

**Foraging on variable resources: the behaviour and decision making
of rufous hummingbirds**

Ida Elizabeth Bacon

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Declaration

This thesis is entirely my own work except where due reference or acknowledgements state otherwise. The work included in it has not been submitted in application for the reward of any other degree.

I.E. Bacon5th January 2010

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Abstract

Supplementary food is less variable than natural food. While feeding from constant food sources tends to be preferred by animals they must sometimes forage from more variable resources. However, the ways in which animals deal with the temporal and spatial variability of more natural food is not entirely understood.

I investigated the decisions free-living rufous hummingbirds made when foraging from variable resources, where variability was encountered over time or within a bout via four field experiments. In addition, I investigated their use of wild flowers and differences in the use of supplementary food by these birds in different regions using surveys and by manipulating the distribution of feeders at feeding sites. I investigated the possibility of a genetic explanation for any differences in feeder use between regions using analysis of microsatellite DNA and banding data.

Hummingbirds seemed to prefer to make foraging decisions based on past behaviour or post-ingestive feedback rather than on sensory information such as taste, which may be harder to assess accurately. Birds choosing between constant and variable rewards with equal means preferred the constant rewards when variability was high but tended to prefer the variable reward when variability was low. This seems to be a result of hidden time and other costs associated with foraging on highly variable resource but not on less variable ones, combined with potential benefits of information seeking from less variable resources. In addition, these preferences between constant and variable resources were affected by preceding foraging conditions.

The number of birds using feeders was affected by population density and air temperature. Microsatellite data showed the rufous hummingbird population to have a fairly panmictic population structure.

Investigating influences on foraging decisions at a large scale (population density) and small scale (resource variability) has provided a much wider understanding of their foraging behaviour than either could alone.

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Chapter 1 Introduction

Supplementary food is very different from natural food. Its occurrence and location are usually very reliable and it is typically available in much larger quantities than food found at natural foraging sites. Where both natural and supplementary food are available, animals must choose which to eat and when (O' Leary & Jones 2006; Robb et al. 2008). To do this and to adjust their behaviour appropriately to whichever sources they choose, they must use a number of different sorts of information, including memories of past experiences and current information such as sight and taste and physiological feed-back (Hall et al. 2007; Hirvonen et al. 1999; Lara 2006; Vasquez et al. 2006).

In general, animals show a preference for resources that provide constant rather than variable reward amounts, suggesting that not only the quantity but also the predictability and constancy of food in artificial feeders may make them attractive (Kacelnik & Bateson 1996). However, the way in which animals deal with the high levels of temporal and spatial variability of more natural food sources is not entirely understood.

In addition to small scale within bout decisions made on the basis of food quality and variability, animals foraging behaviour may also be influenced by larger scale variables such as population density, and therefore competition, as well as genetic tendencies such as aggressiveness (Carpenter 1987; Inouye et al. 1991; Lopez-Sepulcre & Kokko 2005). These two levels of influences on behaviour are usually examined in isolation. However, both are likely to involve fitness consequences that animals should respond to. Therefore, in order to more completely

understand foraging behaviour both large and small-scale influences should be investigated. This understanding could then be used to, for example, develop more affective supplementary feeding practices.

In some locations more animals use artificial feeding stations than in others. Such differences could be due to many things including: differences in population density, the availability of alternative food sources and genetics (Carpenter 1987; Inouye et al. 1991; Lopez-Sepulcre & Kokko 2005). Understanding what influences how many hummingbirds (in my case) choose to compete for predictable food at artificial feeders rather than to forage from wild-flowers should help lead to an understanding of the wider influences and contexts under which they must make finer scale foraging decisions e.g. which flowers to visit or how much to drink within a single bout.

General aims

The main features of my thesis were firstly to investigate birds' foraging decisions when feeding from variable sources, when (a) variation in the food source is experienced over multiple feeding bouts (chapters 2 and 3), or (b) variation is experienced within a single bout (chapters 4 and 5). This compares more closely to their natural food, as opposed to very constant resources like artificial feeders. Secondly, at a larger scale, I was interested in what causes the substantial variation in numbers of animals using artificial feeders (chapters 7-10).

Subjects

Rufous hummingbirds (*Selasphorus rufus*) were chosen as the subject species, because, birds (including hummingbirds) are one of the most popular subjects for artificial feeding by the general public (at least in North America) and because hummingbirds are easier to observe and to experiment on in the wild than are many other bird species (Robb et al. 2008; Williamson 2001). Hummingbirds obtain their energy from nectar or in the case of artificial feeders, sucrose solutions (Healy & Calder 2006; Williamson 2001). It is very easy to manipulate the quantity and quality of this type of food and to calculate the energy provided by it, especially as assimilation by hummingbirds is nearly 100%, compared to more complex foods of other species, such as seeds (Lopez-Calleja et al. 1997). In addition, hummingbirds feed very frequently, every ten minutes or so, and territorial males will defend food sources making it easy to obtain multiple observations of a single individual (Calder 1991; Wolf & Hainsworth 1977). They are also fairly bold and can be observed at close proximity without the need for hides or long periods of acclimatization to human proximity to avoid disturbing them.

Rufous hummingbirds are one of the most common species of sixteen species of hummingbird that breed in North America. They breed along the west coast of North America from Washington state as far north as Alaska and inland as far as Alberta (Healy & Calder 2006). The vast majority overwinter in Mexico but a few individuals have recently started over wintering in the southeast United States (Hill et al. 1998). They are believed to follow a typically circular migration route, following the first opening of spring flowers, first moving north up west coast in the

spring and then, as conditions warm and flowers open at higher altitudes, moving to the east into the Rocky mountains to take advantage of the later flowering mountain flowers on their way back south (Healy & Calder 2006).

Larger-scale influences on foraging behaviour

It appears from banding data that this species is site faithful, at least to its breeding sites, but there are no reliable data on juvenile dispersal and no genetic data on population structure (Finlay 2007). Prior to my thesis, however, there was anecdotal evidence to suggest that, compared to British Columbia, the behaviour of birds around feeders is very different in Alberta, with far fewer birds using feeders simultaneously in Alberta than in British Columbia (Cam Finlay, pers. comm.). This difference may be due to population density differences between the two regions. However, it is also possible that, if the population is highly structured, there may also be a genetic component to this difference. One possibility is that birds in British Columbia are less aggressive than the Albertan birds and are, therefore, more inclined to feed together.

I used two approaches to investigate why more birds are seen at feeders in one region than in another. The first was to collect data on the behaviour of birds around feeders in both regions to assess if there is any variation in aggression or other behaviours (chapters 7 and 8). The second approach was to study their population structure, including evidence from banding and recapture data (chapter 9) and microsatellite DNA analysis (chapter 10), in order to establish if the two populations had diverged sufficiently to suggest a genetic component to the variation in behaviour.

My main interest, however, was to enhance our understanding of how animals make decisions when foraging from variable resources compared to very constant resources like artificial feeders.

Smaller-scale influences on foraging behaviour

Hummingbirds prefer sucrose solutions up to concentrations of around 50%, at which they achieve about maximum energy intake rates (Tamm & Gass 1986). Yet the optimal volume of a feeding bout decreases with increasing concentration, a trade-off between the flight costs of carrying the meal and the energy consumed (DeBenedictis et al. 1978). This trade-off results in hummingbirds with reliable access to food (territory holders rather than intruders) drinking from a tenth to a third of their crop capacity (Carpenter et al. 1991). While the energetics of hummingbird foraging have been addressed using these models, the mechanisms involved in the birds making decisions that lead them to drink less of something they prefer are not.

A single hummingbird-pollinated flower produces on average $22.5 \pm 33.9 \mu\text{l}$ of $23.5 \pm 6.9\%$ sucrose solution per day (Ornelas et al. 2007). This is equivalent to approximately one fifth of the total sucrose consumed during a single foraging bout at an artificial feeder (Carpenter et al. 1991). Hummingbirds foraging at artificial feeders feed about once every ten minutes (around 120 times in a twelve-hour day), equivalent to emptying at least 600 flowers a day. It is not hard to see that finding at least five flowers every ten minutes, remembering how fast these refill and when they were last visited, not to mention the variation among flowers in the volume and concentrations of sucrose they provided, is a much more complex task than returning to the same feeder over and over again. Furthermore, unlike feeders, plants' flowers

are transient and their sugar production is sensitive to air-temperature and other climatic variables (Corbet 2003; Mikhailova 1959). Somehow hummingbirds must weigh up all the information available to them about the available food resource including information about competition, process this information and decide where to feed and how much to drink at any one time.

Resource variability between bouts

When feeding on flowers, a foraging hummingbird is likely to sequentially encounter a range of different concentrations and although it may not have the choice of drinking as much as it likes of any one of these due to the low volumes found in flowers, it must, nonetheless, decide when it has visited enough flowers. This decision may be based partly on memories of past experiences. Past experiences shape animals' expectations of what reward a resource will provide and can significantly affect behaviour even after the reward the resources provides has changed (Biernaskie et al. 2009; Shettleworth & Plowright 1992; Valone 2006). There is also current information such as sight, taste and touch, which may indicate the actual reward size or quality (although not necessarily very accurately; Blem et al. 2000). Finally, there is feed-back from whatever action is taken, for example, the post-ingestive consequences of a meal (Savory 1999). Studying how hummingbirds adjust how much they drink when sucrose concentrations vary over time may allow us some insight in to which of these sorts of information they prefer to use to make foraging decisions to allow them to forage efficiently from variable resources (chapters 2 and 3).

Resource variability within bouts

Yet another sort of variation hummingbirds must deal with is variation within a single bout in either the concentration or in the volume of nectar in the flowers visited. Resource variability has been shown to affect animals' choices between resources in a number of species including hummingbirds (e.g. Caraco 1980; Hurly & Oseen 1999; Kacelnik & Bateson 1996). This is referred to as risk-sensitive foraging, where risk means the degree of variability or uncertainty associated with the outcome of an action e.g. a flower that always provides the same reward is less risky than a flower that unpredictably provides a range of different rewards (Caraco 1980; Kacelnik & Bateson 1996). Risk-sensitive choices are examined by looking at animals' choices between food resources that provide the same mean reward but where the variability about that mean is different (Kacelnik & Bateson 1996). Although risk sensitivity has received considerable interest over the past few decades, it is still not fully understood and none of a number of risk sensitive foraging models correctly predicts all the general trends observed in the data (Kacelnik & Bateson 1996). Thus, there is clearly a need for exploration of other possible mechanisms that may affect this behaviour if it is to be better understood. There seem to be two promising lines of investigation here. First (chapter 4), despite increasing evidence that animals' past experiences significantly affect their current behaviour for sometime after conditions have changed, this has not been investigated in the light of risk-sensitive foraging (Biernaskie et al. 2009; Doherty & Cowie 1994; Lima 1983; Shettleworth & Plowright 1992). Secondly (chapter 5), as risk sensitive models are based entirely around the energetic rewards obtained during foraging, or at least animals' perceptions of these energetic rewards, it seems possible that other

currencies such as time and information may also be involved (Inglis et al. 1997; Kacelnik & Abreu 1998; McNamara et al. 1991; Shapiro 2000; Stephens 1981).

Summary

In summary, in this thesis I explored the decisions animals make when foraging from variable resources. I concentrated on two types of variation, variation in food quality over time and variation in the mean reward provided by different resources in the context of risk-sensitive foraging. This involved the manipulation of food resources provided to wild, free-living rufous hummingbirds. In addition, I looked at larger-scale influences on foraging behaviour. Specifically I investigate hummingbirds' use of supplementary food in different regions and explore possible reasons for any observed difference in the number of birds visiting these resources. The possibility that rufous hummingbirds breeding in different regions may be genetically distinct and behaviour differently accordingly was investigated using analysis of microsatellite DNA and of banding and recapture records.

Chapter 2 **Foraging decisions made by rufous hummingbirds**
Selasphorus rufus

Ida E. Bacon, Andy T. Hurly and Susan D. Healy

I designed the experiment in collaboration with SDH and TAH, collected and analysed the data and wrote the manuscript with comments from SDH and TAH.

Abstract

Animals base their decisions on how to respond to changes in resource quality on several sorts of information: sensory, physiological and cognitive (memory). Each of these types of information is acquired and remains relevant for different periods.

The timing and sequence of behavioural changes, then, can inform us about the types of information an animal uses in decision-making. We investigated the behavioural responses made by territorial male rufous hummingbirds *Selasphorus rufus* to changes in the value of supplemental food provided during the breeding season.

Both territory holders and intruders adjusted their behaviour in response to changes in supplemental food quality: when the sucrose concentration in the feeder was increased territorial males immediately increased meal size and reduced the distance from the feeder at which they perched prior to an increase in intrusion rate.

Conversely, following a decrease in concentration, birds initially drank less while perch distance increased concomitantly with a decrease in intrusion rate. Birds used sensory (e.g. sugar concentration), physiological (e.g. postingestive) and cognitive

(e.g. memory) mechanisms to adjust both territorial and feeding behaviour and their responses varied with experience.

Introduction

Animals make decisions about where and when to feed and which resources to defend on the basis of sensory, physiological and cognitive responses (Wingfield 2003). The rate at which an animal is able to change its behaviour in response to changes in resources is limited by its abilities to detect and to quantify the change, abilities predominantly controlled by sensory information from the external environment and post-ingestive functions (sensory information from the internal environment: Savory 1999). However, current behaviour is also strongly influenced by cognitive mechanisms. Long- and short-term memory enable an animal to recognise and assess the degree and novelty of resource changes and use that information to make decisions about current behaviour. Under stable conditions, where feeding experience over a relatively longer time period most accurately reflects expectations of current conditions, animals tend to base current behaviour on past experience (e.g. Bell & Baum 2002; Shettleworth 1984). But in unpredictable environments animals place less emphasis on past experience and track resource changes more closely as the most recent information is likely to be the best predictor of current conditions (e.g. Bell & Baum 2002; Davison & Baum 2000; Davison & Baum 2002; Schofield & Davison 1997; Shettleworth & Plowright 1992).

