such as those used to examine hovering in hummingbirds (see e.g. Chai & Dudley, 1999), it might be possible to measure the putative energetic cost of foraging in the vertical dimension outlined by Grobéty and Schenk (1992). It is likely that animals foraging in the real world will use whatever cues are available to them to solve the problem they are facing. These are no doubt multiple, varied, and, in contrast to a laboratory situation, inherently difficult to control.
Chapter 6: The use of time as a cue in hummingbird foraging

6.1 Introduction

Many important events in animals' lives - from the availability of food to the arrival of predators - occur predictably in time. Many animals are able to detect and use temporal cues (see e.g. Gallistel, 1990; Church, 1997; Gibbon et al., 1997). Timing abilities range in scale from animals varying activity levels according to events (such as the predictable onset of dawn and dusk) to anticipating the availability of food over shorter intervals. Pigeons *Columbia livia*, for example, respond at the appropriate time of day after learning how often food is available in the morning versus the afternoon (Budzynski & Bingman, 1999). Food-storing scrub jays *Aphelocoma coerulescens*, on the other hand, are able to remember where they stored food, what they stored, and when the storing took place (Clayton & Dickinson, 1998). By integrating both spatial and temporal information, foraging is more effective as the likelihood of visiting a site and finding food there is increased (Olton, 1985; Crystal & Miller, 2002).

Non-food storing animals also benefit from being able to use temporal cues. Hummingbirds can remember which flowers they have visited recently (Brown & Gass, 1993; Healy & Hurly, 1995; Sutherland & Gass, 1995; Henderson et al., 2001). As emptied flowers may take several hours to replenish, hummingbirds could save time and energy by avoiding them. To do this, the bird must remember not only where the flower was, but also when it was last visited.

There are two broad categories of timing of potential relevance to hummingbird foraging: phase and interval timing (see e.g. Gibbon et al., 1997). An example of phase timing is the circadian clock, which signals the occurrence of events at approximately 24-hour intervals. For foraging nectarivores, the time of day can act as a relevant cue regarding the location of food. Gould (1987) trained honeybees *Apis mellifera ligustica* to feed on a petal of an artificial flower at one time of day.
Subjects were then rewarded for feeding on another petal, on the same flower, later on. In later testing, the bees returned to the petal rewarded at that time during training. Other animals are known to return to places at certain times to find food over periods on or around 24 hours. Garden warblers *Sylvia borin* can learn at what time of day food is available at a particular location (Biebach et al., 1989). Such 24 hour time-place learning has been demonstrated in a number of other species under various conditions (wild European kestrels *Falco tinnunculus*, Rijnsdorp et al., 1981; wild oystercatchers *Haematopus ostralegus*, Daan & Koene, 1981; wild herring gulls *Larus argentatus*, Sibly & McCleery, 1983; captive goldfish *Carassius auratus*, Gee et al., 1994; various wild scavenging birds, Wilkie et al., 1996). Many important events in an animal’s life do not, however, occur at predictable points in the day.

The second category of timing is interval timing, which is used to keep track of events with arbitrary start points. Instead of marking a fixed point within a phase, as in circadian timing, interval timing allows successful anticipation of events that occur reliably a fixed amount of time after another event, such as the delivery of food (Carr & Wilkie, 1997). Investigations of interval timing have typically required animals timing intervals ranging in duration from seconds to minutes (Zeiler & Powell, 1994; Plowright, 1996), although occasionally longer intervals (a few hours) have been tested (see e.g. Eckerman, 1999).

In laboratory studies of timing, animals are required to time intervals between signals such as tones and lights, and reinforcement can occur if the subject responds after the "to-be-timed" interval has elapsed (see e.g. Zeiler, 1977). Rats, pigeons and humans have been shown to time intervals using various experimental procedures (see e.g. Bateson, 2001; Malapani & Fairhurst, 2002). One example is the peak procedure, where in food trials animals are rewarded if they respond after a fixed amount of time from a signal (e.g. a light) going on. In later empty trials, where subjects are not rewarded, the signal remains on much longer than before and animals’ responses across time are recorded (Catania, 1970). Few data exist on timing in other species
although of those tested, qualitatively similar abilities have been found (see e.g. Richelle & Lejeune, 1984).

In general, research on animals’ abilities to time short intervals (from seconds to hours) has revealed two main characteristics. Firstly, and unsurprisingly, animals return most frequently to those places where reward is available most often (Miller & Loveland, 1974; Church & Gibbon, 1982). Secondly, longer intervals are harder to time than shorter ones (see e.g. Brodbeck et al., 1998). This latter feature, termed scalar variability, arises from increased uncertainty in estimating longer intervals (Malapani & Fairhurst, 2002).

In contrast to frequently-used laboratory paradigms, the harvesting of nectar by hummingbirds foraging in the field provides a natural example of a fixed-interval (FI) schedule of reinforcement in which the animal responds after a fixed period of time has elapsed since the flower was last visited (Bateson, 2003). An animal following the most efficient strategy, namely minimising its number of responses while maximising its intake, should time the fixed interval and respond as soon as the FI has elapsed (Dews, 1970).

Long-tailed hermit hummingbirds *Phaethornis superciliosus* appear able to predict when sucrose is available at a single source (Gill, 1988). Four free-living, marked individuals fed from an artificial feeder with fixed-interval (FI) schedules of either 10 or 15 minutes. When a bird had almost exclusive use of the feeder, returns were most commonly made at intervals exceeding the FI. In contrast, birds responded immediately to real and simulated competition at the feeder by returning after shorter intervals, sometimes before a refill was due.

Hummingbirds provide a good example, then, of animals that need to forage often and that have presumably been under selective pressure to do so efficiently. One way in which birds appear to achieve this is through timing the intervals between visits to flowers.

In this experiment, I tested whether territorial male rufous hummingbirds can time intervals in multiple locations. Hummingbirds in the field were presented with an
array of flowers that were individually refilled either 10 or 20 minutes since the bird's last visit. I predicted that if birds could track multiple refill schedules they would 1) return more frequently to the flowers refilling after 10 minutes; and 2) that the variation in return time to the 20 minute flowers would be higher than for the 10 minute flowers, as longer intervals are more difficult to time.

6.2 Materials and methods

6.2.1 Subjects and study site

The subjects used in this experiment were three wild, experimentally naïve male rufous hummingbirds. The experiment was run from 0800-2000 hours Mountain Standard Time in June and July 2000. The study area is described in chapter 2.

6.2.2 Initial training

It took 1-2 hours to train the bird to feed from artificial flowers containing sucrose placed in his territory. Initial training involved gradually lowering and then removing the bird's feeder, leaving an artificial "flower" available to feed from. This was a small white disc (6 cm diameter), made of stiff cardboard, mounted horizontally on a wooden stake at a height of 60 cm. At the centre of the flower was placed a filled inverted blue syringe tip (capable of holding 120 µl) which was held in place by a small piece of cork mounted on top of the stake. The flowers used in training contained sucrose solution (concentration 20 %). Once the bird had visited this flower, additional white flowers were presented. These were added, one at a time, after consecutive foraging bouts until a total of four was available. Flowers were moved and refilled after each visit so the bird became used to moving around his territory to locate them. Birds readily learned to feed from these flowers after a few bouts (ca. 10–20). When the bird was consistently feeding from and moving between artificial flowers, his feeder was returned to a tree until the experiment began.
At the start of a day's testing (usually around 0830) the bird's feeder was removed and an array of flowers was constructed in the meadow. The experiment then began.

6.2.3 Procedure

An array containing eight flowers was used (Figure 6.1) in experimental sessions. The nearest-neighbour flower distance was 60 cm. All flowers in the array were of the same type used in training (60 cm tall wooden stake with flower mounted on top) but were all different colours (single colours used: neon green, orange, blue, brown, purple, yellow, green, red). The flowers stayed in the same place for the duration of the experiment.

Figure 6.1  Arrangement of flowers in hummingbird timing experiment.

Configuration of the array used in the experiment. Nearest-neighbour flower distance was 60 cm. Flowers were mounted horizontally on wooden sticks (height 60 cm), and were all different colours. Not to scale.

At the start of each experimental session all the flowers were filled with a 20 μl (concentration 20%) sucrose reward. Once the bird had visited the array for the first
time, two different fixed-interval flower reward schedules were used. Four flowers were designated as being 10 minute flowers, the other four as 20 minute flowers. The locations of flowers within the array were determined in a pseudo-random fashion (the only constraint being that no three nearest-neighbour flowers in a row had the same refill schedule). The positions of 10 and 20 minute refill flowers within the array were the same for the duration of the experiment. I refilled each flower 10 or 20 minutes after the bird had emptied them.

No restriction was placed on the number of flowers that the focal male was allowed to visit or on the duration of individual foraging bouts.