Foraging behaviour is a useful model with which to study behavioural changes because these behaviours have real fitness consequences and because behaviours change across a range of time scales (seconds, hours, days). In patch-choice experiments in the

laboratory, animals may switch patch preference within a very short period of time (e.g. within sixty seconds Bell & Baum 2002) following a change in patch profitability, or respond quickly but continue to sample from the alternative patch, even when that patch is consistently poorer than the current patch (Wildhaber & Crowder 1991). But some aspects of feeding behaviour may change more slowly: for example, when broiler chicks are presented with a new food resource of different quality, they adjust their consumption rate during a single feeding bout but take four feeding bouts to slow their speed of approach to the food source (Haskell et al. 2001). The time taken to complete the changes in behaviour might be because chicks are very slow to learn about the change or, more likely given their rate of change in consumption, they quickly detect the change in resource quality but their expectation of food quality (expressed as rate of approach to the food source) is based on their memory for food quality from multiple foraging bouts prior to the change. This memory might be accorded more value in predicting the current resource value, than the last experience of a resource, as it is an average over multiple bouts. The predictive value attributed to memory of a past feed decreases with each feeding experience until it no longer contributes to the bird's feeding decisions. Pigeons, at least in some circumstances, use a combination of information about the current foraging bout and the previous one or two foraging bouts to make patch leaving decisions (Todd & Kacelnik 1993). These decisions could be predicted using a rate maximisation model based on Scalar Expectancy Theory (SET) for temporal memory. SET combines information from the working memory (current experience) and reference memory (previous experiences) to predict optimal foraging behaviour for rate maximisation. More weight is given to the most recent experiences and one would predict different weightings under different conditions of environmental stability.

Decision-making experiments have largely focussed on investigating features of the information that is available preceding the decision point. However, studies such as the broiler chick experiments show that we can also use the sequence of behavioural responses that follow an alteration of resource value to determine what information animals use and how they use that information to modify their behaviour in response to changes. This has been done, typically, by examining the latency to response initiation or the latency to stabilization of the new behaviour. Often it is this stable end-response that is deemed to be the animal's decision. However, the interval until stability is reached provides insight as to the information to which an animal pays attention and the relative importance it places on different kinds of information. Additionally, multiple responses might be appropriate following a change in a resource and these behaviours may not all be changed simultaneously. Nectarivores, for example, can detect and respond to some changes in resource value with a single sensory input: concentration changes may be detectable with one taste. However, responses to less easily detectable or measurable changes, such as an increase in food quantity, require more than a single taste and may initiate from a physiological process such as digestion. Responses to changes in resource value may be even less direct as the focal animal responds to the behaviour of other animals as those other animals discover the change in resource value. For example, an increase in the amount of food provision to hummingbirds leads to an increased intrusion rate and a reduction of territory size (e.g. Eberhard & Ewald 1994), although it is unclear whether the territory holders adjust their behaviour in response to the change in resource quality or to the behaviour of intruders.

Rufous hummingbirds are useful animals for investigating the sequences of response to a change in resource value as: (1) the defence by males of artificial feeders enables ready

manipulation of food resources; (2) behavioural responses are readily quantified (e.g. food intake, feeding rate, territorial size, intrusion rate); (3) they can immediately detect very small changes in resource quality, when the quality is concentration (Blem et al. 2000; Stromberg & Johnsen 1990b); (4) there is a lot known about hummingbird crop volumes, digestion rates and so on (e.g. Diamond et al. 1986; Lopez-Calleja et al. 1997; Tiebout 1989); and (5) they can use spatial and temporal memory to make foraging decisions (Healy & Hurly 1998; Henderson et al. 2006; Hurly & Healy 2002).

In this experiment, we manipulated the quality of food provided to male rufous hummingbirds so as to examine the sequence of responses to those changes and subsequently infer the recruitment of sensory, physiological and cognitive information used in assessing the changes. Hummingbirds have very high metabolic rates and can ill-afford to make poor decisions regarding food intake and energy expenditure. Thus, we hypothesized that they would employ sensory, physiological and cognitive information to promptly adjust their foraging and defence activities. Males had already set up feeding territories around artificial feeders containing 14% sucrose, which we then increased to 25% for two days, before returning the feeder contents to 14% sucrose. We predicted that birds would respond immediately to the increase in sucrose concentration by drinking more than on previous visits. The territorial male was, then, expected to stay away longer in order to digest the larger meal. At stability, however, we expected a male to drink an amount that filled his crop to the same degree, irrespective of concentration. The bird would then be able to increase the time before he had to return to his feeder or to increase his activity level between visits (e.g. courtship displays: J-flights). We also predicted that the territorial male would reduce his territory size as soon as he detected an increase in sucrose concentration rather than reducing it slowly in response to an eventual change in intruder behaviour. Higher

concentrations were expected to be more attractive to intruders and so intrusion rate was expected to increase following increases in concentration.

Materials and Methods

Subjects and Site

Rufous hummingbirds breed in Western North America and migrate to overwintering grounds in Mexico and the southern United States (Healy & Calder 2006). This experiment was conducted from the 11th to 19th of June 2006 in the foothills of the Rocky Mountains, Alberta, Canada, near Beaver Mines (Lat: 49:27:57N Lon: 114:11:31W altitude 1400m). Before the birds' arrival in late May, artificial feeders supplying approximately 220ml of 14% sucrose solution were put out in traditional locations (clearings in mixed woodland). By June males had already been defending territories around these feeders for 1-2 weeks. Five territorial males occupying neighbouring or nearby territories were selected for the study.

Preliminary observations were made to determine where each resident male commonly perched. The distance between the feeder and these perches was then measured to enable an estimation of territory size or local defended area (related to the maximum perch distance from the territory core: Armstrong, 1987). Males were caught in a mesh trap, banded and colour marked with non-toxic ink on their upper breast to allow individual identification. The experiment began at least three days after marking.

Experimental design

The experiment consisted of three main phases:

Phase 1: On the first day of the experiment, residents were observed for two separate periods (each 45-60 minutes), one in the morning and one in the afternoon. The number and time of visits to the feeder by resident or intruding birds, duration spent at the feeder, number of display flights by the resident and the perches he used were recorded.

Phase 2: The sucrose concentration in the male's feeder was increased to 25%. The observation period began with the resident's first visit to the feeder following the change in concentration and continued for 45-60 minutes. Three subsequent observation periods of the same duration were made over two days per bird, after this first post-change observation period.

Phase 3: The sucrose concentration in the feeder was returned to 14%. Observations began with the first visit of the resident to the feeder following the decrease in concentration and continued for 45-60 minutes. Three subsequent observation periods of the same duration were made over two days per bird, after this first post-change observation period.

Territories were observed in a fixed sequence so each bird was always observed at the same times of day. Each bird was observed on ten occasions: twice in Phase 1 to obtain base line data for birds feeding on 14% sucrose and four times each in Phases 2 and 3. This was so as to distinguish between initial responses (in the first hour) when the resident had just encountered the altered sucrose

concentration and later responses by which time it had considerable experience of the new concentration.

We recorded the inter-bout interval, which was the time between the end of one feeding bout to the start of the next, and feed-bout length, which was the time between the start and the end of a single feeding bout. The degree of resource defence was approximated as the distance birds perch from the feeder and we calculated the average of the maximum perch distances (MPD) observed during each hour of observation. MPD was the distance between the feeder and the most distant perch used. We also recorded J-flights.

The data for analysis were the mean of Phase 1 and the first feed and means of all subsequent feeds for each concentration for Phases 2 and 3. Data were analysed using ANOVA in the statistical package R and using two-tailed paired t-tests.

Results

Supplemental food sources in all three phases were strongly preferred to naturally occurring nectar. During the entire 50 hours of the experiment a hummingbird was only once observed to feed from a flower, and on that occasion it was immediately after his first feed in Phase 3 when the sucrose concentration had just been reduced.

Feed bout length

We used two-way ANOVAs to assess the influence of sequence (first feed vs. mean of subsequent feeds) and concentration (25% Phase 2 vs. 14% Phase 3) on feed-bout length (Figure 1). Feed bout lengths on 25% were significantly longer than

they were on 14% ($F_{1,9} = 13.97, p < 0.01$). Across concentrations, the durations of the first feed and later feeds were similar ($F_{1,9} = 1.78, p = 0.21$). However, there was a significant interaction between sequence and concentration ($F_{1,9} = 14.80, p < 0.01$). Within 25% first feeds were longer than average (means: first feed= 22.75 sec, later feeds= 13.02 sec; $t_3 = 3.37, p = 0.04$, Figure 1) and within 14% first feeds were shorter than average (means: first feed= 8.50 sec, later feeds= 13.22 sec; $t_3 = 5.47, p = 0.01$).

While feed bout lengths tended to be longer on 25% than 14% this is mostly due to the effect of the first feeds, as the means for later observations for 25% and 14% were not different (means: later 25% = 13.01 sec, later 14% = 13.22 sec; $t_4 = 0.24, p = 0.83$). The difference between the first and later feeds was greater for all birds in 25% than in 14%, however this difference was not significant (means: 9.73 vs. 4.72 sec; 13.02 sec; $t_4 = 1.93, p = 0.15$).

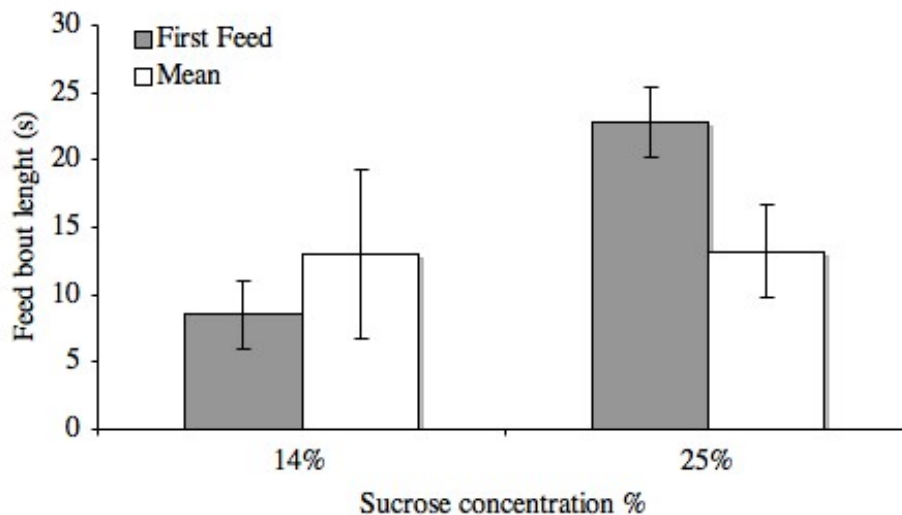


Figure 1: The length of the first feed bout and the mean feed bout length of observation periods 2-4, for each concentration. Data are means and standard errors.

Inter-bout intervals

Both sequence and concentration influenced inter-bout intervals (Figure 2). Intervals were significantly longer on 25% than on 14% ($F_{1,9} = 91.81, p < 0.01$), but differed by sequence ($F_{1,9} = 14.01, p < 0.01$). Critically, the interaction term was significant ($F_{1,9} = 43.98, p < 0.01$). The first inter-bout intervals on 25% were longer than the average for 25% (means=17.70 vs. 10.33 mins; $t_3 = 5.39, p = 0.01$) and first inter-bout intervals on 14% were shorter than the average for 14% (means= 5.22 vs. 7.26; $t_3 = 3.79, p = 0.03$). The mean of later 25% intervals was significantly greater than the mean of later 14% intervals (means=10.33 vs. 7.26; $t_4 = 3.60, p = 0.037$).

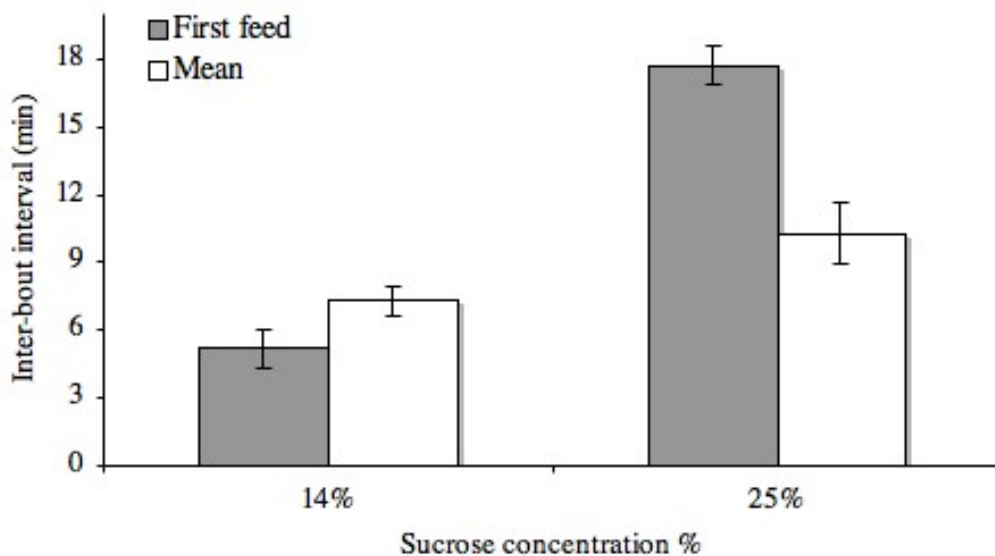


Figure 2: *The length of the first inter-bout interval and the mean inter-bout interval of observation periods 2-4, for each concentration. Data are means and standard errors.*

Intrusion rate and perch distance from the feeder

As intrusions were relatively rare events we thought it inappropriate to split the first observation period into the first inter-bout interval and the rest of that period. Instead, sequence is represented by mean intrusion rate during the first hour vs. mean intrusion rate during subsequent observations for both 25% (Phase 2) and 14% (Phase 3) (Figure 3a). Again, interpretation of the main effects is not straightforward (sequence, $F_{1,9} = 3.05$, $p = 0.11$; concentration, $F_{1,9} = 0.39$, $p = 0.55$) but, rather, relies upon the significant interaction between the two ($F_{1,9} = 21.04$, $p < 0.01$). The intrusion rate was much lower during the first than later periods of 25% but conversely was higher during the first than later periods of 14% (Figure 3a). The very low intrusion rate in the first hour of Phase 2 requires further assessment. This value was not significantly lower than the preceding baseline intrusion rate in Phase 1 (means= 1.78 vs. 4.78 intruders/h; $t_4 = 1.38$, $p = 0.26$) as there was a large decrease in intrusions in half the territories but no change in others, suggesting a change in defence behaviour of only some of the resident males. Note that the intrusion rate in Phase 3 returned to baseline levels (means= 4.02 vs. 4.78 intruders/h; $t_4 = 0.57$, $p = 0.61$).