Between bouts birds typically remained visible in their territory – usually perched on a nearby high tree where they could remain vigilant for intruders and potential mates. The refilling of flowers took place at the 10 or 20 minute interval, which was usually in the bird’s absence or not in direct view of it. After each bout the flowers visited in that bout were checked (the syringe tips of visited flowers were removed from the stake and shaken to ensure that no sucrose remained). This was done to ensure that the birds were not rewarded if they returned to a flower before its refill schedule. At the end of each session the array was removed and the bird’s feeder replaced. Small markers were placed in the ground to ensure that in subsequent sessions the array was put back in the same place.

A series of continuous observations was performed on each bird, mostly on successive days. On occasions, however, inclement weather interrupted data collection. In these instances data collection was continued as soon as the weather improved - either later on in the same day (beginning as a new session), or the next day.

The time of the bird’s visits to the array, and the flowers visited in each bout, were recorded throughout each session.
6.3 Results

6.3.1 Experimental sessions

Sessions varied in duration [Bird 1: 13 sessions; mean duration 344 ± 41 (minutes ± S.E.), range 155 – 563 minutes; Bird 2: 11 sessions; mean duration 454 ± 29 minutes, range 208 – 584 minutes; Bird 3: 12 sessions; mean duration 419 ± 50 minutes, range 84 – 576 minutes].

6.3.2 Flower visitation rates and reinforcements

I compared the timing of visits made to both 10 and 20-minute flowers. Visits were defined as probes made by the bird to individual flowers within a foraging bout. The intervals between visits to each flower were calculated for each experimental session. Distributions of all “post-reinforcement pauses” (PRPs), the intervals between a bird’s current visit to a flower and its last rewarded visit to that flower, were then constructed (Figures 6.2 and 6.3).

A further measure of only examining the first PRP was also used. This measure, considering only the time to the first probe after reinforcement, took account of the possibility that multiple visits to individual flowers before their refill time could cause a bias in the distribution of pause data. If this were to occur often the recording of one or more additional PRPs closer to the time at which the flower was refilled could lead to a potentially erroneous conclusion that the birds were more accurate at timing flower refill rates than they actually were.

The rate of responding to the 10 minute flowers was expected to be twice that to the 20 minute flowers. All birds visited 10 minute flowers more often than 20 minute flowers [mean number of visits per session ± s.e. to flowers, pooled over all trials, Bird 1: 26.3 ± 2.9 (10 minute flowers), 21.2 ± 2.3 (20 minute flowers); Bird 2: 31.9 ± 2.7 (10 minute flowers), 26.9 ± 2.4 (20 minute flowers); Bird 3: 41.3 ± 5.2 (10 minute flowers), 29 ± 3.8 (20 minute flowers)]. This difference was significant but 10 minute flowers were not visited twice as often as 20 minute flowers (two-sample t-test: \( t_{63} = 2.53, p = 0.014 \); ratio of visits to 10:20 minute flowers 1.32:1).
Longer fixed interval schedules are expected to generate longer post-reinforcement pauses. Modal PRP values were used to determine when the birds returned to flowers. The modal PRP values (all pauses) suggest that the birds returned to flowers coincident with the FI schedule (figures for 10 / 20 minute flowers, pooled across all sessions: Bird 1: 11 / 21 minutes; Bird 2: 10 / 20 minutes; Bird 3: 10 / 20 minutes).

Performance – all postreinforcement pauses

I used one-sample Wilcoxon tests to test whether median PRPs (pooled across sessions for each of the two flower types) were different from, respectively, the expected medians of 10 and 20 minutes. At 10 minute flowers, all of the birds had median PRPs significantly greater than 10 minutes (two-tailed one-sample Wilcoxon tests, \( H_0 \) median = 10: Bird 1: \( n = 1149, p < 0.001 \); Bird 2: \( n = 1224, p < 0.001 \); Bird 3: \( n = 1734, p < 0.001 \); see Figure 6.4 for overall medians). At the 20 minute flowers, one bird was later than 20 minutes (Bird 2: \( n = 1074, p = 0.001 \); Figure 6.4) while the other two birds had PRPs which did not differ from 20 minutes (Bird 1: \( n = 1008, p = 0.51 \); Bird 3: \( n = 1243, p = 0.07 \)) (Figure 6.4).

Performance - first postreinforcement pauses

In terms of median first PRP data, a similar pattern was observed at the 10 minute flowers as with all PRP data. Two birds had median PRPs significantly greater than 10 minutes (two-tailed one-sample Wilcoxon tests, \( H_0 \) median = 10: Bird 1: \( n = 858, p < 0.001 \); Bird 2: \( n = 937, p < 0.001 \)) while the remaining bird had first PRPs not differing from 10 minutes (Bird 3: \( n = 1230, p = 0.475 \)). Using the same measure, all the birds had median PRPs of less than 20 minutes at the 20 minute flowers (two-tailed one-sample Wilcoxon tests, \( H_0 \) median = 20: Bird 1: \( n = 570, p < 0.001 \); Bird 2: \( n = 632, p < 0.001 \); Bird 3: \( n = 673, p < 0.001 \); see Figure 6.4 for median values and Figures 6.5 and 6.6 for the 10 and 20 minute flower first PRP distributions). Despite the pattern of earlier returns to the 20 minute flowers when examining first PRPs, for each bird these were all significantly longer than 10 minutes (two-tailed one-sample Wilcoxon tests, \( H_0 \) median = 10: Bird 1: \( n = 549, p < 0.001 \), Bird 2: \( n = \)
The birds appeared, then, to have learnt a different temporal reward schedule.

Figure 6.2 Distribution of all PRPs at 10 minute flowers.

Post-reinforcement pause (PRP) data (in minutes) for each of the subjects at 10 minute flowers.

Bird One

Bird Two

Bird Three

Post-reinforcement pause (minutes)
Figure 6.3  Distribution of all PRPs at 20 minute flowers.

Post-reinforcement pause (PRP) data (in minutes) for each of the subjects at 20 minute flowers.
Figure 6.4  Median post-reinforcement pauses at both flower types.

The bars show the overall medians (all and first post-reinforcement pauses) for each of the subjects at the 10 minute and 20 minute flowers.
Figure 6.5  Distribution of first PRPs at 10 minute flowers.

First post-reinforcement pause (PRP) data (in minutes) for each of the subjects at 10 minute flowers.
Figure 6.6  Distribution of first PRPs at 20 minute flowers.

First post-reinforcement pause (PRP) data (in minutes) for each of the subjects at 20 minute flowers.
6.3.3 The effect of learning

To examine how consistently birds were returning to the flowers, I looked at PRP data across sessions in the experiment. These data are shown in Figures 6.7 (all PRPs) and 6.8 (first PRPs). The following discussion refers to all PRPs to reflect all the flower visits made by the birds (as shown in Figure 6.8 the main effect of examining only first PRPs was to lower the 20 minute flower values).

With no exceptions the median PRPs at the 10 minute flowers always matched, or exceeded, 10 minutes. Returns made to the 10 minute flowers, then, were consistently rewarded. In contrast, all three birds made some premature returns to the 20 minute flowers (Figure 6.7). Taken together, these results suggest that the birds treated the 10 and 20 minute flowers differently. The birds did not simply wait for 20 minutes or more (when all the flowers would be full) before returning to 10 minute flowers. The intervals between actual visits show that the birds did not simply come to the array every ten minutes (mean intervisit interval ± s.e: Bird 1: 15.02 ± 0.2 minutes; Bird 2: 15.49 ± 0.2 minutes; Bird 3: 11.79 ± 0.12 minutes).

Performance at the 20 minute flowers was poorer as occasionally at one or more flowers within sessions birds would visit too soon. Overall, however, the birds were rewarded more often than not even at the 20 minute flowers (there was a range of 22.7–39.6% in premature visits to 20 minute flowers across the three subjects at 20 minute flowers). Non-parametric two-tailed sign tests showed that one bird (Bird 2) visited significantly later than 20 minutes (n = 11, p = 0.03). The effects for the two other subjects were not significant (Bird 1: n = 13; Bird 3: n = 12, both NS). However, the number of sessions where returns were made later than the FI either equalled (Bird 1) or exceeded (Bird 3) the number of sessions where returns were made sooner than 20 minutes.

There were small differences in the rate of learning between birds and flower types. At 10 minute flowers overall the median PRP was never less than 10 minutes from the first session onwards for any of the subjects. In contrast two birds (Birds 1 and 3) tended to visit 20 minute flowers a little early over sessions 1 and 2 (overall
median PRPs of 18/19 minutes and 16/18 minutes, respectively), thereafter visits were made on or after 20 minutes on the vast majority of occasions.
Figure 6.7 Learning across the duration of the experiment (all PRPs).

The graphs show all PRP data for each bird across the experiment at both flower types. Each point represents the mean of the median PRPs for each of the four flowers of each type (filled symbols: 10 minute flowers, open symbols: 20 minute flowers). The solid horizontal line denotes 10 minutes, and the hatched horizontal line denotes 20 minutes.
Figure 6.8  Learning across the duration of the experiment (first PRPs).