We assessed maximum perch distance (MPD) to determine if residents significantly altered their defence behaviour. Certainly, the tendency for a rapid decrease in intrusion rate between Phase 1 and the first hour of Phase 2, in some territories, is matched by a corresponding tendency for a decrease in MPD (means= 22.00 vs. 33.88m; $t_4 = 2.82$, $p = 0.07$; figure 3b). Within Phases 2 and 3 we see further influence of sequence and concentration. Overall MPD at 25% and 14%

were similar ($F_{1,9} = 0.93$, $p = 0.36$). However, the MPD of later observations for 25% was significantly lower than for later 14% (means= 22.93 vs. 32.46m; $t_4 = 2.78$, $p = 0.05$). First observations periods tended to be lower than later observation periods ($F_{1,9} = 4.46$, $p = 0.06$). During the latter part of the Phase 3 14% treatment, MPD returned to baseline levels (means= 33.58 vs. 33.87m; $t_4 = 0.10$, $p = 0.46$).

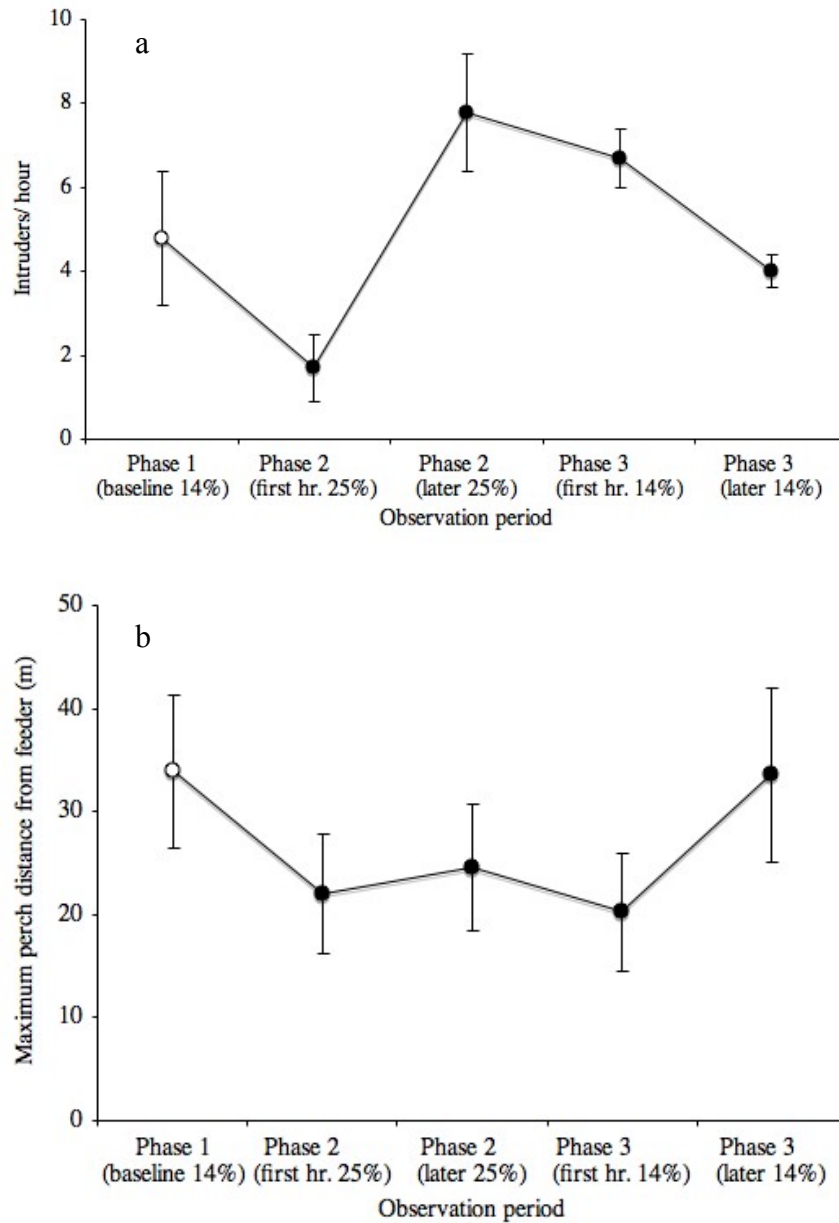


Figure 3: The intrusion rate (a) and maximum distance birds perched from their feeder (b) for the baseline period on 14% and the first and later observation periods following concentration changes to 25% and then to 14%. Data means and standard errors and are presented in time sequence. Dark points represent data used in 2 x 2 ANOVA whereas the open point represents the baseline values.

J-flight displays

Our manipulation did not obviously influence either the encounter rate with intruding females or the rate of energetically-demanding courtship displays made by males. Males performed 0-12 J-flights/hr, with an average of 2.73 (\pm 0.65) J-flights/hr. There were no significant differences in J-flight rates between concentrations ($F_{1,9} < 0.01$, $p=0.98$) or with sequence ($F_{1,9} = 0.09$, $p=0.77$). There was no interaction between concentration and sequence ($F_{1,9} = 0.67$, $p=0.42$).

Discussion

Rufous hummingbirds, both territory holders and intruders, adjusted their behaviour in response to manipulations in supplemental food supply. Some of these responses occurred at, or immediately following, the first feeding bout: birds tended to spend longer drinking during the first bout of 25% than they did on later visits to feeders containing 25% and conversely, birds spent less time drinking on their first visit to the feeder when 25% sucrose had been switched to 14% (Figure 1). These decisions can have been based only on the taste of the change in sucrose concentration. Continuous updating of the hedonic value associated with the taste of either of these two concentrations, over multiple bouts, could have modulated birds 'desire' for that concentration such that they consumed the usual appropriate amount. However, at first experiences of the other concentration, this value may have lead birds to overvalue 25% sucrose or undervalue 14% and thus over- or undershoot the optimal amount to drink for those concentrations respectively (Costa et al. 2007).

The fact that males stayed away from the feeder for longer after the first feed of 25% is likely to be due to physiological feedback from processing a meal that was larger than usual in both volume and concentration. As hummingbirds tend to empty their crop and digest most of the sucrose from one meal before feeding again, a larger meal results in longer inter-bout intervals (DeBenedictis et al. 1978; Diamond et al. 1986; Wolf & Hainsworth 1977). Digestion time depends on the amount of sucrose in a meal (concentration and meal size) and crop-emptying rates usually reflect digestion rates ((Hainswor.Fr & Wolf 1972; Hainsworth.Fr & Wolf 1972; Karasov et al. 1986; McWhorter et al. 2006; Tiebout 1989). However, while the crop empties between meals (within 5-20 mins) it takes several hours (many feeds) for a particular feed to be completely digested and all traces of it (a marked feed) excreted (Karasov et al. 1986). Thus, while an individual is free to choose the size of its meals, inter-bout intervals are subject to the size of that meal and to some extent the volume and concentration of several previous meals. The presence of small proportions of previous meals in the guts of our hummingbirds may explain the larger response of their inter-bout intervals following changes to 25% sucrose than 14% sucrose.

Models of optimal meal sizes for hummingbirds that account for meal volume, sucrose concentration, transit time to the feeder, feeding time, and inter-bout intervals, predict that territorial individuals (birds with free access to an ad-lib food source) should typically fill their crops to as little as a tenth of the crop volume, and rarely beyond a third, to optimise energy intake (meal volume) and expenditure (the cost of flying for a certain amount of time with the additional weight of that meal; DeBenedictis et al. 1978). Laboratory data and field data from territorial hummingbirds confirm that they appear to prefer to adjust the inter-bout interval and keep feed volume relatively constant

(Carpenter et al. 1991; DeBenedictis et al. 1978). Our hummingbirds did this too: feed bout length was different only on the very first feed of a new concentration while inter-bout intervals were always longer when birds fed on 25% than when they fed on 14%.

It is not clear why the meal size changes at all in response to a change in concentration. One possibility is that the safest assumption the bird can make with regard to a change in concentration is that it is a unique event such that when the concentration increases, so the bird should drink more and when concentration decreases, the bird should drink less. This was, indeed, the way in which the birds in this experiment responded. Following a change in concentration it appears that memory for the previous concentration did affect the size of the first feed.

Unlike feed bout length, the distance the bird sat from his feeder seemed to be partially dependent on the sucrose concentration in his feeder: the higher the concentration, the closer he sat. This response occurred immediately after the first feed for half the individuals. As only the first few feeds in an hour were abnormally large, it seems unlikely this was due to any physiological constrain such as increased flight costs. Intrusion rate, on the other hand, did not change as quickly. Despite intruders occasionally managing to feed at the feeder, it was not until over an hour after the change in concentration that intrusion rate increased in Phase 2 and decreased in Phase 3. The reduction in perch distance to the feeder by the male in response to 25% sucrose might have been physiological (i.e. because he was too heavy to fly farther with more sucrose) but it is also possible that he moved closer to make it easier to defend his enhanced resource (Adams 2001). His proximity was not a response to a need for defence but, seemingly, a prediction of a need for greater defence (Switzer et al. 2001). We do not know whether proximity is correlated with changes in resource quality but it

seems likely that the bird's change in proximity to his feeder was based on prior experience of fluctuating resources and associated changes in intrusion pressure. Apparent prediction on the part of the territorial male was seen only when the resource increased in value. Even if he had predicted that intrusions would decrease with the return to the lower concentration, the territorial male's proximity to the feeder in the first few feeds of 14% are likely to be a response to actual territorial intrusions. Only as those intrusions declined did the male perch farther from his feeder. To our knowledge, ours is the first manipulation to clearly separate the effect of changed food resources from the associated change in intrusion pressure on the degree of resource guarding /territory size.

Except in the first hour following each change in sucrose concentration the intrusion rate increased in response to increased sucrose concentration and vice versa. The initial drop in intrusions in most territories after provision of 25% sucrose corresponds with the territory owner tending to perch closer to the feeder and, as intruders at that time had not had time to learn of the increased concentration in the feeders, they were likely to avoid an increased risk of an aggressive encounter for no obvious additional benefit (Carpenter & Hixon 1987). The reverse is true following the switch to 14%: territorial males slightly decreased their defences but intrusion rate remained high, presumably because intruders were unaware of the decreased resource value. This suggests that changes in resource value necessitate a learning period before non-territorial individuals can optimise the balance between intrusion risk and energy gain.

Our experiment demonstrates that changes in behaviour in response to the changes in resource quality vary across time and in response to the kind and amount

of the information used to make a decision. For some food types, such as nectar, immediate sensory information about concentration is sufficient for the animal to know the resource has changed and to what degree. Animals cannot respond as rapidly to resources that change in amount as the animal will have to use physiological processes of gut filling, digestion and so on to determine the changes. A clear example of a cognitive, rather than a physiological decision, was the reduction in territory size immediately after the first feed of 25%.

Most work on foraging decisions is conducted under a patch choice paradigm in which animals may continue to sample from the less preferred option (or at least have access to the less preferred option). Such choice experiments allow investigation of preference for various options but in some situations, as in our experiment, animals have no choice (although our birds could always forage on natural flowers) but to adjust behaviours appropriately to the new resource level. In this situation, one can then use the very first response to investigate the kind and amount of information required to reach a decision and to determine the different rate of change in subsequent behaviours. In nectarivores, at least, this approach allows differentiation of different kinds of information used (e.g. sensory, physiological, cognitive) as well as quantification of the number of experiences required for a behavioural response. Finally, we note that while long-term responses to changes in resource value are often of most interest, there is variation in the rate at which behaviours change and some of those changes are conspicuous only transiently.

Chapter 3 **Hummingbirds choose not to rely on good taste**

Ida E. Bacon, Andy T. Hurly and Susan D. Healy

I designed the experiment in collaboration with SDH and TAH, collected and analysed the data and wrote the manuscript with comments from SDH and TAH.

Abstract

To increase their chances of survival and reproduction, animals must detect changes in food quality and then decide if, and how quickly, to adjust their behaviour. How quickly an animal responds to change will depend on the information available (cognitive, sensory or physiological) and how it weights those types of information. Surrogate measures of meal size suggest that sensory information is used to make initial choices about how much to eat following changes in resource quality, choices that are subsequently changed and refined as further information becomes available. Using direct measures, we investigated the amount of food consumed, the time taken to feed and inter-bout intervals of rufous hummingbirds before and after changes in sucrose concentration. The hummingbirds used both cognitive and physiological information to decide how much to drink but appeared to ignore sensory information such as taste. We conclude that detailed information about the early responses animals make to changed resources can provide crucial insights as to the types of information they rely on most in their decision-making.

Introduction

Food availability (amount and quality) varies in both space and time and we expect animals to respond to changes in food quality in a timely manner. Food quality poses a particular problem for animals, because while they tend to prefer higher quality food, they require less of it to maintain their body condition than of poorer quality food so animals must control their intake (e.g. Scalfani 2004; Tamm & Gass 1986). Like weight loss, weight gain can have negative consequences including, in the case of birds, increased flight costs, reduced manoeuvrability and increased predation risk (DeBenedictis et al. 1978; Gosler et al. 1995; Macleod et al. 2005). Therefore, where access to food is unrestricted, animals should consume relatively less when preferred high quality food is available than they do when food quality is lower.

Animals base decisions about where to forage and how much to eat on both expectations of resource quality and current information. A combination of recent and more distant memories are used to form expectations about current conditions, with more weight given to more recent information (Hirvonen et al. 1999; Shettleworth & Plowright 1992). By comparing these memories and expectations with current information, foragers are able to detect changes in patch quality and change their behaviour appropriately, neither too hastily nor too slowly (Hall et al. 2007; Hirvonen et al. 1999; Lara 2006; Vasquez et al. 2006).

An animal gains information about the current situation in two ways, sensory input and physiological feedback. Each of these may vary in how readily the animal can access the information: orosensory information, such as taste, is available

immediately on encountering an item of food while physiological information from post-ingestive feedback becomes available only after a temporal delay as the first meal is digested (Sclafani 1994; Yearsley et al. 2006).