The graphs show first PRP data for each bird across the experiment at both flower types. Each point represents the mean of the median first PRPs for each of the four flowers of each type (filled symbols: 10 minute flowers, open symbols: 20 minute flowers). The solid horizontal line denotes 10 minutes, and the hatched horizontal line denotes 20 minutes.
6.4 Discussion

In this study, rufous hummingbirds were presented with flowers refilled after one of two intervals. Birds tended to return to flowers soon after they had been refilled. Hummingbirds thus appeared able to recall when they last visited flowers and then visit those flowers likely to be full the next time a visit was made.

The predictable renewal of nectar over time means that a foraging hummingbird may either return to a flower too soon, resulting in no reward, or after longer periods. Late returns result in the flower being full, but increase the risk that other birds (and some insects such as Hymenopteran pollinators) may steal the nectar. A bird that can learn when rewards are likely to be available should enjoy increased foraging success over a bird that cannot.

These hummingbirds also appear able to track two different refill schedules at multiple locations. This was the case when considering all postreinforcement pause data and only the first postreinforcement pauses. Comparing these two measures, little difference was apparent at 10 minute flowers, whereas a leftward shift in the distribution occurred at the 20 minute flowers relative to all post reinforcement pauses. Despite more premature visits to 20 minute flowers, the pauses were still different to (and greater than) 10 minutes. The birds did not seem, then, to treat both flower types identically. Similarly, the birds did not simply wait for long periods between consecutive foraging bouts as they visited a mixture of 10 and 20 minute flowers on each bout. By remembering when particular flowers were last visited, they were more likely to return to visit full flowers on subsequent bouts. In Gill’s (1988) study on long-tailed hermit hummingbirds, birds able to largely monopolise a feeder similarly adjusted their visits to the fixed-interval schedule at that feeder. On other occasions the flowers were emptied by competing conspecifics. In contrast, I monitored the movements of known territorial male hummingbirds, and other hummingbirds were excluded by the resident. I was able, then, to look at the consequences of individuals’ choices of flowers over continuous sessions of several hours duration. However, it is clear that the hummingbirds in this study learned the
timing characteristics of the flowers within the first few days. The 10 minute flowers seemed to have been learned by the end of the first day (see Figure 6.7).

A prediction from timing theory is that longer intervals should be harder to time than shorter intervals. The scalar property of timing states that the standard deviation of timing an interval is proportional to the magnitude of that interval (see e.g. Church & Gibbon, 1982). Greater variability in subjects' responses is expected, then, when longer intervals are being timed. The hummingbirds in this study did make more premature visits to 20 minute flowers than 10 minute flowers, yet they were still more often rewarded than not at the longer FI. Single-interval timing tasks, as commonly used in operant laboratory paradigms, allow direct comparisons to be made of animals' abilities to time intervals of various lengths. Black-capped chickadees Parus atricapillus, for example, pecked with greater variability when timing intervals of 37.5s than when timing 12.5s (Brodbeck et al., 1998). In the current experiment the hummingbirds were presented with a simultaneous timing task with two FI schedules instead of a single interval tested over hundreds of discrete trials as in Brodbeck et al's study. In any one bout, the birds in this study could choose from a mixture of full and empty flowers. They were able to track the schedules of refill at these flowers throughout the experiment.

Timing theory states that animals are expected to increase their rate of responding where (and when) the frequency of reinforcement is greatest (see e.g. Rider, 1987). Although these hummingbirds did not visit 10 minute flowers twice as often as 20 minute flowers, visits were made to 10 minute flowers significantly more often than to 20 minute flowers. Matching is most commonly observed in experiments performed in the laboratory using intervals in the seconds range, and occasionally a few minutes (Davison & McCarthy, 1988). Under highly controlled conditions, however, postreinforcement pause times are not necessarily proportional to FI duration even when subjects otherwise show evidence of timing (Talton et al., 1999). The ways in which animals respond to concurrent choice situations (where two or more options are simultaneously available, as in this experiment) has not been investigated to the same extent as single intervals. Usually only two options are
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used, and with short schedules in the seconds range (see e.g. Baum, 1974; Grace & Nevin, 1999). An early experiment on rats led to the formulation of the now dominant paradigm to explain the patterns of responding observed under concurrent choice reinforcement schedules. Herrnstein (1961) found that when rats were presented with variable interval schedules of reinforcement, they allocated their behaviour such that the percentage of responses made to a key during a session matched the percentage of food rewards obtained from that key. This became known as the matching law. Deviations from strict matching are common, however, in studies of animals' choice behaviour (Bacotti, 1977; Davison & McCarthy, 1988).

The hummingbirds in this study did not always return to both flower types immediately after the FI. Overall, however, returns were made most frequently after the FI. In the laboratory, studies using concurrent schedules often incorporate a changeover delay. This imposes a short delay period between a response at any one of the options and the next available reinforcement (see e.g. Pliskoff & Brown, 1976; Temple et al., 1995). This may mean, for example, that a subject has to respond twice at a key before a reward can be received. The inclusion of a changeover delay potentially imposes a cost (e.g. in time and energy) on switching from one alternative to the other and can result in subjects' responses matching more closely the schedules in use (Davison & McCarthy, 1988). In rats, increasing the cost of responding (by for example adding weights to food cup covers) and of making errors (by imposing a time-out period between trials) increases the likelihood of subjects making appropriate time-place discriminations (Widman et al., 2000). In this study, no limits were placed on where and when the hummingbirds could feed from the flowers. At multiple locations, the birds were able to track the availability of rewards. Pigeons under laboratory conditions show evidence of matching when presented with five schedules of reinforcement ranging in duration from 1-5 minutes (Miller & Loveland, 1974). A challenge in future studies of timing in hummingbirds will be to present individuals with more schedules to assess their ability to time visits to flowers appropriately. Despite rudimentary knowledge of nectar production rates in many plants, ultimately it might be possible to correlate the timing of visits to flowers with known refill rates of natural flowers (see e.g. Klinkhamer et al., 2001).
Timing abilities ought to be influenced by factors such as animal's ecological niche and other features of the environment in which it routinely makes decisions. Although recognised by psychologists (see e.g. Killeen, 1975; Zeiler, 1991) and ethologists (Krebs & Kacelnik, 1984) alike, there are still few examples of timing studied under naturalistic conditions. In this study, rufous hummingbirds fed regularly from artificial flowers, and did so throughout continuous days of testing. I was able to show that hummingbirds make use of temporal cues when foraging. Further studies to investigate animals' use of time in naturalistic contexts, and for a number of different behaviours, will perhaps emerge in the future.
Chapter 7: Can parids time short intervals?

7.1 Introduction

Some species, including tits and corvids, store food items (e.g. seeds) in scattered locations. In winter, perhaps weeks or months after initially storing the food, individuals return to their caches to retrieve items (see e.g. Vander Wall & Balda, 1977; Shettleworth, 1990). Successful cache recovery depends not only on the animal remembering what type of food was cached, where, but also when caching took place, depending on the cache’s perishability (Olton, 1985). Limited data exist, however, on birds’ timing abilities and how these may relate to behaviours such as food-storing.

In chapter 6, I described an experiment in which rufous hummingbirds Selasphorus rufus appeared to learn appropriate delays before returning to refilling flowers. The aim of the current experiment was to carry out a pilot experiment to assess whether a coal tit Parus ater, a food storer, might be able to time short intervals. Black-capped chickadees Parus atricapillus, tested with a single interval peak procedure paradigm in the laboratory, can time intervals of 12.5 and 37.5 seconds (Brodbeck et al., 1998). In order to determine whether a European congener, the coal tit, might also time intervals, in this pilot study I tested one bird on a simultaneous timing task involving the presentation of two identical stimuli. These were associated with FI schedules of reinforcement of either 30 or 60 seconds. Intervals in the 30-60s range are common in timing experiments (see e.g. Zeiler & Powell, 1994; Staddon, 1969). I predicted that if the coal tit in this experiment was timing the intervals in this range, it would delay pecking each stimulus until the appropriate time. Furthermore if the bird were to match its responses to availability of reward, I expected it to visit the 30s stimulus around twice as often as the 60s stimulus (Herrnstein, 1961).
7.2 Experiment One: Can a coal tit time 30s and 60s?

7.2.1 Methods

Subject

As a first step to examine the possibility that coal tits may be able to time, I tested one bird on a timing task. The coal tit was kept and tested in a wire mesh cage (dimensions: 77 x 44 cm, 44 cm high) containing wooden perches (Figure 7.1). Other conspecifics were kept in the same room, in both visual and vocal contact. Water was provided *ad libitum* and a food bowl containing Orlux (a commercially available insectivorous food mix), a vitamin supplement, small pieces of peanut and a wax-moth larva was provided daily following testing. The room was maintained on a light/dark cycle of 13.5:10.5h and under controlled conditions (14-16°C).

Figure 7.1 Laboratory touch screen set-up.

A touch screen was placed at the front of the bird’s cage. Small peanut rewards were dispensed through a plastic tube into the cage. The cage dimensions were 77 x 44 x 44cm. In this picture the bird shown is a great tit, and not a coal tit as used in this particular experiment.
Ethical notes

The bird used in this experiment was caught in the wild under permit in April 2000 from deciduous woodland on the outskirts of Edinburgh. Following veterinary approval, the bird used in this experiment (and those used in later experiments) was released in the summer of 2002.

Apparatus

Images were presented on a computer screen immediately behind a touch screen (touch screen dimensions: 27.5 x 20 cm), to which the bird could direct pecks. The equipment, resembling a standard computer monitor in shape and appearance, was mounted on a trolley and moved in front of the bird’s cage at the time of testing (Figure 7.1). The computer ran a program coded in BASIC, and this controlled the presentation of stimuli on the touch screen and saved data directly to disc.

On the front of the bird’s cage a sliding wire mesh door could be removed. Rewards were small pieces of peanut (ca. 3mm squares) and these were delivered one at a time from a pellet dispenser mounted on the front of the cage, via a plastic tube, to a small horizontal tray within the bird’s cage. The position of this tray (and the food dispenser) was to the left of the monitor in this experiment. Three horizontal perches situated on the front wall of the bird’s cage (at heights of 11, 19 and 29 cm) allowed the bird to reach the images presented on the screen.

Prior training

The bird used in this experiment had been tested previously in a spatial memory experiment for which it had been trained to peck at stimuli on the screen so no initial training was required. It was naïve with respect to timing tasks.

Experimental protocol

The bird was presented with a touch screen on which two stimuli (white squares: 2.5 cm²) were presented simultaneously (Figure 7.2). The first peck to each square was rewarded. Thereafter, there was a different FI schedule for each square. One square
had a reward schedule of 30s, and the other a 60s schedule. Pecks to either square on or after the appropriate time had elapsed were rewarded. The bird had to move away from the touch screen to collect its food reward. It did this immediately, usually flying to a perch towards the back of the cage to eat the small piece of peanut. The stimuli remained visible on the screen after every peck and occupied the same positions on the screen between sessions. Each stimulus location was associated with the same reward schedule throughout the experiment.

**Figure 7.2** Arrangement of stimuli on touch screen.

This schematic diagram illustrates the touch screen arrangement. Stimuli (white, 2.5 cm²) were presented on a dark background. The stimuli occupied the same positions in each session. Each was associated with a different reward schedule: left = 30s, right = 60s. Diagram not to scale.
Two hours before testing the bird's food bowl was removed. The bird was given one hour-long session per day, beginning around 1400, for a total of 23 days. Occasional checks were made to maintain a supply of peanut rewards in the feeder; disturbance was, however, kept to a minimum throughout testing. The bird was given fresh food at the end of each session.

Data collected

The data analysed were the times at which stimuli were pecked, as this permitted an examination of post-reinforcement pause (PRP) data. PRPs are the interval of time from the current visit to a stimulus and the last rewarded visit to that stimulus, and are a reliable measure of whether animals tend to return to stimuli on or around the times at which reward is available (Dukich & Lee, 1973; Hanson & Killeen, 1981). For a subset of the data I also used a further measure of examining only first PRPs (the interval between the current reinforced peck and the next peck, see also chapter 6). The first PRP measure ignores any additional (unrewarded) pecks before the next rewarded peck.

7.2.2 Results

Activity data

The coal tit pecked at both of the stimuli in every session (Figure 7.3). The number of pecks in any one session was highly variable (30s stimulus: range 8-149 pecks/session, 60s stimulus: range 4-82 pecks/session). Across the experiment, more pecks were directed to the 30s stimulus than to the 60s stimulus except in sessions 1 and 16 (30s: mean of 47.8 ± 7.8 pecks/session; 60s: 28.8 ± 4.7 pecks/session). The 30s stimulus was not however pecked twice as often as the 60s stimulus, rather the ratio of pecks to the 30s:60s stimuli was 1.51:1.

Performance data

Postreinforcement pause (PRP) data were use to determine whether the bird matched its peck rate to the FIs. I combined all PRP data across sessions for both the 30s and
the 60s stimuli. The distribution of all PRPs was not normal for either stimulus (Anderson-Darling tests for normality: $p < 0.001$ for both stimuli) and as attempts to normalise the distributions through transformation (e.g. $\log_{10}$) were unsuccessful, I used nonparametric tests. The PRP distributions for both stimuli were different from each other (Mann-Whitney test: $W = 885755.5, p < 0.0001$) and were more closely matched to the FI at the 30s stimulus than the 60s stimulus.

The median PRP values were 33s and 52s respectively for the 30s and 60s stimuli (Figure 7.4). The bird tended to visit the 30s stimulus shortly after the appropriate time but appeared to visit the 60s stimulus a little early. One-sample Wilcoxon tests (two-tailed) were used to compare the observed PRP values (data pooled from all sessions) to the expected medians of 30s and 60s. At the 30s stimulus, the median PRPs were significantly greater than 30s ($n = 1075, W = 389515, p < 0.001$). The PRPs at the 60s stimulus were not significantly different to 60s ($n = 702, W = 123398.5, p = 0.997$; see Figure 7.4).

I also analysed first PRP data from the last day of testing. The last day was chosen as by this point the bird was most accustomed to performing the timing task. Visual inspection of the bird’s pecking activity confirmed that by this point the bird was pecking consistently throughout the hour of testing, in contrast to earlier sessions when, on a few occasions, fewer pecks were made.

Comparing the distributions of all and first PRPs at both stimuli on the last day of testing revealed no significant differences (Mann-Whitney tests: 30s stimulus: $W = 1185.5, p = 0.97$; 60s stimulus: $W = 578.6, p = 0.66$). At the 30s stimulus, examining all and first PRPs revealed that in both instances the pauses were significantly different to, and longer than, 30s (two-tailed one-sample Wilcoxon tests, both $p < 0.001$). The same pattern was evident at the 60s stimulus, where both all and first PRPs were significantly different to, and greater than, 60s (two-tailed one-sample Wilcoxon tests, both $p < 0.015$). The actual median first PRPs were 64s and 97s respectively at the 30s and 60 stimuli on the last day (and 64s and 86s for the corresponding all PRPs).
Figure 7.3  Activity across the experiment.

The graphs show activity data (pecks) for the 30s stimulus (top) and the 60s stimulus (bottom) across the experiment.
**Figure 7.4** Postreinforcement pauses across the experiment.

The median postreinforcement pauses (all PRPs) across the experiment, in seconds, for the 30s stimulus and the 60s stimulus.

![Graph showing median PRPs](image)

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**Effect of learning across the experiment**

As the bird appeared to have learned the two intervals, I wanted to determine when this occurred. I therefore split the experiment into three approximately equal blocks of days: 1-8, 9-16 and 17-23. The median PRPs (all pauses) to the 30s stimulus in the first and last blocks were not different to 30s (two-tailed nonparametric sign tests: sessions 1-8: \( n = 423, p = 0.433 \); sessions 17-23: \( n = 382, p = 0.073 \)). The bird tended to return a little later in the middle of the experiment (sessions 9-16: \( n = 270, p = 0.001 \); Figure 7.5). Pecks to the 60s stimulus were often made before 60s (sessions 1-8: \( n = 326, p < 0.001 \); Figure 7.5) at the start of the experiment. In the later blocks, the timing of the pecks was not significantly different from the FI...
The graphs show PRP data (all pauses) for each block at both stimuli (top: 30s stimulus, bottom: 60s stimulus). At the 30s stimulus, the bird consistently returned after 30s. At the 60s stimulus early returns were more frequent.
Spatial and temporal cognition in birds

(sessions 9-16: n = 166, p = 0.816; sessions 17-23: n = 210, p = 0.189). This suggests that the bird found it more difficult to learn the 60s FI than the 30s FI.

7.2.3 Discussion

This pilot study was designed to test whether a coal tit might be able to time short intervals. The bird was presented with two stimuli offering reward after fixed intervals (FIs) of 30s and 60s. The bird was matching its pecking to the 30s stimulus by the end of the first block of testing and this continued for the remainder of testing (Figure 7.5). In the last two blocks at the 30s stimulus the bird was again returning after the FI, and hence was consistently being rewarded. Responding at the 60s stimulus was less accurate, with pecks before the FI being more frequent. During the second block, however, pecks to the 60s were not significantly different to the FI. An analysis of first versus all PRP data from the last day of testing revealed no significant differences (30s stimulus: p = 0.97, 60s stimulus: p = 0.66), and at both stimuli the bird returned after the time of scheduled reward. These results provide preliminary evidence that a coal tit can learn short intervals that are longer than those black-capped chickadees P. atricapillus, another storing species, are known to be able to time (Brodbeck et al., 1998).