How much an animal decides to consume depends on how much it wants the food on offer. Animals develop an internal representation of the value of food (often referred to as its hedonic value) based on the sensory attributes of the food and the animal's body condition. Indeed, both vertebrates and invertebrates tend to prefer stimuli they encountered when in poorer condition a behaviour known as state-dependent learning (Pompilio et al. 2006). In general, foods that are better than normal are more desirable (hedonically positive) and *vice versa*. Following changes in food quality, the internal value of the food is up-dated as physiological feedback via 'incentive learning', which may take several experiences under the new changed conditions (Costa et al. 2007). The hedonic value is updated such that animals take an appropriate amount, (i.e. the hedonic value associated with a new food, that tastes worse than the animal's previous food, is increased with increasing experience so the animal does not under eat). However, reductions in preferences for (the hedonic value of) higher quality foods do not always happen which can lead to obesity (Sclafani 2004).

By knowing the previous experience and the delay between meals we can use an animal's behavioural responses following a change in resource quality to determine the relative importance of different kinds of information, as they become available over time, in animals' decisions about how much to consume of a changed resource (Sclafani 2004). This allows us to determine if animals will regulate their food intake using learned or innate preferences for certain flavours (e.g. sweetness)

rather than from direct information about food quality obtained from physiological feedback (Ackerman et al. 1992; Sclafani 1987; Sclafani 1994). For example, in rats, preferred flavours can induce increased consumption and weight gain independently from the actual nutrient content of the reward received (Sclafani 2004).

While models of decision-making suggest that in general animals should use more reliable types of information such as post-ingestive feedback in preference to information such as taste that is only indirectly related to a meal's physiological consequences (Koops 2004; McLinn & Stephens 2006), little is known about the sequence of adjustments animals may make to their behaviour as different sorts of information become available following changes in resource quality. For first experiences of new food, when only sensory information is available, it is conceivable that the food's hedonic value, relative to the previous diet, may significantly affect an animal's decision about how much to consume such that it takes more of better tasting foods than it would after it had fed on those foods for sometime.

In this experiment, we examined the behavioural responses of territorial male rufous hummingbirds to changes in resource quality (sucrose concentration), in order to determine whether the kind of information a bird uses to decide how much to drink on encountering a change in resource quality differs with increasing experience of the changed resource. Rufous hummingbirds are particularly suitable for this experiment as they have optimal meal sizes that differ with food quality such that they should drink less of preferred higher concentration sucrose solutions than of less preferred lower sucrose concentrations (DeBenedictis et al. 1978; Tamm & Gass 1986). This is a result of trade offs between the energy consumed and the additional

flight costs of carrying the meal (DeBenedictis et al. 1978). The sucrose concentration we provided was either 14% or 25%, a difference that these birds can easily detect using taste (discrimination is considered to be possible with 1-3%: Blem et al. 2000).

The first time birds encountered a new sucrose concentration they would only have had two sorts of information available to them to decide how much to drink, the taste of the sucrose solution and the memories of the taste and how much they had drunk of previous bouts, they may also remember their physiological responses to past combinations of concentration and volume. As hummingbirds can learn the position of more rewarding flowers, we assume that that the birds' memory for the taste of the more rewarding flower(s) is used to determine the expected taste of food at subsequent bouts (Hurly & Healy 1996).

Using taste and memory, hummingbirds must then decide how much to drink of the new concentration. Hummingbirds prefer stronger concentrations (Blem et al. 2000; Gass et al. 1999), at least up to 40% to 50% sucrose (Tamm & Gass 1986), thus, higher concentrations are expected to be more rewarding than the preceding concentration (would have a higher hedonic value). After first experiences of higher concentration the hedonic value associated with it may be lowered to prevent over consumption (Costa et al. 2007). Therefore, if they use taste alone to decide how much to drink, at the first experience of 25% sucrose after feeding on 14% sucrose, they should drink more of the 25% sucrose as it is preferred to, and offers a higher reward than the 14% sucrose they had been drinking previously. Likewise, at the first experience of 14% sucrose, taste-alone decisions would lead to birds drinking less than they had been drinking of the preceding 25% solution as 14% is poorer and

less rewarding (Costa et al. 2007). Additionally, on a first encounter, which the bird would not know was not just a one-off event, he may be expected to drink less of the 14% solution so as to return sooner and feed from 25%. Conversely, he should drink more of the 25% solution in case on the subsequent visits the concentration returns to 14%. This would be fairly typical of animals choosing among different quality rewards, here the acceptance of poor prey types is typically lower when they are less common and it pays the animal to wait for a better prey item (Berec & Krivan 2000). As hummingbirds prefer higher concentrations and can obtain a higher daily energy intake from them, it is possible that such behaviours would allow them to maximise feeding from high concentrations and avoid feeding from low concentrations when feeding from highly variable resources such as wildflowers (Blem et al. 2000; Gass et al. 1999). Both of the preceding mechanisms (decisions based on the hedonic value associated with taste and avoidance of unexpectedly poor rewards and vice versa), would lead to the amount drunk at first experiences of poorer concentrations being lower than the optimal meal size for that concentration and the amount drunk at first experience of higher concentrations being larger than optimal for that concentration.

Conversely, as animals readily form associations between foods tastes and their post-ingestive consequences, it is possible that hummingbirds could learn that they should drink less of higher than of lower concentrations. For example, chicks (*Gallus gallus*) can learn to avoid a colour of chick crumbs that are bitter tasting and slightly toxic more strongly than crumbs of a colour that just tastes bad within a foraging bout of 20 chick crumbs, forming an association between the different tastes and the colours (Skelhorn et al. 2008). It is important to note that responses to some

tastes such as avoidance of toxic tastes may be innate as well as learnt. As hummingbirds have a lifetime of experiences of different concentrations in which to learn the association between sucrose concentration and optimal bout volumes, we might then expect hummingbirds to change how much they drank at a first experience of a new concentration, in the direction of the optimal volume for that concentration.

However, if birds do not use taste to decide how much to drink but use only post-ingestive feedback, then they would not be expected to respond to the change in concentration until post-ingestive information became available after the first experience of a new concentration. Post-ingestive feedback informs animals of their energetic state, (Sclafani 2004; Yearsley et al. 2006). When there is little delay between ingestion and its post-ingestive consequences (e.g. feedback between meals), as in hummingbirds (Karasov et al. 1986; Tiebout 1989), information about an individual meal becomes available over fewer bouts, than when there is a longer delay to feedback (e.g. feedback only after several subsequent meals). This makes it easier for animals to attribute the characteristics of that meal with its post-ingestive consequences, allowing relatively rapid behaviour adjustments in response to a new food type (Yearsley et al. 2002). For example, sunbirds adjust their foraging behaviour in response to changes in concentration of sucrose within five to ten minutes (equivalent to two to five feeding bouts: Kohler et al. 2008). Like sunbirds, hummingbirds have rapid meal transit times of around 5-15 minutes (Downs 1997; Tiebout 1989). As over 50% of the hummingbirds first meal should have been fully digested between successive meals hummingbirds would have had at least some post-ingestive information by the second meal, following a change in concentration

(10-15 minutes; Tiebout 1989). Thus, if post-ingestive feedback but not taste was used to decide how much to drink following a change in concentration there would be no change in the size of the first meal but a change in the direction of the optimal meal size for that concentration at around the second meal onwards. However, this seems unlikely as taste is a very reliable indicator of food quality so we would expect some change in the size of the first meal to be made based on its taste.

At bouts after the first experience of a new concentration, post-ingestive feedback would start to become available. Regardless of whether hummingbirds choose to drink more, less or the same amount at the first experience of a new concentration than the previous concentration, we would then expect them to fine-tune that amount over subsequent experiences in the direction of the optimal amount for that concentration. Based on the time Sunbirds take to adjust their meal size, and similarly rapid gut transit times in hummingbirds (Kohler et al. 2008; Tiebout 1989), hummingbirds would be expected to be drinking roughly optimal volumes by around five bouts after a change in concentration.

Overall, we predict that hummingbirds will use a combination of taste and post-ingestive feedback to respond to changes in concentration. Any immediate responses must be based on taste whereas later responses are expected to be based on post-ingestive feedback. If responses are based on taste alone, birds are expected to either: 1) respond according to the food's hedonic value (drink more at first experiences (F1) of 25% than typical for 14% and less at F1 of 14% than typical for 25%); or 2) respond according to anticipated energy budget changes (drink less at F1 25% than typical for 14% but drink more at F1 of 14% than typical for 25%). While we expect hummingbirds to adjust how much they drink immediately using taste, we

have no clear prediction as to the direction of this change as both hypotheses seem equally plausible. By subsequent encounters post-ingestive feedback will be available and birds are expected to fine-tune the amount they drink to around an optimal for that concentration within about five feeding bouts.

Methods

Twelve male rufous hummingbirds (six in 2007 and six in 2008), which had been defending territories containing an artificial feeder (filled with 14% sucrose) for at least a week, were trapped, colour-marked and banded for individual identification. The field site was the Westcastle river valley in the Rocky Mountains, Alberta, Canada (Lat: 49.349024, Lon: -114.410902).

Not less than three days after trapping birds were trained to feed from an artificial flower containing 14% sucrose during the course of a day (6-9 hours). The 'flower' was a red cardboard disk (diameter 4.5cm) with a syringe cap inserted through its centre to act as a well. The flower was taped with red tape to the top of a cane (1m), which was pushed into the ground within five metres of the usual position of the feeder. Observers sat at least ten meters from the flower.

Following training, there were two experimental days, Treatment 1 and Treatment 2. Weather permitting, the two experimental days immediately followed the training day. As on training days, the feeder was removed and replaced with the artificial flower. In Treatment 1, the flower contained 14% sucrose for the first three hours, 25% sucrose for the next three hours and 14% sucrose for the final three hours. In Treatment 2, birds were presented with a flower containing 25% sucrose

for three hours, followed by three hours containing 14% sucrose and then finally three hours containing 25 % sucrose. Half the birds received Treatment 1 first and the other half Treatment 2 first.

The volume drunk and the duration of each of a bird's visit to the flower were recorded during both training and treatment days. Volumes were measured by refilling the syringe cap using a repeating pipette accurate to 10 μ l.

Results

We first determined whether birds' foraging behaviour (volume drunk, inter-bout intervals and drinking duration) differed between the two years of testing with a maximum-likelihood mixed-model in the statistical package JMP. The data were averaged for each bird for each experimental period. The model included bird (as a random effect), year and concentration. There was no effect of year on the amount the birds drank (ANOVA: $F_{1,10} = 1.42$, $p = 0.26$), inter-bout intervals ($F_{1,10} = 1.22$, $p = 0.29$) or duration of the feeding bout ($F_{1,10} = 0.26$, $p = 0.62$). Given no effect of year in these analyses, year was drop from subsequent analyses.

To investigate initial responses and the speed of behavioural adjustments following changes in concentration we required more than just the first and later feeding bouts following changes in concentration. As birds were expected to adjust their behaviour within five bouts, we split the data into: the first feeding bout (F1), when only sensory information would be available; a mean of feeding bouts two, three and four (F2-4), when post-ingestive feedback from the previous meals of the new concentration would be available; and thirdly, the mean of feeding bouts five

onwards (F5+), when behaviour was expected to be relatively stable.

The following data were analyzed with MANOVA repeated-measures tests in the statistical software package JMP. The Mauchly-criterion test was used to test for sphericity and, where data were non-spherical, we used Greenhouse–Geisser-adjusted degrees of freedom and p-values. Paired t-tests (two-tailed) were used to make further comparisons.

Volume consumed

To investigate if birds changed the amount they drank during their first experience of a new concentration, we compared the volume of the first feeding bout to the volume consumed during feeding bouts 5+ of the previous concentration. Birds did not change the amount they drank on their first visit to the feeder containing the new concentration. On both 25% and 14%, the birds drank a similar volume during F1 compared with the volume drunk during F5+ of the previous concentration (means= 131.70 vs. 122.28 μ l; $t_{11} = 1.8$, $p = 0.10$) and (means=113.12 vs. 102.41 μ l; $t_{11} = 0.64$, $p = 0.53$) respectively.

We then investigated whether birds changed how much they drank with increasing experiences of a new concentration both for changes from 14% to 25% sucrose and from 25% to 14% sucrose, we compared the means of F1, F2-4 and F5+ for each concentration. There was no effect of concentration ($F_{1, 11} = 0.23$, $p = 0.63$) or the number of feeding bouts since the concentration change ($F_{2, 22} = 0.76$, $p = 0.48$). There was a significant interaction between the concentration and the number of feeding bouts since the change in concentration such that the volume of 25% sucrose the birds drank decreased from F1 to F5+ on 25%, while the amount of 14%

sucrose consumed increased from F1 to F5+ ($F_{2,22} = 5.72$, $p = 0.01$; Figure 1).

Decreases in bout volume with increasing experience between F1 of 25% and F5+ of 25% were significant (means = 131.70 vs. 109.07 μl ; $t_{11} = 3.43$, $p < 0.01$), increased in bout volume between F1 and F5+ of 14% were nearly significant (means = 113.12 vs. 139.18 μl ; $t_{11} = 2.04$, $p = 0.06$).