It was expected that the bird might match visits to the two stimuli such that twice as many pecks would be directed to the 30s stimulus than the 60s stimulus (see e.g. Baum, 1974). Although this ratio was not observed, more pecks were made to the 30s than the 60s stimulus. This is at least in accordance with the expectation that animals ought to show increased rates of responding at shorter FIs than longer FIs (Zeiler, 1977).

It is also possible, however, that the bird preferred returning to the 30s stimulus due to some form of spatial bias. Data from more subjects tested with the two intervals in different locations are needed to exclude this possibility.
7.3 Experiment Two: Can coal tits time three intervals?

7.3.1 Introduction

The aim of this experiment was to investigate whether coal tits might be able to track the availability of food at three locations, each associated with different FI reward schedules. In the first experiment, one coal tit tracked the availability of rewards at two stimuli, associated with FI schedules of 30s and 60s. In this experiment, I added a third stimulus with an FI of 120s. Presenting animals with concurrent schedules has, traditionally, been limited to two alternatives (see e.g. Davison & McCarthy, 1988; Shull, 1992). In natural circumstances animals tend however to visit multiple patches throughout the day, and this has been likened to a “many-component” schedule analogous to depletion and renewal at different food patches throughout the day (see e.g. Mellgren & Brown, 1988). The rationale for choosing a longer interval was that if birds use temporal cues when recovering caches in the wild, they would have to remember longer intervals of time (although in the context of the periods birds would have to remember in the wild the schedules used in these experiments are of course much shorter). Pigeons *Columba livia* can time 120s (Zeiler & Powell, 1994) and, when presented with multiple intervals, track the availability of rewards in different places (Miller & Loveland, 1974). I predicted, then, that coal tits would be able to time intervals of 120s while simultaneously tracking the availability of rewards in three places.

7.3.2 Methods

Subjects

Six coal tits were tested. All of these birds had previously taken part in touch screen experiments and hence did not require to be trained to peck images. They were, however, naïve with respect to timing tasks. The laboratory housing, conditions and the apparatus used for testing were the same as in the first experiment.

Three subjects were tested beginning at around 1100 in the morning, a minimum of two hours after the removal of their food bowl. The other three birds were tested
after the first three birds' sessions were completed (around 1300). The birds in the second group were given a small piece of pine nut at around 1000. Subjects were given one hour-long session per day, for 18 days, 5 days a week. Disturbance during testing was kept to a minimum, with feeders being replenished around every fifteen minutes throughout testing. All birds were provided with fresh food as soon as their session was finished.

Experimental protocol

At the start of each session, subjects were presented with a touch screen slotted onto the front of their cage. The set-up was similar to that used in the first experiment; here, however, there were three stimuli available for the bird to peck (Figure 7.6). The initial peck to each of the three stimuli was rewarded. Thereafter, each of the three squares was associated with a FT reward schedule of either 30s, 60s or 120s. The stimuli occupied the same positions on the touch screen between sessions, and each was associated with the same FI throughout the experiment for each subject.

Data collected

As in the first experiment, I analysed postreinforcement pause data. Two measures were used – all and first postreinforcement pauses.
Figure 7.6 Arrangement of stimuli on touch screen.

The schematic diagram illustrates the touch screen arrangement. Stimuli (white, 2.5cm²) were presented on a dark grey background. The stimuli occupied the same positions in each session, and each was associated with a reward schedule of 60s (left), 120s (middle) and 30s (right). The position of the feeder (to the left or right of the screen) was counterbalanced across birds. Diagram not to scale.

7.3.3 Results

Activity data

Every stimulus was pecked to at least some degree by all of the birds throughout the experiment (Figure 7.7). The 30s stimulus was always visited by each bird in every session; there were however some sessions in which the 60s and 120s stimuli were
not pecked. Every bird pecked more at the 30s stimulus than the 60s stimulus (range 2.1 – 30 times more pecks to 30s stimulus than to the 60s stimulus). Five of the six subjects pecked the 120s stimulus more often than the 60s stimulus (up to 5.43 times more often to the 120s stimulus). Overall, then, the birds favoured returns to the 30s stimulus.

**Performance data**

To assess performance I first examined all postreinforcement pause (PRP) data, pooling all subjects’ data at each stimulus across all sessions. None of the distributions (Figure 7.8) were normal (Anderson-Darling tests for normality: all p < 0.0001). The data were not normalised through transformation (e.g. log_{10}). I therefore calculated median PRP values for each of the stimuli. One-sample Wilcoxon tests (two-tailed) were used to compare the PRP values to the expected medians of 30s, 60s and 120s. The PRPs at each stimulus were all significantly different to the FIs (30s stimulus: n = 3114, W = 3350000, p < 0.0001; 60s stimulus: n = 715; W = 192719.5, p < 0.0001; 120s stimulus; n = 689, W = 145064.5, p < 0.001). The overall median PRP values for the 30s, 60s and 120s stimuli were 35s, 90s and 117s respectively (Figure 7.9).

Postreinforcement pause data (all pauses) for each bird were also compared to the expected medians of 30s, 60s and 120s; the results can be seen in Table 7.1. The PRP data for each subject at each stimulus (displayed as medians) are shown in Figure 7.10. In most instances the birds did not have PRPs that closely matched the FI schedules (Table 7.1) as the birds usually pecked later than the FI. Representative PRP distributions for three birds can be seen in Figure 7.11; the other birds showed similar patterns of responding.
Figure 7.7  Activity across the three-stimulus experiment.

The graphs show the mean number of pecks per session (± s.e.) for all six birds at each stimulus (FIs of 30s, 60s, 120s).
Figure 7.8  Postreinforcement pause distributions in three stimulus experiment.

The histograms show the pooled PRP distributions (all PRPs) at the 30s, 60s and 120s stimuli. The vertical lines correspond to the trained FIs. The x axis was truncated at 300s for clarity. Individual time bins were 5s.
Figure 7.9  Postreinforcement pauses across the three stimulus experiment.

Columns show the pooled median PRPs (all pauses, in seconds) at each of the stimuli in the experiment.

![Figure 7.9](image)

Figure 7.10  Individual postreinforcement pause data at the three stimuli.

The bars show the median PRPs (all pauses) for each subject at the 30, 60 and 120s stimuli.

![Figure 7.10](image)
Table 7.1 Results of one-sample Wilcoxon tests for each bird at each stimulus. The p values in bold indicate median PRP values not significantly different to the Fl. Refer to Figure 7.10 for the median values.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Stimulus</th>
<th>n of PRPs</th>
<th>W statistic</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT 97</td>
<td>30s</td>
<td>368</td>
<td>56575</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>60s</td>
<td>30</td>
<td>305</td>
<td>$p = 0.139$</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td>193</td>
<td>11441.5</td>
<td>$p &lt; 0.005$</td>
</tr>
<tr>
<td>CT 99</td>
<td>30s</td>
<td>367</td>
<td>45836.5</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>60s</td>
<td>111</td>
<td>3415.5</td>
<td>$p = 0.366$</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td>140</td>
<td>4897.5</td>
<td>$p = 0.939$</td>
</tr>
<tr>
<td>CT 108</td>
<td>30s</td>
<td>408</td>
<td>74060.5</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>60s</td>
<td>182</td>
<td>14950</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td>183</td>
<td>11652.5</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td>CT 109</td>
<td>30s</td>
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<td>74623</td>
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</tr>
<tr>
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<td>60s</td>
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<td>9749.5</td>
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</tr>
<tr>
<td></td>
<td>120s</td>
<td>72</td>
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</tr>
<tr>
<td>CT 119</td>
<td>30s</td>
<td>938</td>
<td>197311</td>
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</tr>
<tr>
<td></td>
<td>60s</td>
<td>223</td>
<td>15946.5</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td>74</td>
<td>1598.5</td>
<td>$p = 0.25$</td>
</tr>
<tr>
<td>CT 120</td>
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<td>618</td>
<td>117600</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>60s</td>
<td>17</td>
<td>76</td>
<td>$p = 0.698$</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td>27</td>
<td>181</td>
<td>$p = 0.857$</td>
</tr>
</tbody>
</table>
As in the previous experiment, I also analysed performances using only first PRP data. Repeated (unrewarded) visits between scheduled rewards either did not occur at all (CTs 108 and 109 at the 120s stimulus) or were rare. However, as they did occur on occasion, it is possible that the analyses with all the PRP data provide a less accurate picture of the birds' timing ability than do the first PRP data.

Due to high variability in the number of pecks made by each subject, with occasions of either no or very few (2-3) pecks being directed towards one or more of the three stimuli within a session, it was not possible to examine only the last day's data (as in the previous experiment). However, for each subject I was able to compare the first and all PRP measures for the last day of testing in which all three stimuli were pecked to an appreciable extent (at least 5 pecks). These data are shown in Table 7.2. This analysis revealed no significant differences between the two PRP measures, and there was only one example of a performance difference among all combinations of stimuli and birds tested (Table 7.2). There is no evidence, then, that occasional extra pecks had a marked effect on birds' pause data.