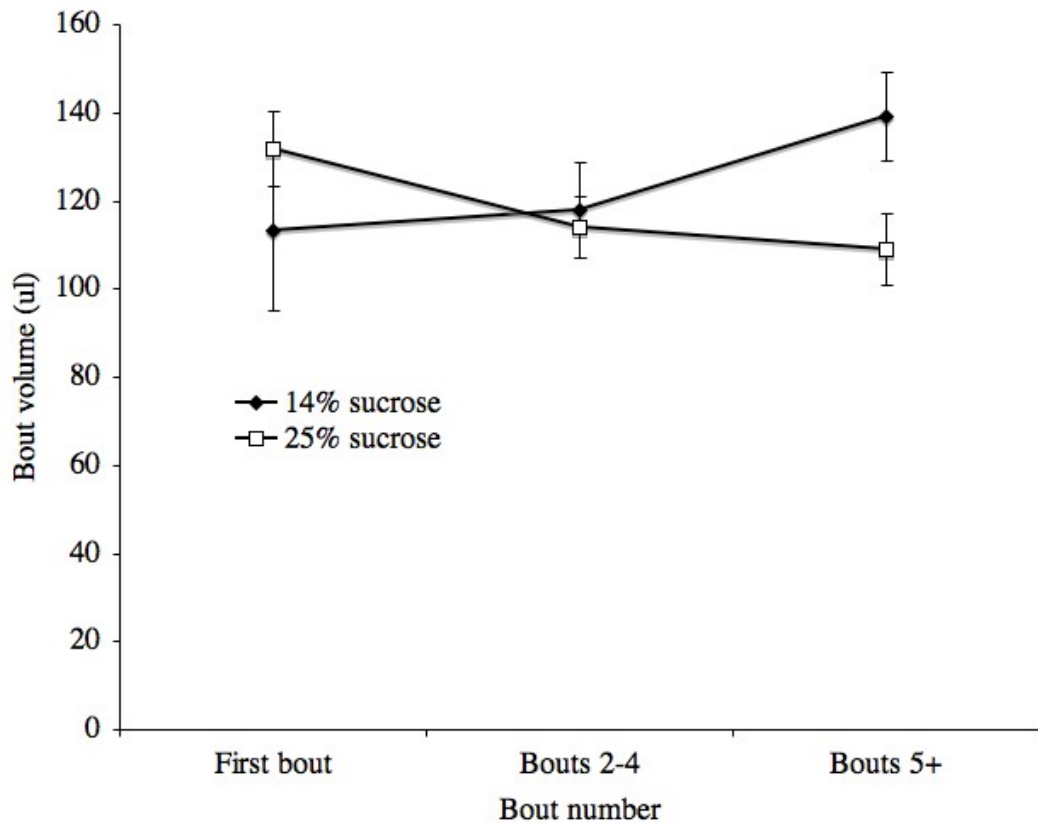


Figure 1. The average volume of the first feeding bout, the 2nd to 4th bouts and feeding bouts five onwards, following changes from 25% to 14% sucrose (black diamonds) and 14% to 25% sucrose (open squares). Data are means and standard errors. $N=12$

Having found that following changes in concentration birds changed how much they drank with increasing experiences of the change, we looked at this pattern in more detail to determine how many bouts it took birds to fully adjust the volume they drank to a fairly stable amount for that concentration. To do this, we compared the volume of F2-4 when birds may still have been adjusting how much they took to F5+ when they were expected to have reached a stable decision. If birds adjusted their behaviour in less than five bouts, then the volume of F2-4 were expected to be similar to the volume of F5+. On 25%, the adjustment of the amount drunk was complete by F2-4 (comparison F2-4 to F5+: means= 113.99 vs. 109.07 μ l; $t_{11} = 0.66$, $p = 0.52$). However, the adjustment of the amount drunk on 14% was slower: the volumes of F2-4 were still significantly less than the volumes drunk during F5+ (means= 117.98 vs. 139.18 μ l; $t_{11} = 2.48$, $p = 0.03$).

Time of day effects

To test if birds behaved differently throughout the day we compared the mean volume the birds drank in F5+ for each concentration at each time of day (14% a.m., noon, p.m. and 25% a.m., noon, p.m.). Birds drank larger volumes of 14% sucrose than of 25% sucrose (mean 14% = 126.60 \pm 12.85 μ l, mean 25% = 112.47 \pm 10.52 μ l; $F_{1,11} = 5.21$, $p = 0.04$), and they drank more of both concentrations (mean morning = 99.25 \pm 2.15 μ l, mean afternoon = 138.10 \pm 5.50 μ l; $F_{2,22} = 10.69$, $p < 0.01$), as the day progressed.

Feeding bout duration

We investigated the duration of feeding bouts to determine the direction of change, if any, following a change in concentration. We compared the means of feeding bout duration for F1, F2-4 and F5+ within each concentration. Following changes to 25% the duration of feeding bouts decreased from F1 to F5+, but conversely, increased from F1 to F5+ following changes to 14% ($F_{2,22} = 5.87$, $p = 0.01$; Figure 2). The duration of F5+ for 14% and 25% were similar (means = 7.63 vs. 7.76 sec; $t_{11} = 0.35$, $p = 0.73$).

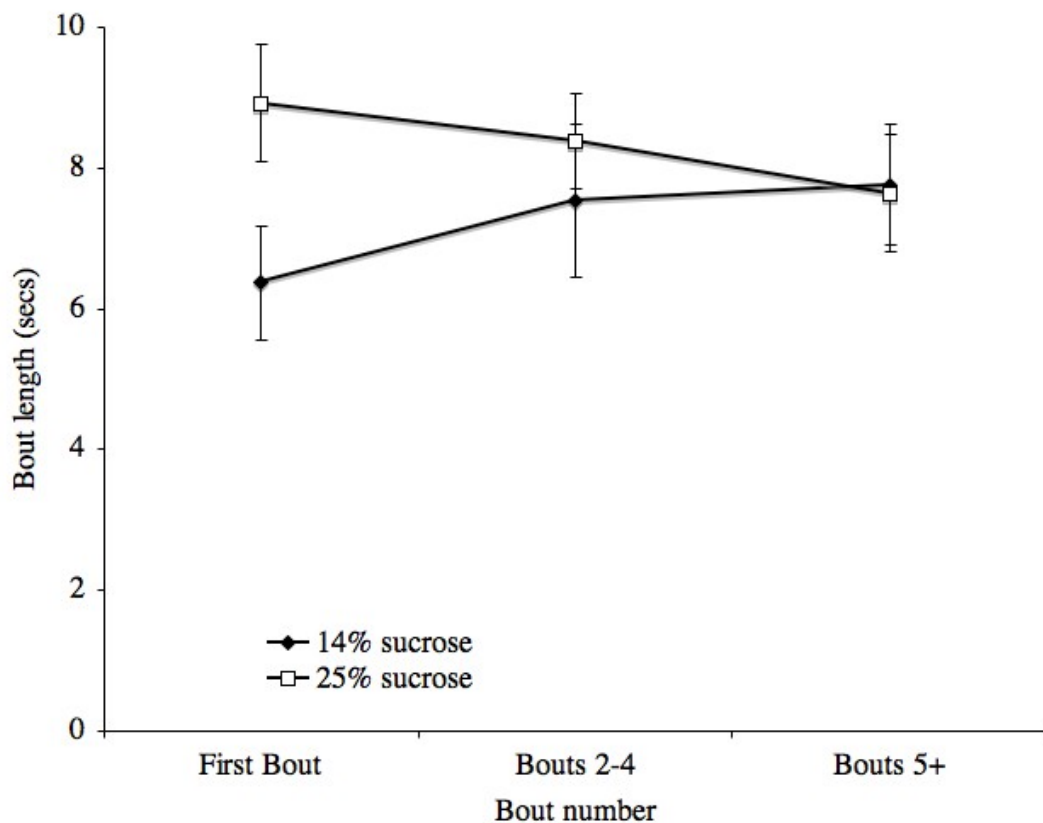


Figure 2. The average duration of the first feeding bout, the 2nd to 4th bouts and feeding bouts five onwards, following changes from 25% to 14% sucrose (black diamonds) and 14% to 25% sucrose (open squares). Data are means and standard errors. $N=12$

To see if birds changed the length of their feeding bouts at the first bout on a new concentration we compared the duration of the first feeding bout to the mean of feeding bouts 5+ of the previous concentration. The duration of F1 on 14% sucrose was significantly shorter than it was for F5+ of the previous 25% period (means= 6.38 vs. 7.64 sec; $t_{11} = 2.68$, $p = 0.02$). The duration of F1 on 25% sucrose was significantly longer than it was for F5+ of the previous 14% period (means= 8.92 vs. 7.76 sec; $t_{11} = 2.14$, $p = 0.05$).

Intake rates

We calculated the mean intake rate of sucrose (bout volume/bout duration) for F1, F2-4 and F5+ within each concentration. Intake rates did not differ with increasing experience of either concentration ($F_{2,22} = 0.82$, $p = 0.45$). However, intake rates tended to be higher when birds were drinking 14% rather than 25% sucrose (mean 25%= 14.93 ± 1.00 $\mu\text{l/s}$, mean 14%= 19.14 ± 2.93 $\mu\text{l/s}$; $F_{1,11} = 3.89$, $p = 0.07$).

Inter-bout intervals

We compared the means of inter-bout intervals following F1, F2-4 and F5+ for each concentration. Inter-bout intervals decreased from F1 on 25% to F5+, but increased from F1 on 14% to F5+ (Interaction between concentration and number of feeding bouts: $F_{1,25,13.72} = 9.73$, $p < 0.01$; Figure 3). Inter-bout intervals on 14% were shorter than when birds were feeding on 25% ($F_{1,11} = 78.55$, $p < 0.01$).

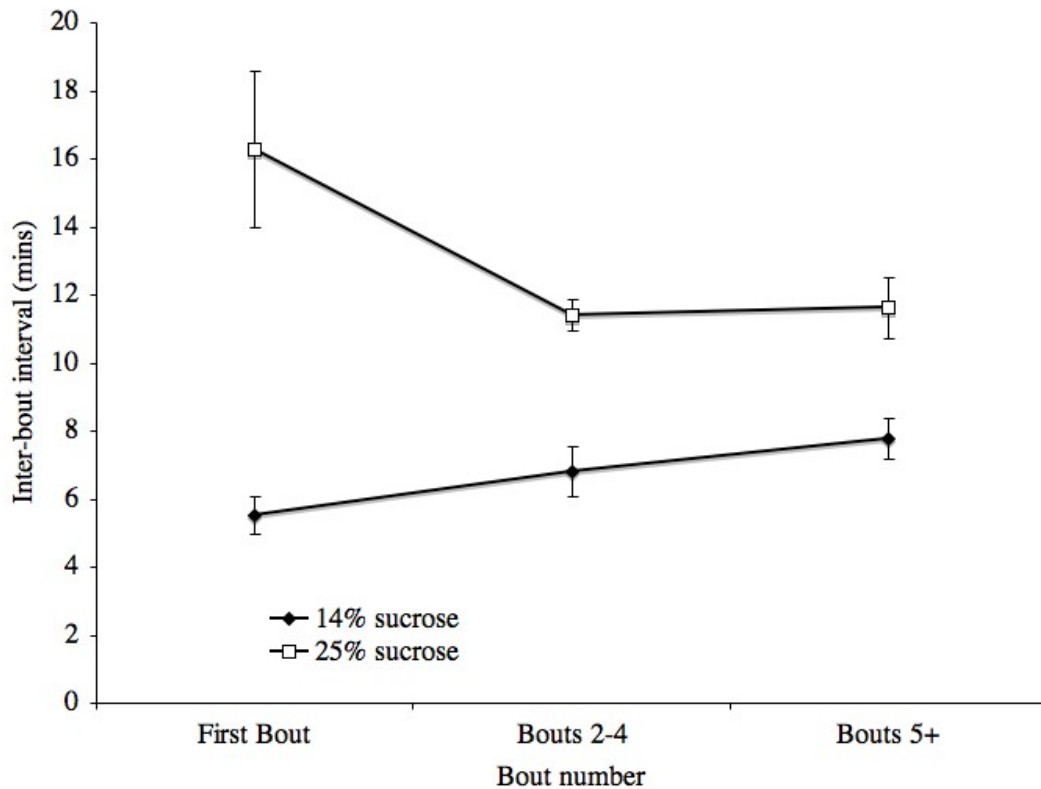


Figure 3. The average inter-bout interval following the first feeding bout, the 2nd to 4th bouts and feeding bouts five onwards, for changes from 25% to 14% sucrose (black diamonds) and 14% to 25% sucrose (open squares). Data are means and standard errors. $N=12$

We then looked to see how rapidly birds adjusted their behaviour following changes to 25% or 14% sucrose. If birds had adjusted how much they drank before feeding bout five then the inter-bout intervals of F2-4 would be similar to the inter-bout-intervals of F5+. On 25% the decrease in inter-bout intervals occurred during F2-4 (comparison of intervals 2-4 vs. 5+: means= 11.40 vs. 11.62 mins; $t_{11} = 0.31$, $p = 0.76$). On 14% the adjustment of inter-bout intervals was slower, as inter-bout intervals 2-4 were significantly shorter than were inter-bout intervals 5+ (comparison of intervals 2-4 vs. 5+: means= 6.80 vs. 7.77 mins; $t_{11} = 2.74$, $p = 0.02$).

Time of day effects

We used inter-bout intervals 5+ for each concentration at each time of day to investigate if inter-bout intervals changed across the day. Inter-bout intervals were shorter when the birds were drinking 14% sucrose than when drinking 25% sucrose ($F_{1,11} = 56.70, p < 0.01$). Inter-bout intervals on both concentrations increased across the day ($F_{2,22} = 8.85, p < 0.01$).

To estimate whether changes in inter-bout interval and the amount the birds drank across the day were affecting the birds' rate of energy intake, we calculated the average joules available to birds per unit time for each time of day:

Joules/per minute = (mean meal size (litres) / mean inter-bout interval (min))

*(mols sucrose/litre (14% sucrose = 0.431 mol/l and 25% sucrose = 0.806 mol/l))

* mass of 1 mol of sucrose = 342g

*joules/g sucrose = 16480j.

Birds consumed the same number of joules per minute on both concentrations ($F_{1,11} = 1.55, p = 0.24$; Figure 4) and all times of day ($F_{1,38,15.21} = 1.44, p = 0.25$) respectively.