Effect of learning across the experiment

In order to assess whether timing became more accurate as the experiment progressed, I split the experiment into six three-day blocks. Performance (all PRPs) was most consistent at the 30s stimulus. As shown in Table 7.3, in 22 of the 25 occasions (88%) at the 30s stimulus when the median PRP was different to the FI, the birds pecked after the FI. At the 60s stimulus the comparable figure was 90%, whereas at the 120s stimulus birds always returned before the FI.
Table 7.2 The table shows a comparison of first and all PRP data for each bird on the last day ("day") on which each stimulus was pecked at least 5 times. Only one bird (CT108) pecked enough on the last day of the experiment (day 18) for actual last day data to be used. The median PRPs (in seconds) are shown for the measures of all and first PRPs. The first and all PRP distributions were compared using nonparametric Mann-Whitney tests; “N” indicates that the distributions were not statistically different to each other; whereas “n/a” denotes that first and all PRP measures were identical (i.e. the bird made no additional unrewarded visits to the stimulus). In the final “PRP analysis” column I compared the two PRP measures to the expected medians of 30, 60 and 120s respectively using one-sample Wilcoxon tests. Only in one instance (CT97 at the 30s stimulus) was there a significant difference in the performance measure; using the all PRP measure the bird returned after an interval that was not significantly different to 30s ($W = 138$, $p = 0.08$, 32s) whereas using the first PRP data only the bird returned significantly later than 30s ($W = 65$, $p < 0.001$, 65s).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Day</th>
<th>Stimulus</th>
<th>first PRPs median (s)</th>
<th>all PRPs median (s)</th>
<th>Distributions different (Y/N)?</th>
<th>PRP analysis</th>
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<td>30s</td>
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<td></td>
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<td>56</td>
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<tr>
<td></td>
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<td>15</td>
<td>34</td>
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<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120s</td>
<td>395</td>
<td>181</td>
<td>N</td>
<td>x</td>
</tr>
<tr>
<td>CT108</td>
<td>18</td>
<td>30s</td>
<td>76</td>
<td>63</td>
<td>N</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60s</td>
<td>85</td>
<td>81</td>
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<td>x</td>
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<tr>
<td></td>
<td></td>
<td>120s</td>
<td>481</td>
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<tr>
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<td>17</td>
<td>30s</td>
<td>121</td>
<td>117</td>
<td>N</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60s</td>
<td>149</td>
<td>96</td>
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<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120s</td>
<td>221</td>
<td>221</td>
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<td>30s</td>
<td>34</td>
<td>26</td>
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<td>x</td>
</tr>
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<td></td>
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<td>20</td>
<td>410</td>
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</table>
Figure 7.11  Distribution of all postreinforcement pauses for three birds.

The histograms show PRP distributions for three birds (CTs 97 (top), 99 (middle) and 108 (bottom)) in the experiment. The left hand column shows data for the 30s stimulus, the middle column for the 60s stimulus and the right hand column the 120s stimulus. The data are truncated at 300s for clarity of presentation. Individual time bins are 5s, and the graphs are all drawn using the same scale. The vertical lines denote the FI.
Table 7.3
Sign tests were used to assess timing in blocks across the experiment. The bird could return to a stimulus before or after the trained FI. Only visits made after the FI were rewarded. Non-significant (NS) values indicate that the median PRP was not statistically different to the FI. Blank areas indicate a lack of responding in that block.

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<th>Bird</th>
<th>30s stimulus</th>
<th></th>
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<td>Block 2</td>
<td>Block 3</td>
<td>Block 4</td>
<td>Block 5</td>
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<tr>
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<td>p &lt; 0.05 after</td>
<td>p &lt; 0.05 after</td>
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<td>p &lt; 0.05 after</td>
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<td>NS</td>
<td>NS</td>
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<tr>
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<td>p &lt; 0.05 after</td>
<td>p &lt; 0.05 after</td>
<td>p &lt; 0.05 after</td>
<td>p &lt; 0.05 after</td>
<td>p &lt; 0.05 after</td>
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<td>NS</td>
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<td>CT 99</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
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</tr>
<tr>
<td>CT 108</td>
<td>NS</td>
<td>p &lt; 0.05 after</td>
<td>p &lt; 0.05 after</td>
<td>p &lt; 0.05 after</td>
<td>p &lt; 0.05 after</td>
<td>p &lt; 0.05 before</td>
</tr>
<tr>
<td>CT 109</td>
<td>p &lt; 0.05 after</td>
<td>p &lt; 0.05 after</td>
<td>p &lt; 0.05 after</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CT 119</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td>NS</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>CT 108</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CT 119</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CT 120</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
7.3.4 Discussion

In this study, coal tits appeared able to track three simultaneous FI schedules of 30, 60 or 120s.

The birds in this experiment (as did the bird in Experiment 1) pecked at the 30s stimulus most often corresponding to the expectation that an animal should respond most often to the shortest FI (Dews, 1970). However, five of the six birds pecked the 120s stimulus more often than the 60s stimulus. The birds appeared to have learned the three FIs, as assessed by examining all PRPs. Where the median PRP was significantly different to the FI, it was always longer than the FI. An analysis of all PRPs versus first PRPs from the last day on which subjects pecked all three stimuli did not reveal any consistent differences between the two measures. There was only one instance in which the two measures produced a different result. This was in the predicted direction (the bird tended to peck later than the FI, and not sooner).

Weber’s Law predicts estimation of time should be proportional to the magnitude of the FI so animals ought to find longer intervals harder to time than shorter intervals. This has often been found in timing studies (see e.g. Zeiler & Powell, 1994; Machado, 1997; Malapani & Fairhurst, 2002). In this experiment, the variation in response times was greater at the 60s and 120s stimuli than at the 30s stimulus (Figure 7.8). Despite greater variability in responding at the 60s and 120s stimuli, however, returns tended to be made after the FI. In a peak procedure task carried out in the laboratory, black-capped chickadees *Parus atricapillus* showed greater variability in timing a longer interval than a shorter one. The spread of responses when birds were timing 37.5s was greater than when timing 12.5s, although did not correspond precisely to Weber’s Law (Brodbeck et al., 1998).

It appears, then, that coal tits at least are able to time intervals in the 30s – 120s range. In the next experiment, I wished to extend these findings and to compare timing abilities in two closely related species.
7.4 Experiment Three: Timing in coal tits and great tits

7.4.1 Introduction

Food-storing birds have frequently been used in laboratory tests of learning and memory, often in comparison with nonstorers in order to determine whether storing is correlated with better spatial memory (e.g. Brodbeck, 1994; Clayton & Dickinson, 1998; McGregor & Healy, 1999; Biegler et al., 2001). Caching and subsequent retrieval of food items by storing species is assumed to place demands on spatial memory greater than those faced by nonstorers.

The avian hippocampal formation - situated in the dorsomedial area of the forebrain - is known to be important for spatial memory and is relatively larger in food storing birds than in nonstorers (Krebs et al., 1989; Bingman et al., 1995). This can be correlated with observed behaviours. In a delayed non-matching to sample (DNMTS) task in the laboratory, coal tits remembered the location of a rewarded site for longer periods (up to 20s) than did nonstoring great tits P. major (Biegler et al., 2001). The storing coal tits, then, enjoyed an advantage over related nonstorers in a way that might confer an advantage under natural conditions where food items are stored for longer periods of time. In addition to spatial tasks, however, the hippocampus is also implicated in animals’ abilities to time (Bingman, 1992).

Lesion studies in rats have shown that a damaged hippocampal system impairs not only spatial working memory, but also their ability to time. In a peak procedure task, rats with fimbria-fornix lesions consistently responded sooner than the scheduled reinforcement (20s) in unreinforced trials than did control rats (Meck et al., 1984). Similarly, rats with lesions to the hippocampus and the amygdala made fewer appropriate choices in trained temporal discriminations. Instead of responding at the trained interval, they consistently responded too early (Olton et al., 1987). Similar effects have been observed in pigeons (Spetch & Wilkie, 1983). Food-storing birds, then, may differ in their ability to make temporal discriminations compared to nonstorers.
In this study, I investigated the use of temporal information by great tits (non-storers) and coal tits (storers). I predicted that coal tits would outperform great tits on a timing task due to the likelihood that, in order for accurate cache recovery to take place, a storing species would rely more on temporal cues than a nonstoring species.

7.4.2 Methods

Subjects

Six coal tits and six great tits were used as subjects. All birds were naïve to timing tasks (although had previously been trained to peck at a touch screen) and were housed and maintained under conditions described in the previous experiments. Due to other experiments taking place in the laboratory and equipment availability a total of three birds could be tested each day. The birds were randomly allocated to four groups. The coal tits were tested first in the experiment, as at the beginning of the study some of the great tits were still being used in a different experiment.