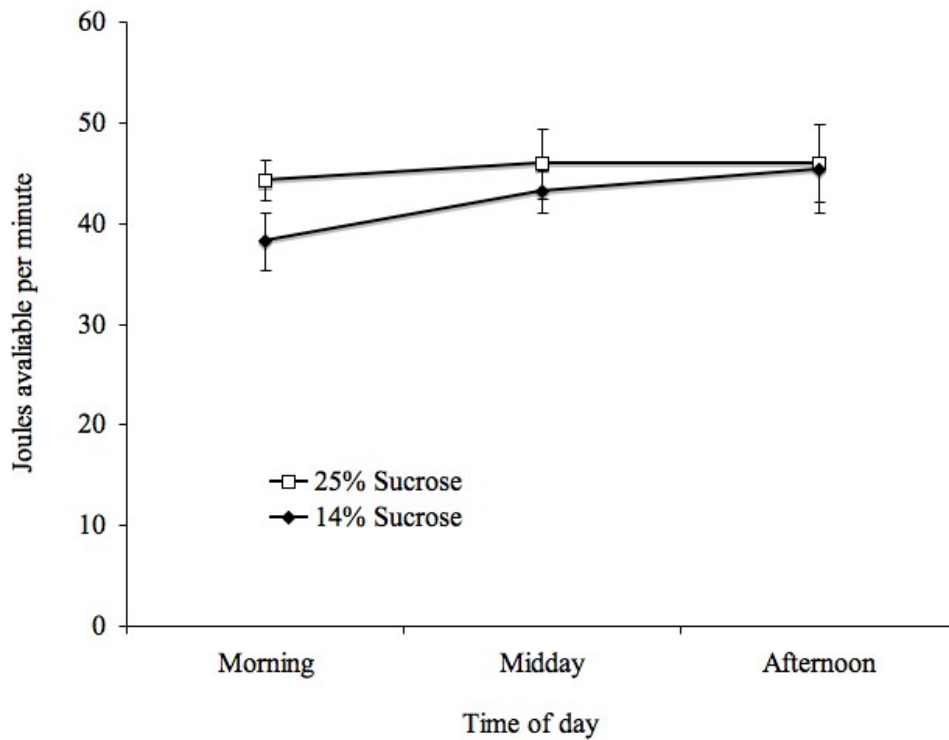


Figure 4. The average joules available per minute at different time of day for 14% sucrose (black diamonds) and 25% sucrose (open squares). Data are means and standard errors. $N=12$

In order to test whether increasing experience of a new concentration affected the energy available to birds in subsequent inter-bout intervals, we calculated the mean energy available to them per minute following F1, F2-4 and F5+ for changes to 25% and 14% sucrose. There was no affect of increasing experience ($F_{2,22} = 0.33$, $p = 0.72$) or of concentration ($F_{1,11} = 0.16$, $p = 0.70$).

The relationship between inter-bout interval and the volume drunk

We used a mixed effects model fitted by maximum likelihood in the statistical package R (LMER) to investigate whether the volume birds drank was better explained by the time since their last meal or the time until they fed again. The data were log transformed for normality and bird was included as a random effect. Both intervals were longer when the bird drank more (prior to feeding: $F_{1,1382} = 17.89 = 1.55$, % var = 2.5 $p < 0.01$; after feeding: $F_{1,1382} = 114.99$, % var = 9.9, $p < 0.01$). However, the interval between the feeding bout of interest and the following bout explained more than twice the variance in the volume consumed in that bout than the time since the last bout. There was a significant interaction between the inter-bout interval before a feeding bout and the inter-bout interval following that bout, such that, if birds stayed away longer before a feeding bout they subsequently fed again slightly sooner than would otherwise have been expected ($F_{1,1382} = 11.79$, % var = 1.9, $p < 0.01$).

Discussion

Unexpectedly, birds did not change the amount they drank at their first experience of a new concentration. However, as we know they can detect much smaller changes in concentration using taste, we are sure that the birds will have detected the change (Blem et al. 1997; Blem et al. 2000; Roberts 1996; Stiles 1976; Stromberg & Johnsen 1990a). Birds did, however, change how much they drank of a new concentration after the second experience. There are two possible explanations for this delay in changing how much they drank following a change in concentration. Firstly, from a

single experience the bird cannot be sure that the change in sucrose concentration is more than transitory. There may be little cost associated with taking one drink of a sub-optimal volume so birds may simply have ignored the change. This possibility we consider unlikely because the birds are expected to capitalize on one-off, or first, experiences of higher concentrations by drinking more, or to decrease the amount they drink, depending on the optimal drink volume for that concentration. Animals' acceptance of poor food items is low if the energy gained from those food items is less than that gained by waiting for a larger reward (Stephens & Krebs 1986). This possibility, then, does not explain why they did nothing in response to a change.

The second, more plausible explanation is, that although birds can detect the change using taste, they need further information to assess the magnitude or reliability of the change. In this situation we would expect them to drink the same amount as previously and to wait for post-ingestive feedback from that meal to adjust the amount they drink at the next meal in the right direction relative to the size of the first and thus avoid over-shooting the optimal meal size of the new concentration. Indeed, starlings will only use taste to inform their foraging decisions when it reliably informs them about the food's content (Skelhorn & Rowe 2010). The post-ingestive information available from the new concentration would have been considerable by the time the bird came to feed for a second time (Tiebout 1989). It is possible that birds further delayed reaching a final decision on the appropriate amount to drink so as to confirm that the change was stable (Shettleworth & Plowright 1992). This may be why birds took up to four experiences to adjust how much they drank following changes to 14% sucrose.

The way in which birds responded depends on which behaviour was measured. Birds drank for longer on their first encounter of the higher concentration and for less time when they encountered the lower concentration for the first time. Had bout duration been the sole measure we used, we would have concluded that birds, in fact, drank more on first experiences of high concentrations and drank less on first experiences of lower concentrations and, consequently, that birds did not wait for post-ingestive feedback before adjusting the amount they drank. The discrepancy between our direct and surrogate measures of drink volume may be explained by differences in the viscosity of the two solutions: the higher the viscosity, the longer it takes a hummingbird to obtain the same volume (Roberts 1995). Surrogate measures of food intake, such as bout-lengths and average bite masses, are frequently used and are fairly accurate over multiple bouts. However, they may be inaccurate as measures of the amount consumed at a bout-by-bout level, particularly where several food types are being eaten (Agreil et al. 2005; Magrath et al. 2007). This inaccuracy can then lead to misinterpretation as to the information on which animals base their decisions with regard to how to respond to a changed resource.

The amount drunk, and the frequency at which birds came to the feeder, took several visits before they settled to a new rate. The first inter-bout interval following an increase in concentration was the longest and that following a decrease in concentration the shortest. This was expected, as the time between meals is largely dependant on the time required to digest most of the sucrose in the previous meal (DeBenedictis et al. 1978; Hainsworth 1989; Wolf & Hainsworth 1977). Indeed, the amount birds drank was strongly correlated with both the time since the previous

feeding bout and the time until they fed again and the energy available to them per minute did not change with increasing experience of a new concentration. Of these two time periods, however, the variability in the amount drunk was accounted for better by the interval following the current feeding bout. This relationship opens up the possibility that hummingbirds might be able to plan their near-future activities (Clayton et al. 2003; Raby et al. 2007).

Examining the pattern of behavioural responses associated with changes in food quality allows determination of the information hummingbirds use to make foraging decisions. They probably use taste to detect changes but do not necessarily then respond, memory to compare past and present conditions (to drink the same amount as before at first experiences of new concentrations) and physiological post-ingestive feedback to fine-tune how much they drink over several experiences. Decision-making models are typically based on the assumption that animals use the most reliable types of information available (Koops 2004; van Bergen et al. 2004). However, we have shown that animals may, at least initially, ignore what is a very accurate indicator of the relative quality of their food. It may be that some animals generally prefer to delay changing their behaviour until they have several types of information on which to base their decisions. If so, then detailed information about the amount and sorts of information animals require and prefer to use to make decisions may greatly improve the resolution with which we are able to model and interpret animal decision-making not only with regard to initial responses to change but also to the time animals may take to reach a stable response level. Finally, it appears that care should be taken when using surrogate measures of meal size

especially where the questions being addressed concern an animal's responses to changes in food resources.

**Chapter 4 Both the past and the present affect risk-sensitive
decisions of foraging rufous hummingbirds**

Ida E. Bacon, Andy T. Hurly and Susan D. Healy

I designed the experiment in collaboration with SDH and TAH, collected and analysed the data and wrote the manuscript with comments from SDH and TAH.

Abstract

There is substantial evidence that an animal's current energy budget affects its preference for food patches that provide a constant reward relative to patches that provide a variable reward, when both patches have the same mean reward. Animals currently on a positive energy budget are expected to choose the constant option whereas animals on a negative budget are expected to use the variable option.

However, there is increasing evidence that prior experience can affect an animal's current decisions. We investigated choices made by rufous hummingbirds when they were tested with strong or weak sucrose solutions following several days of foraging on those strong or weak solutions. Foraging from weak concentrations prior to and during testing led to a higher preference for the variable option whereas foraging from strong concentrations led to an increased preference for the constant option.

We suggest that the energetic conditions experienced by animals prior to testing, had a significant impact on the animals' risk-sensitive decisions and their memories of those prior conditions may have played an additional role.

Introduction

Given two food options where reward quality and mean reward amount over time are equal for both options but where the variability in reward amount differs, animals often prefer one option over the other (Drezner-Levy & Shafir 2007; Heilbronner et al. 2008; Kacelnik & Bateson 1996; Logan 1965; Real et al. 1982; Schuck-Paim & Kacelnik 2007; Young 1981). For example, blue jays (*Cyanocitta cristata*) given the option of foraging in a variable patch that provided either two or four half mealworms (a mean of three halves) or a constant patch that provided three half mealworms, preferred to feed from the constant patch (approx. 70% of feeds, Clements 1990). This sensitivity to variation in reward can be explained by the energy budget rule (Stephens 1981), which stipulates that when an animal is on a positive energy budget and is not in danger of starvation, it should choose the constant option in order to minimize the chance that it will encounter numerous small rewards and risk starvation. However, an animal in danger of starvation should choose the variable option as it increases the chance that the animal will encounter sufficiently large rewards to avoid starvation. The energy-budget rule does not fully explain risk-sensitive choice as it predicts exclusive preference for either the constant or variable option, whereas partial preferences are observed in the vast majority of cases (Hurly 2003; Kacelnik & Bateson 1996). One possible explanation for partial preferences is that animals continue sampling all options in order to enable resource tracking (Krebs et al. 1978; Schuck-Paim & Kacelnik 2007). Another possible reason for partial preferences is that animals forage among rewards

in proportion to how rewarding they find them rather than entirely avoiding less preferred options (Schuck-Paim & Kacelnik 2007; Shapiro 2000).

Manipulations of energy budgets either before or during choice trials show that an animal's sensitivity to reward variability is, indeed, state-dependant (Kacelnik & Bateson 1996). For example, Dark-eyed juncos (*Junco hyemalis*) preferred the constant reward when on a positive energy budget, were indifferent when on a balanced energy budget and preferred the variable reward when on a negative energy budget (Caraco 1981). Likewise, Yellow-eyed juncos (*Junco phaeonotus*) preferred the variable option under conditions of low ambient temperature and the constant option under warmer, less energetically demanding, conditions (Caraco et al. 1990). Typically, in this kind of experiment, manipulations are designed so that animals experience a particular energy budget, which is then expected to be the main influence on their risk sensitivity. However, the duration of the energy budget manipulations, which varies considerably among experiments, may play a significant role in the outcome of the experiments as previous foraging experiences often affect current foraging decisions. For example, young canaries fed for eight weeks on a single seed type (hemp, niger, millet or linseed), and then on a mixed seed diet for fifteen weeks, preferred the seed type on which they were reared. Birds reared on a mix of all four seeds, on the other hand, preferred hemp seed (Doherty & Cowie 1994). If the context animals experienced weeks ago (such as the canaries' previous diet) can affect current preferences, then it seems likely that previous experiences may also affect an animal's sensitivity to variability. Prior knowledge of patch types and their frequency in the environment can be used by animals alongside current sampling information to form an estimate/expectation of patch quality and inform,

for example, patch leaving decisions (e.g. McNamara 1982). This is referred to as Bayesian or Bayesian like decision-making and has been described in many species including: The Arizona pocket mouse, *Perognathus amplus*; Merriam's kangaroo rat, *Dipodomys merriami*; and the Round-tail ground squirrel, *Spermophilus tereticaudus* (Valone & Brown 1989); Inca doves, *Columbina inca* (Valone 1991); Black-chinned hummingbirds, *Archilochus alexandri* (Valone 1992), and Bumblebees, *Bombus impatiens* Cresson (Biernaskie et al. 2009).

Expectations can be affected by experiences from throughout the animal's life (Simitzis et al. 2008a; Simitzis et al. 2008b). Effects of expectations about the quality of specific foraging locations, on foraging behavior have been demonstrated both in vertebrates and invertebrates (Gil et al. 2007; Lima 1983; Schilman & Roces 2003). Based on expectations, animals return more often to, and invest more effort in, investigating locations or food types associated with higher rewards, but if those reward values are decreased animals continue to show more interest in those resources than their current value would predict. Thus, past experience is clearly often used in foraging decisions and to aid the assessment of current conditions. In addition, current conditions may also be assessed in terms of contrast: change relative to past conditions. Marsh and Kacelnik (2002) demonstrated that starlings foraging in a risk-sensitive task selected options according to whether the current conditions were perceived as better or worse than past conditions. In this instance the past affected current decisions as if a change in energy budget/ resource value had occurred when in fact it had not. When changes in resource value are real such contrast effects may also be at work but as they change behavior in the same way as actual changes in resource quality, they would be hard to detect.

Here we tested whether the foraging context preceding test conditions and the foraging context during test conditions affected rufous hummingbirds' (*Selasphorus rufus*) preference for high or low variability rewards. We manipulated food concentration in the feeder (low vs. high) before testing and during testing (low vs. high) in a factorial design. As far as we are aware this is the first time that the effects of both past and current foraging conditions on risk-sensitive foraging have been tested simultaneously. Hummingbirds are useful subjects for studies involving energy manipulations as their high metabolic rate means they must feed frequently throughout the day and balance their energy budget daily or even hourly. Their natural food (flower nectar) varies considerably in the concentration and volume within and between plants (Ornelas et al. 2007) and in experimental manipulations of reward variability hummingbirds are, indeed, sensitive to reward variability, usually avoiding flowers providing the highest variability in reward volume (Biernaskie et al. 2002; Hurly & Oseen 1999). Our hypothesis was that both past and current context would affect birds' sensitivity to variability. Assuming that higher energy budgets lead generally to risk aversion, birds should choose the constant option more often when they experienced the higher sucrose concentration both prior to and during testing. They should choose the constant option least often when they experienced the lower concentration prior to and during testing. The factorial design allowed us to determine whether the pre-testing and testing influences are additive or whether they interact in a more complex fashion. There were no clear predictions as to the effects of expectations that may be carried over from the pre-testing to the testing period, nor with regard to the effects of contrast between the periods (e.g. the perception of the foraging situation improving from low concentration food during

the pre-testing period to high concentration food during testing or vice versa). Unlike most experiments investigating risk-sensitivity we were not concerned with significant departures from risk-neutrality, but rather with how the conditions prior to and during testing influenced the relative preferences for constant and variable options.