On each experimental day a supplementary pine nut was given to the birds around 1100, and testing began around 1400 each day. Each group was tested for a total of 10 concurrent days, with each testing session lasting for two hours. The experiment ran for a total of forty days (testing on Group 1 commenced on February 15th 2002, and was complete on Group 4 by 25th March 2002).

A touch screen with three stimuli was presented to each bird. The stimuli were white squares (2.5cm²) and remained on the screen at all times. Fixed-interval schedules of 40, 70 and 70 seconds were used (see Figure 7.12 for stimulus configurations). Each stimulus was associated with the same refill schedule throughout the experiment, and these were randomly allocated to each bird. Data on the timing of pecks to stimuli were saved directly to disc for later analysis.
The schematic diagrams illustrate the touch screen arrangement. Stimuli (white 2.5cm² squares) were presented on a dark grey background. The stimuli occupied the same positions in each session. In a), the three configurations used are indicated by three different combinations of squares (one per coal tit and one per great tit). In b), the other three configurations are shown. The position of the feeder (to the left or right side of the screen) was counterbalanced across the birds.
7.4.3 Results

Activity data

All of the coal tits (Figure 7.13) and great tits (Figure 7.14) pecked each stimulus to some extent throughout the experiment. Only one coal tit (CT111) pecked the 40s most frequently; in contrast, four great tits (GTs 86 / 92 / 104 and 112) did.

Performance data

Postreinforcement pauses (PRPs) were used to assess timing accuracy. As the distributions of PRPs (all pauses) were all non-normal (Anderson-Darling tests for normality: all \( p < 0.003 \)) and were not normalised through standard transformations, I used median data and compared them to each FI using one-sample Wilcoxon tests. The great tits were significantly late pecking at the 40s stimulus but responses to the two 70s stimuli were not significantly different from 70s (all \( n = 6 \); 40s stimulus: \( W = 21, p = 0.036 \); 70s stimulus: \( W = 16, p = 0.295 \); 70s stimulus: \( W = 7, p = 0.99 \); see Figure 7.15). The coal tits had median PRPs that were not significantly different to the FIs (all \( n = 6 \); 40s stimulus: \( W = 14, p = 0.529 \); 70s stimulus: \( W = 7, p = 0.530 \); 70s stimulus: \( W = 6, p = 0.402 \); see Figure 7.15).

In this experiment a direct comparison of all versus first PRPs (as in Experiment 2) was not possible: there were multiple occasions within- and between days when stimuli were not pecked, or were pecked fewer than 5 times. This was the case both for great tits and coal tits. There were no consistent patterns with regard to a lack of response in both species; all three stimuli were affected and at no particular stage in the experiment.
Figure 7.13  Peck activity of coal tits across the experiment.

The graphs show the mean number of pecks per session (± s.e.) for all six coal tits at each stimulus (FIs of 40s, 70s and 70s). Individual birds’ codes are shown on each graph. All the graphs are drawn to the same scale.
Figure 7.14  Peck activity of great tits across the experiment.

The graphs show the mean number of pecks per session (± s.e.) for all six great tits at each stimulus (FIs of 40s, 70s and 70s). Individual birds’ codes are shown on each graph. Subject GT112’s graph is drawn to a different scale.
Species differences in performance

I used a general linear model equivalent of a repeated-measures analysis of variance to compare the performances of great tits and coal tits. The following model was fitted:

\[ \text{SCORE} = \text{SPECIES} + \text{TREATMENT} + \text{INDIVIDUAL (SPECIES)} + \text{SPECIES*TREATMENT} \]

where "score" was the median PRP (all pauses) for each bird at each stimulus across the experiment, "species" was coal tits and great tits and "treatment" were the FIs of 40s, 70s and 70s. Appropriate nesting and interaction terms were included; and the model was deemed valid after inspecting the distribution of residuals. There were no
significant effects of species ($df = 1, F = 3.07, p = 0.075$) or treatment ($df = 2, F = 1.48, p = 0.251$) or any other term in the model. However, the coal tits tended to take longer to make responses than did the great tits. Although there is no evidence of one species outperforming the other in the timing task, it would appear from the data in Figure 7.15 that the great tits tended to be timing more accurately than the coal tits, even though the longer delay to peck by the coal tits meant that they received a greater proportion of rewards than did the great tits.

I used one-sample Wilcoxon tests to compare individuals’ performances to the FIs. The results can be seen in Table 7.3 (coal tits) and Table 7.4 (great tits). All subjects of both species pecked at the FI at the 40s stimulus. Pecks to the 70s stimulus were sometimes made before the FI by both species. These data are shown in Figure 7.16.
Table 7.3 Results of one-sample Wilcoxon tests for each coal tit at each stimulus. The p values in bold indicate median PRP values not significantly different to the Fl. Refer to Figure 7.15 for the median values.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Stimulus</th>
<th>n of PRPs</th>
<th>W statistic</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT 89</td>
<td>40s</td>
<td>89</td>
<td>3587</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>187</td>
<td>11734.5</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>142</td>
<td>6662</td>
<td>( p = 0.001 )</td>
</tr>
<tr>
<td>CT 94</td>
<td>40s</td>
<td>38</td>
<td>614</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>117</td>
<td>2345</td>
<td>( p = 0.003 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>36</td>
<td>306</td>
<td>( p = 0.677 )</td>
</tr>
<tr>
<td>CT 107</td>
<td>40s</td>
<td>52</td>
<td>1157</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>43</td>
<td>876</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>228</td>
<td>15759.5</td>
<td>( p = 0.007 )</td>
</tr>
<tr>
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<td>102</td>
<td>4666</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>50</td>
<td>989</td>
<td>( p = 0.001 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>213</td>
<td>10493.5</td>
<td>( p = 0.317 )</td>
</tr>
<tr>
<td>CT 111</td>
<td>40s</td>
<td>164</td>
<td>11999</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>121</td>
<td>6096</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>114</td>
<td>5503</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td>CT 117</td>
<td>40s</td>
<td>227</td>
<td>18924</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>188</td>
<td>10871</td>
<td>( p = 0.008 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>447</td>
<td>40431.5</td>
<td>( p &lt; 0.001 )</td>
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Table 7.4  Results of one-sample Wilcoxon tests for each great tit at each stimulus. The \( p \) values in bold indicate median PRP values not significantly different to the Fl. Refer to Figure 7.15 for the median values.

<table>
<thead>
<tr>
<th>Subject</th>
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<th>( n ) of PRPs</th>
<th>( W ) statistic</th>
<th>( p ) value</th>
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<tr>
<td>GT 86</td>
<td>40s</td>
<td>741</td>
<td>140924</td>
<td>( p = 0.552 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>348</td>
<td>24451.5</td>
<td>( p = 0.002 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>419</td>
<td>29388</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td>GT 87</td>
<td>40s</td>
<td>467</td>
<td>66102</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>260</td>
<td>18125.5</td>
<td>( p = 0.339 )</td>
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<tr>
<td></td>
<td>70s</td>
<td>634</td>
<td>89451</td>
<td>( p = 0.015 )</td>
</tr>
<tr>
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<td>40</td>
<td>25</td>
<td>270</td>
<td>( p = 0.004 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>409</td>
<td>45261</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>374</td>
<td>33758.5</td>
<td>( p = 0.533 )</td>
</tr>
<tr>
<td>GT 92</td>
<td>40s</td>
<td>827</td>
<td>192815</td>
<td>( p = 0.002 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>314</td>
<td>27811.5</td>
<td>( p = 0.055 )</td>
</tr>
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<td></td>
<td>70s</td>
<td>602</td>
<td>88652.5</td>
<td>( p = 0.623 )</td>
</tr>
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<td>GT 104</td>
<td>40s</td>
<td>642</td>
<td>137798.5</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>497</td>
<td>78365</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>91</td>
<td>3099</td>
<td>( p &lt; 0.001 )</td>
</tr>
<tr>
<td>GT 112</td>
<td>40s</td>
<td>42</td>
<td>722.5</td>
<td>( p = 0.003 )</td>
</tr>
<tr>
<td></td>
<td>70s</td>
<td>23</td>
<td>221</td>
<td>( p = 0.012 )</td>
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<td></td>
<td>70s</td>
<td>22</td>
<td>168</td>
<td>( p = 0.183 )</td>
</tr>
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</table>
Figure 7.16  Postreinforcement pauses of individual birds.

The bars show the median PRPs (all pauses) for each subject at the three stimuli. The solid horizontal line denotes 40s, and the hatched line 70s.

7.4.4 Discussion

The main finding of this study was that great tits and coal tits did not differ in performance on a simultaneous timing task with intervals in the range of 40 to 70 seconds. I am unable to conclude, then, that coal tits rely on temporal cues to a greater extent than nonstorers.