Methods

Subjects were eight male rufous hummingbirds who had been defending territories for several weeks in the Westcastle River Valley, Alberta, Canada (+49° 20' 56.41", -114° 24' 35.08"). Each male's territory contained a 14% sucrose *ad libitum* feeder that he defended and from which he obtained almost all of his nectar. To allow individual identification birds were marked with waterproof, colored, non-toxic ink on the upper breast.

Subjects were trained to feed from arrays of 20 wells and to associate the color of wells with a particular sucrose concentration and volume variability reward type. The wells (10 mm deep x 3.5 mm diameter) drilled in to a rectangular plexiglas plate (28 x 21.5 x 1.2 cm) were arranged in a hexagonal pattern such that neighboring wells were 5.2 cm apart. The plate was attached to a stake that held it approximately 60cm from the ground. Surrounding each well was a paper reinforcement colored pink, purple, blue or orange, each representing a different reward type. Two of the reward types, 14% constant and 25% constant always contained 20 μ l sucrose solution, of 14% and 25% sucrose, respectively. The different concentrations were indicated by the color of paper reinforcement surrounding the wells. The other two

reward types were 14% variable and 25% variable represented by two colors that differed from those used for the constant wells. Wells in the variable reward types contained either 10 or 30 μ l sucrose solution in equal number giving a mean reward of 20 μ l sucrose solution. The color of the reinforcement did not indicate the volume of sucrose in the variable wells. No two birds had the same well-colour/well-type combinations.

Training consisted of presenting the bird with a board on which all 20 wells were marked with a single color i.e. all 20 wells were of the same reward type. The bird was allowed to visit and feed from the array five times before being presented with a board on which all the wells were of one of the other reward types. Once all four reward types had each been presented on five occasions, birds were presented with all four types again but with three successive visits for each type. The last four sessions of training consisted of birds visiting a board of a different reward type just once before another reward type was presented. The sequence of presentation of well types was different for each bird. Training was completed in a single day. Feeders were removed both during training and testing. Birds obtained virtually all of their daily energy requirements from either the feeder or test arrays and were seen to visit natural flowers only on one or two occasions.

The training day was followed by two to three days (mean 2.50 ± 0.19 days) in which the bird's feeder contained either 14% or 25% sucrose. On the morning following the days of feeder access, the bird was presented with a choice test. Each choice test was followed by two to three days during which the birds were provided with only their feeders (mean 2.04 ± 0.04 days). Each bird experienced all four treatments, and the order of treatments differed across the eight birds:

Treatment 1: (25|25): Two days feeding from 25% sucrose in the feeder was followed by a variable/constant choice test using 25% sucrose.

Treatment 2: (25|14): Two days feeding from 25% sucrose in the feeder was followed by a variable/constant choice test using 14% sucrose.

Treatment 3: (14|25): Two days feeding from 14% sucrose in the feeder was followed by a variable/constant choice test using 25% sucrose.

Treatment 4: (14|14): Two days feeding from 14% sucrose in the feeder followed by a variable/constant choice test using 14% sucrose.

During choice tests all wells on the board contained sucrose of the same concentration but half of the wells were designated variable flowers (i.e. five contained 10 μ l and five contained 30 μ l) while the other half the flowers were designated constant wells (i.e. 10 flowers containing 20 μ l). Each well type was marked with the appropriate color from the training period. So that the birds did not learn the position of the wells containing 30 μ l, the location of 10 μ l and 30 μ l wells was changed every five feeding bouts (visits to the board). Additionally, the board was moved at least 15cm and rotated 90 degrees between every feeding bout. The sequence of each well visited and the volume consumed from each was recorded for each feeding bout. The volume of sucrose consumed from each well was measured by collecting and measuring the volume of any residue using a micro-capillary-tube and then subtracting that volume from the initial volume in the well.

We recorded 56-150 feeding bouts in total for each of the eight individuals (feeding rates were determined by the free-living birds). We used mean values for each treatment for each bird. Data were tested for normality using a Shapiro Wilk

test. The Mauchly-criterion test was used to test for sphericity. If variances were non-homogeneous, data were square-root transformed.

Results

Choice of constant or variable flowers - number of each type visited

We calculated the proportion of all wells visited that were the constant option for each bird for each treatment. We used one-sample t-tests to determine birds' preferences in each treatment (the proportion of constant wells chosen compared to an expected 50% if choices were random). Birds preferred the constant option in the 25|25 treatment (mean=62.22%; $t_7 = 3.72$, $p < 0.01$), but had no statistically significant preference in all other treatments (means= 46.41 to 55.49%; $t_7 = 1.26$ to 0.79 , $p = 0.24$ to 0.45).

The within-subject design of the experiment permits us to examine patterns of choices across the two treatments (feeder concentration and test concentration). In the following analyses choices to the constant option were compared across treatments using a maximum-likelihood mixed-model in R. Model simplification based on AIC (Akaike Information Criterion) was used to determine the significant factors and interactions. We are aware of concerns about using model simplification but feel it was justified in these circumstances and that the p-values are meaningful due to both the very limited number of comparisons made and only slight differences between the p-values values of factors in the full and reduced models (Mundry & Nunn 2009). The full model included: test concentration, feeder concentration, an interaction term between the two and bird as a random factor. The interaction term

was not significant and was removed from the model ($F_{1,21} < 0.01$, $p = 0.95$). Both in the full model and model with the interaction removed, the sucrose concentration used in the choice tests had a significant effect on the birds' choices: birds chose the constant option more often when the test concentration was 25% than when it was 14% (Full model: $F_{1,21} = 5.49$, $p = 0.03$, Reduced model: $F_{1,22} = 5.69$, $p = 0.03$, Figure 1). Feeder concentration also played a role. Although preference for the constant option did not quite change significantly with feeder concentration (Full model: $F_{1,21} = 3.44$, $p = 0.08$, Reduced model: $F_{1,22} = 3.56$, $p = 0.07$), removal of feeder concentration from the model increased AIC and the resulting model explained the variation significantly more poorly than did the model including feeder concentration (ANOVA comparison of the model including feeder concentration with the model excluding feeder concentration: $L.ratio_{2,4} = 3.70$, $p = 0.05$). We conclude, therefore, that the concentration of sucrose in birds' feeders for the two days preceding tests did have a significant effect on the choices they made during tests about which wells to visit. Specifically, when birds had fed for two days from a feeder containing 25% sucrose they were more likely to choose the constant option in the choice tests (Figure 1). Residuals were normally distributed (Shapiro Wilk W test: $W = 0.97$, $p = 0.60$).

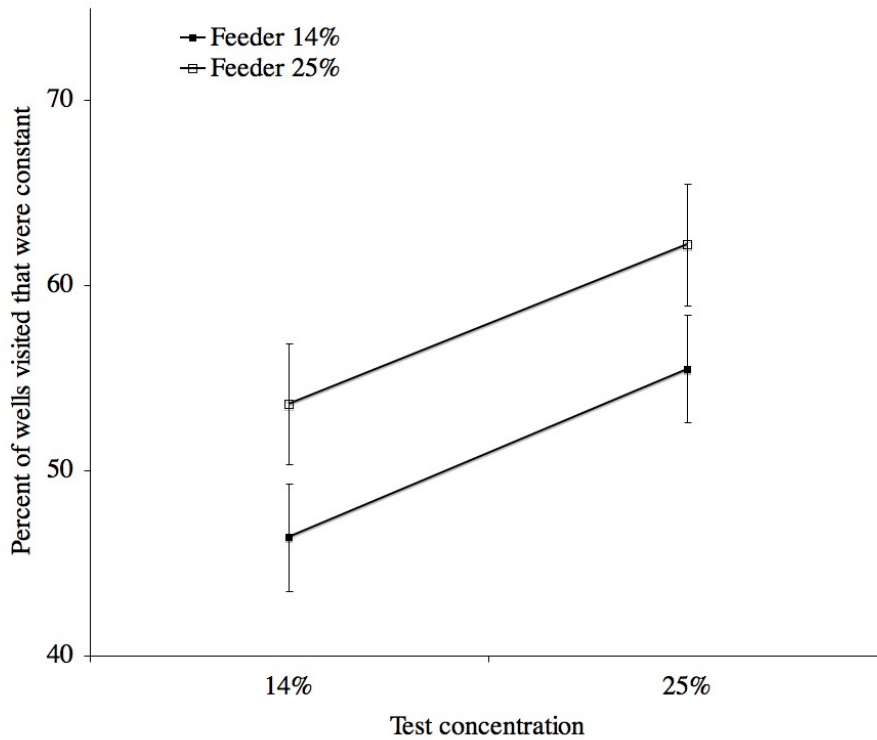


Figure 1: The proportion of the total number of wells visited that were the constant option for the four combinations of feeder and test concentration. Data are means and standard errors.

Choice of constant or variable flowers - volume of sucrose consumed

An alternative way to quantify preference is to measure the volume of sucrose consumed from each flower type because birds did not necessarily consume all of the nectar from each flower they visited. We used one-sample t-tests to determine the birds' preferences in each treatment (the proportion of sucrose drunk per feeding bout from constant wells compared to an expected 50% if choices were random). Birds drank more from the constant option in the 25|25 treatment (mean= 63.11%; $t_7 = 3.61$, $p < 0.01$) but showed no preference in all other treatments (means= 47.58 to 56.11%; $t_7 = 1.71$ to 0.58, $p = 0.93$ to 0.28).

The influence of treatments (feeder and test concentrations) on how much birds chose to drink from each flower type was then modeled (as above for total number of wells). Data were means of the total proportion of sucrose consumed from constant wells within feeding bouts, for each bird/treatment ($N = 8$). The full model included: test concentration, feeder concentration an interaction term between the two and bird as a random factor. The interaction was not significant ($F_{1,21} < 0.01$, $p = 0.99$) and was removed from the model. Both in the full model and model with the interaction removed, the sucrose concentration used in the choice tests had a significant effect on the birds' choices: birds drank more from constant wells than from variable wells when the test concentration was 25% (Full model: $F_{1,21} = 6.14$, $p = 0.02$, Reduced model: $F_{1,22} = 6.36$, $p = 0.02$). Feeder concentration also had a significant effect (Full model: $F_{1,21} = 4.13$, $p = 0.06$ Reduced model: $F_{1,22} = 4.27$, $p = 0.05$), such that when birds had fed for two days from a feeder containing 25% sucrose they drank more from constant wells during the subsequent choice tests. Residuals were normally distributed (Shapiro Wilk W test: $W = 0.96$, $p = 0.33$).

Choices of the variable option

To ensure that the constant/variable tests were not biased by birds learning which specific variable wells held $30\mu\text{l}$ of sucrose, we calculated the total number of $10\mu\text{l}$ and $30\mu\text{l}$ wells visited by each bird in the variable option. Birds did not visit more of either sort of well, indicating they did not learn the position of the $30\mu\text{l}$ wells (means: $10\mu\text{l} = 59.56$ wells vs. $30\mu\text{l} = 57.25$ wells; paired t-test: $t_7 = 1.06$, $p = 0.32$).

Role of increasing experience within and among bouts

We assessed the role of experience on decision-making during the test trials in three ways. First, we compared preferences for the constant option within feeding bouts. We calculated the percentage of first well choices that were to the constant option for each visit to the board and compared this value to the average value across all subsequent wells visited within the feeding bout. Choices made to the first wells visited during feeding bouts and the mean choice across all later wells in feeding bouts did not differ ($F_{1,50} = 2.16, p = 0.15$), indicating that birds' preferences at the beginning of a feeding bout did not change. The main effects of test concentration ($F_{1,50} = 15.65, p < 0.01$) and of feeder concentration ($F_{1,50} = 7.47, p < 0.01$) remained. There were no significant interactions ($F_{1,50} = 0.53$ to $0.03, p = 0.87$ to 0.47).

Second, we compared choices to the constant option in the first feeding bout with choices in all subsequent feeding bouts. Choices made during the first bout were similar to those made during later bouts ($F_{1,50} = 0.06, p = 0.80$), indicating that birds preferences did not change systematically between the first bout and later bouts. Again, the main effect of test concentration was significant ($F_{1,50} = 5.33, p = 0.03$) and that of feeder concentration nearly so ($F_{1,50} = 3.65, p = 0.06$). There were no significant interactions between the feeder concentration or test concentration and bouts, ($F_{1,50} = 0.80$ and $0.789, p = 0.36$ and 0.38 respectively). The interaction between feeder and test concentration was nearly but not quite significant ($F_{1,50} = 3.79, p = 0.06$).

Third, we tested whether preferences changed progressively across the course of each treatment. We split the data into three blocks of five feeding bouts within each treatment (bouts 1-5, bouts 9-14 and bouts 18-22). For each block we

calculated the mean proportion of wells visited that were to the constant option (Figure 2). There was no effect of feeding block ($F_{2,46} = 0.79$, $p = 0.46$). Birds did not systematically change their preferences with increasing experience within test days.