Individual birds’ PRP data (all pauses) confirm that returns to the 40s stimulus were frequently rewarded (Figure 7.16). At the 70s stimuli, the coal tits frequently returned on or after the FI. Despite more erratic performance at the 70s stimuli, at least two birds (GT104 and GT112) were very close to the FI.

There was no significant difference between the species in their performance, as assessed by examining all PRPs. The coal tits did not outperform great tits in timing.
tasks. Ideally, I wished to compare the birds’ timing abilities using both measures of first PRPs and all PRPs (see Experiments 1 and 2). Although infrequent pecking meant that I was unable to make a direct comparison between these two measures (all and first PRPs at the same stage in the experiment), I was able to establish that extra pecks between rewarded visits were uncommon. I am therefore confident that my main findings, as assessed using all pauses, fairly reflect the birds’ pecking and timing abilities.

7.5 General Discussion

In timing studies, animals are commonly presented with single interval tasks. Concurrent schedules, as used in this set of experiments, offer an arguably more realistic scenario of simultaneously presenting an animal with two or more different schedules as would commonly occur in the wild. Although investigating animals’ choices in the laboratory is arbitrary with respect to many features of normal foraging conditions, demonstrating any ability that animals have to learn and remember temporal intervals does show that these cues are at least used in certain contexts. In chapter 6, I investigated rufous hummingbirds’ interval timing abilities and found evidence that they can time the availability of rewards in up to eight locations with intervals in the 10-20 minutes range. In this set of experiments, both coal tits and great tits appeared able to learn about when rewards were available at three different locations at intervals ranging from 30s to 120s.

A criticism sometimes made of laboratory tests of learning and memory is that they lack ecological relevance. In nature, it is argued, the spatial and temporal scales over which animals make decisions is much greater than the dimensions of operant apparatus in the laboratory. Despite this rather obvious statement, tests of spatial memory performed in the laboratory have nevertheless revealed consistent differences in species’ abilities (e.g. in the spatial abilities of birds pecking a touch screen or flying around a larger room: Brodbeck & Shettleworth, 1995; Brodbeck, 1994; for review see Shettleworth, 1995). In another study, humans and pigeons *Columba livia* performed in a similar manner in a touch screen task involving both spatial and temporal information being manipulated over scales of up to 13cm and
40s (Cheng et al., 1996). Although freely living animals move over much greater scales than are feasible to test in the laboratory, it is likely that precise spatial localisation of specific targets occurs after animals have made decisions over relatively small spatial scales, which can meaningfully be investigated in the laboratory (Shettleworth & Westwood, 2002).
Chapter 8: General Discussion

In this thesis, it was my aim to test the general hypothesis that hummingbirds and parids may learn and remember information specific to the problems they face in nature. In the early 1900s work on learning and memory was concerned with experimental psychologists working on a limited number of species in the hope of uncovering general laws of learning. Now, over a hundred years later, a variety of approaches is used in investigations of animals' learning abilities due, in large part, to the recognition that a "one size fits all" approach is unlikely to be sufficient to explain the array of behaviours observed in nature. With this change in emphasis a rich array of specialised behaviours is emerging in a much greater number of species. Before considering my own findings it is worthwhile to illustrate in brief further examples, hitherto largely ignored, on how animals' abilities can be fine-tuned to their specific environments.

An example of this comes from work on the spatial abilities of rodents. The orientation behaviour of the blind mole rat *Spalax ehrenergi* (an exclusively subterranean dweller) was compared to that of Levant voles *Microtus guentheri* and laboratory rats *Rattus norvegicus* in a laboratory study (Kimchi & Terkel, 2001). Designed to test the hypothesis that animals living exclusively below ground with limited auditory and visual cues would have enhanced spatial orientation abilities relative to those species only partly used to living underground, this study involved animals performing a task involving learning and remembering the location of food within a complex maze. The mole rats not only learned the task faster, but also remembered it for longer (up to 4 months) than the voles and rats, lending support to the hypothesis that the subterranean mole rats had indeed evolved highly specialised spatial orientation abilities. In another study, three-spined sticklebacks *Gasterosteus aculeatus* from relatively still pond habitats were more likely to use visual landmarks as reliable cues in orientation than fish from fast-flowing rivers, where factors such
as increased water flow may render many visual cues less reliable (Odling-Smee & Braithwaite, 2003).

I will now consider my own findings, and provide suggestions for future work.

8.1 Route based information in hummingbird foraging

In chapter 3 I investigated hummingbirds’ use of route-based information whilst foraging at an array of artificial flowers. The flowers were contained within an arena, to which I could manipulate the birds’ possible entrance and exit points. My results did not support the hypothesis that hummingbirds rely on using route-based information (e.g. flight paths) while foraging. However, in situations where birds were only able to use one door to access flowers there was some evidence that they visited flowers in similar sequences. Whether or not this might also apply to birds foraging over larger spatial scales, for example at large inflorescences containing many flowers, is unclear. It would be possible, perhaps, to test birds in larger arrays containing more flowers (see e.g. Baum & Grant, 2001).

8.2 Relational learning and maze foraging in hummingbirds

Rufous hummingbirds were found to transpose flower height, at least under some conditions, in chapter 4. Hummingbirds thus appear to learn both relative and absolute properties of stimuli and respond in a context-dependent manner. In my experiment, birds’ responses may have been affected by a preference for taller flowers. When trained such that a taller flower offered reward during a learning phase, the birds were more likely to transpose this spatial relationship in tests and favour visiting a tall flower in a test situation. This did not happen when during the learning phase birds had to visit the shorter of two flowers to be rewarded. In this latter case, the birds showed no preference in tests. Again, it would be interesting to not only test birds using for example different heights but also perhaps in more than one dimension. Birds’ responses to variations in colour, for example, might be useful in understanding foraging choices in a pollination context.
Contrary to expectation I did not find that birds foraging in 3-D mazes performed better than in comparable 2-D mazes (chapter 5). Although hummingbirds will readily feed from mazes containing flowers at different heights, future studies might usefully address different flower configurations coupled with detailed descriptions of birds’ choices at and between flowers, perhaps by filming their movements. In a similar manner to the arena experiment (chapter 3), this might allow a more thorough understanding of the role of various cues in foraging (e.g. global or extramaze cues versus those intrinsic to flowers available once at an inflorescence).

8.3 Timing in hummingbirds and parids

In chapters 6 (hummingbirds) and 7 (parids) I tested the general hypothesis that birds may use temporal cues in foraging tasks. By presenting birds in the field with eight flowers with two different FI schedules, I found that rufous hummingbirds track the availability of rewards at multiple locations and can learn intervals in the range of 10-20 minutes. In laboratory investigations of timing, the inclusion of “empty” trials in single-interval timing tasks allows specific predictions of scalar timing theory (e.g. proportional timing of short and long intervals) to be tested. Although I was not able to include empty trials in the field, where birds were feeding from more than one flower and would readily move elsewhere if not rewarded, I was nonetheless able to show that rufous hummingbirds do make use of temporal cues.

The next step might be to attempt to test birds using more realistic time periods for the FI schedules (in the order of several hours). This was not done here as the logistical difficulties of training the animals to long FIs were not feasible in the time the birds are resident at the field site. The number of flowers tested in my experiment was also many fewer than birds feed from in a day within their territory. Although I showed that they could track up to eight flowers, it is not clear that they can, or are, tracking all the flowers they visit throughout the day. Assessing this aspect of their timing abilities in a realistic fashion is also logistically difficult.

Parids were found to time intervals in the 30-120 seconds range in an operant laboratory task analogous to that used on the hummingbirds in the field. Although
shorter than the intervals used in the field study, both coal tits and great tits were found to be able time intervals longer than those used in the only other study to specifically address timing in parids (Brodbeck et al., 1998). Furthermore, birds were able to simultaneously track the availability of rewards in multiple (up to 3) locations. Here, as with the hummingbirds, the challenge now is to test birds over longer periods such as those the birds may commonly have to remember in the field.

8.4 Further questions

All of the work in this thesis has examined learning and memory abilities in birds over relatively short periods (seconds to minutes within the context of an individual field season). Recent exciting findings on warblers suggest, however, that finely-tuned learning and memory abilities can exist over much longer periods of time. In a semi-natural experimental set-up, migratory garden warblers *Sylvia borin* were found to remember particular feeding sites for a year, whereas a closely related non-migrant species, the Sardinian warbler *Sylvia melanocephala momus*, could do so for only 2 weeks (Mettke-Hofmann & Gwinner, 2003; see also Godard, 1991). Although logistically difficult to monitor known individuals over long periods, migrant rufous hummingbirds may be capable of similar feats. If it were possible to integrate more fully findings from neuroanatomy (e.g. breeding season differences in cowbird hippocampal volume: Clayton et al., 1997) with investigations of spatial memory (e.g. persistence of parids’ spatial memory: Biegler et al., 2001) and advances in knowledge of animals’ use of temporal cues (e.g. Roberts, 2002), our knowledge of behaviour would be more complete.
References


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