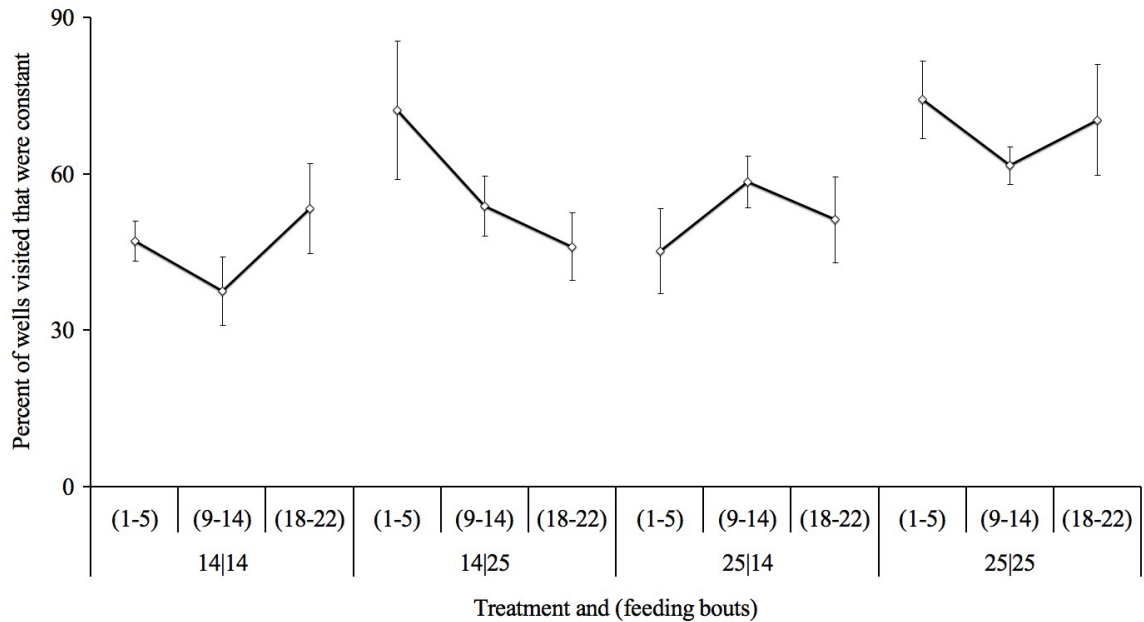


Figure 2: The proportion of the total number of wells visited that were the constant flower type for three equally-spaced blocks of five feeding bouts (bouts 1-5, 9-14 and 18-22) throughout the day of each treatment. Data are means and standard errors.

Amount of sucrose consumed per well

We calculated the mean reward volume consumed from variable and constant wells for each bird for each treatment. Birds drank a mean of $17.5 \pm 0.21 \mu\text{l}$ (mean \pm SE) from each well, taking slightly more ($0.62 \pm 0.31 \mu\text{l}$) from constant wells than from variable wells ($F_{1,50} = 4.84$, $p = 0.03$). There was no effect of feeder concentration ($F_{1,50} = 1.74$, $p = 0.19$) or of test concentration ($F_{1,50} = 0.78$, $p = 0.38$). There was a significant interaction between the feeder concentration and test concentration such that birds drank more ($0.81 \pm 0.34 \mu\text{l}$) per well in the 25|25

treatment than they took in the other treatments ($F_{1,50} = 4.56$, $p = 0.04$). There were no significant interactions between well type (constant or variable) and either feeder concentration ($F_{1,50} = 0.27$, $p = 0.60$) or test concentration ($F_{1,50} = 1.07$, $p = 0.31$).

The amount of sucrose the birds left behind in the wells was dependent on the initial volume of sucrose in the wells ($F_{2,79} = 116.88$, $p < 0.01$). This was unaffected by either the test concentration ($F_{1,79} = 0.903$, $p = 0.34$) or the feeder concentration ($F_{1,79} = 0.29$, $p = 0.59$). Birds left significantly more sucrose in 30 μ l than in 20 μ l wells ($4.95\mu\text{l} \pm 0.65$ vs. $2.08\mu\text{l} \pm 0.25$; post-hoc paired t-test: $t_7 = 5.92$, $p < 0.01$) and significantly more in 20 μ l wells than in 10 μ l wells ($2.08\mu\text{l} \pm 0.25$ vs. $0.62\mu\text{l} \pm 0.12$; $t_7 = 8.21$, $p < 0.01$). The volume left was not an equal proportion of the three well volumes (6.24 ± 1.17 % of 10 μ l wells, 10.38 ± 1.23 % of 20 μ l wells and 16.49 ± 2.15 % of 30 μ l wells: $F_{1,22,8.57} = 23.96$, $p < 0.01$; degrees of freedom corrected for violation of sphericity).

Energy availability per minute

We calculated the mean energy available to the birds per minute from what they consumed from our experimental apparatus:

Joules/per minute = (mean meal size (litres) / mean inter-bout interval (min))

*(mols sucrose/litre (14% sucrose = 0.431 mol/l and 25% sucrose = 0.806 mol/l))

* mass of 1 mol of sucrose = 342g

*joules/g sucrose = 16480j.

Birds consumed more energy when the test concentration was 25% than when the test concentration was 14% (Mean energy available per minute; 25%= 29.81 ± 0.97 joules, 14%= 23.14 ± 1.34 joules: $F_{1,16} = 40.57$, $p < 0.01$), but there was no effect of feeder concentration ($F_{1,16} = 1.77$, $p = 0.20$) and no interaction between the feeder and test concentrations ($F_{1,16} < 0.01$, $p = 0.97$).

Discussion

The concentration of sucrose used for testing and that in birds' feeders prior to testing had a significant effect on the degree to which they were risk sensitive. The birds' preference for the constant option over the variable option was relatively lower when faced with 14% sucrose in the test than it was when they were presented with 25% sucrose in the test. Their preference for the constant option was also relatively lower when their feeder had contained 14% sucrose rather than 25% sucrose prior to testing, irrespective of the concentration used during testing. This effect was seen both in the number of each well type from which the bird chose to drink during testing as well as in the total volume they drank from each well type. The effects of test concentration and the context of prior feeder contents on choices were not due to birds drinking different amounts of sucrose from one or other well type nor because risk sensitivity changed systematically within the day (they did not).

The effects of feeder concentration and test concentration were consistent with each other and seemed to operate independently and additively. This implies that the risk-sensitive choices exhibited in past experiments may have been influenced not just by the energy budget manipulations, but also by the difference

between the pre-test and test conditions. For example, none of the birds tested in the Caraco et al. (1981) experiment was deprived of food for the first hour and a half of each day of testing. Based on our results, we contend that the food available early in the day may have caused these birds to be more risk-averse than if the energy-manipulation period had been maintained consistently throughout the day. Similarly, our results suggest that hummingbirds in previous studies provided with 14% sucrose and tested with 20% were more risk averse than they would have been if they had been tested with 14% sucrose (Biernaskie et al. 2002; Hurly & Oseen 1999). It is possible that past experiments in which no effect of energy-budget manipulation was seen may have been affected by energy budget conditions during the pre-testing period (e.g. (Clements 1990; de Jonge et al. 2008; Wu & Giraldeau 2004).

The probability of the past affecting the experimental data in an unanticipated fashion is reduced in laboratory studies of risk sensitivity in which the experimental energy budget manipulation is extended for several weeks prior to testing (e.g. Caraco et al. 1990), or when testing occurs regularly within a prolonged energy budget manipulation (e.g. e Abreu & Kacelnik 1999; Ha et al. 1990; Hamm & Shettleworth 1987). In this study birds had been using 14% sucrose feeders since their arrival on the feeding grounds several weeks prior to testing and only rarely visited wildflowers, preferring to use the feeders. Thus, their energy budgets were likely to have been positive and stable for a prolonged period prior to our manipulations. Here we are interested in situations in which animals are tested under conditions different from those upon which they were maintained. In addition to effects of past energy budgets, animals' expectations/estimates of the present conditions formed using past experiences are violated when a resource changes,

creating a contrast between the expectation and actual conditions. This contrast can affect behavior even in the absence of a change in resource quality. For example, starlings trained for 10-14 days to expect either one or seven food pellets after pecking reward keys were then tested for risk sensitivity using a constant reward of four pellets and a variable reward of two or six pellets. If expectations were based on a contrast effect, starlings trained to expect seven pellets might view the mean reward of four pellets as a loss whereas starlings trained with a single pellet might regard four pellets as a gain, even though energy obtained over time was kept equal for both groups. Indeed, significantly more starlings were risk prone in the 'loss' treatment than in the 'gain' treatment (Marsh & Kacelnik 2002).

If expectations about the quality of a known patch carry over from the past based upon a certain memory window (Shettleworth & Plowright 1992), then hummingbirds' responses to new conditions (14|25 or 25|14) and current sampling information, may be mediated by this carry-over. For example, conditions that currently provide a poor reward but recently provided a high reward may appear better than when both past and present conditions were poor. Bumblebees foraging in a Bayesian-like way (using prior knowledge of food distributions along side current information), continue to use prior information about resource distributions to inform patch leaving decisions even when tested in an environment with a very different resource distribution (Biernaskie et al. 2009). Bees used to relatively poor patches leave high quality patches after finding fewer rewards than do bees with prior experience of high quality patches. It is not yet clear how long such cognitive effects commonly persist, but there is evidence that some types of effect can be very long-lived (Simitzis et al. 2008a; Simitzis et al. 2008b). In some cases using a period

of acclimatization with the aim of allowing re-stabilization of an animal's energy balance prior to an experiment may not sufficiently reduce the influence of past experience on the experimental data. Alternatively, the contrast effect may be a short-lived cognitive rule of thumb that allows animals to adjust their behavior to changes in reward quality before sufficient post-ingestive feedback is available for them to assess more accurately how the change has affected their energy-budget.

Although we present convincing evidence that both past and current conditions influence risk sensitive choices in hummingbirds, the design of our experiment cannot easily provide insight into the influence of contrast vs. carry-over effects. When test and feeder concentrations differ the same response is predicted both by the energy budget rule and the contrast effect. In both cases birds should be less risk averse if they think conditions have got worse, or if they really have got worse, and *vice versa*. Thus, on top of the energy budget rule the contrast effect would cause the lines in Figure 1 to be steeper, whereas the carry-over effect would cause the lines to be shallower. Distinguishing between these different expectation effects would require multiple manipulations of exposure time and food quality.

Our birds' choices are consistent with predictions from the energy budget rule: birds were more risk-averse when they were or had been foraging on 25% sucrose than when they were or had been foraging on 14%. Our data are also consistent with predictions that come from a contrast expectation model. That is, animals perceiving a decrease in sucrose concentration would be expected to be more risk prone even if their energy budget was unaffected (Kahneman & Tversky 1984; Kuhberger 1998; Marsh & Kacelnik 2002). Territorial hummingbirds' mass gain during the day is limited to 1 to 2% to reduce flight costs (as opposed to 35-40%

increase mass in 20 minutes before dark), intake rate tends to be fairly constant across the day even following periods of fast and compensatory feeding is limited (Calder 1991; Tooze & Gass 1985). Major adjustments of energy budgets occur via manipulations of metabolic costs during the night (Tooze & Gass 1985). As birds fed from their feeders for several hours in the morning before testing, birds feeding from a 25% feeder would have accumulated more energy than when feeding from a 14% feeder by the time the testing began, even if they started the day on fairly equal energy budgets. If they were then tested using 14% sucrose their highest energy budget during recording should have been during the first few test feeding bouts. Therefore, if the birds' decisions were entirely governed by an energy budget rule, we would have expected that birds that had had a feeder containing 14% sucrose would have been least risk-averse during the first few test feeding bouts when tested with 25% sucrose. Conversely, we would have expected that birds that had had a feeder containing 25% sucrose would have been most risk-averse during the first few test feeding bouts when tested with 14% sucrose, unless any difference in intake rate between 14% and 25% sucrose were compensated for by changes in behavior e.g. reducing flying time. We found no evidence, however, that the birds changed their preferences across a test day. We cannot, therefore, rule out that at least in the 14|25 and 25|14 treatments, contrast expectation may have played a role in the birds' decision-making.

One unexpected result was that when birds did not empty wells, they neither left the same amount per well nor did they leave an amount proportionate to the well's contents. If birds were trying to maximize foraging efficiency then they would be expected to empty 30 μ l wells but they did not. A plausible explanation for

this result is that the birds were constantly updating their estimate of the number and location of the different well types, as they took the same total volume and were not volume limited, in all the test conditions. As wells were not completely emptied, it seems unlikely that birds were attempting get a better estimate of the actual well volume. The situations in which animals continue to sample a patch that is consistently poorer than another is known as contrafreeloading (Damato 1974; Osborne 1977a). Information gathering of this kind appears to occur only when the resource or patch contents cannot readily be assessed visually, as is the case for the sucrose-containing wells we presented to our hummingbirds (Bean et al. 1999).

In summary, we show that prior experience can significantly impact current preference for variability although we were not able to distinguish between the effects due to the animal's energy budget and the animal's expectations. As prior experience is gathered throughout an animal's life, we predict that, in addition to effects due to differences in animals energy budgets, prior experience and expectations may well have a more substantial effect on risk-sensitive preferences than is often considered.

Chapter 5 **Information seeking affects risk-sensitive foraging decisions in rufous hummingbirds**

Ida E. Bacon, Andy T. Hurly and Susan D. Healy

I designed the experiment in collaboration with SDH and TAH, collected and analysed the data and wrote the manuscript with comments from SDH.

Abstract

Risk sensitive foraging is where animals choose between two resources of equal mean but where one resource is constant and the other provides a variable reward. Animals typically show a partial preference in favour of the constant resource. This preference tends to be stronger if the variable resource is more variable. Traditionally this behaviour has been explained using purely energetic and cognitive mechanisms. However, none of these models fully explain all aspects of observed risk-sensitive foraging and in contrast to other areas of foraging behaviour do not explore the possible trade offs between net-energy gain and other currencies such as information. Furthermore, they no account is taken of tradeoffs between decision speed and accuracy, that can lead to animals choosing to make less accurate decisions in more complex situations. It is possible avoiding more complex (variable resource) could help avoid making poor foraging decisions in the context of risk-sensitive foraging.

Using different levels of resource variability in either sucrose concentration or volume, I investigated the choices rufous hummingbirds made between a variable and constant resource. Highly variable resources appeared to involve some time as

well as possible energetic costs and where avoided. Whereas, low levels of variability had no obvious costs and were preferred to the constant resource possibly as foraging for information is general adaptive where there is no or little cost of doing so.

Introduction

Studies of risk-sensitivity examine situations in which animals choose between rewards that differ in their variance about a common mean (Kacelnik & Bateson 1996). Rewards are usually food but are sometimes water, while the variance is either in the delay associated with obtaining food or in the amount or quality of food. Where variance is in the delay animals typically prefer the variable or risky option (i.e. are risk prone). However, where variance is in amount or in quality animals typically prefer the constant option (i.e. are risk averse; Kacelnik & Bateson 1996). The degree of these preferences can be altered by changing animals' energy budgets and the variability of the variable reward.

Risk sensitivity has been explained traditionally in terms of energetics, either directly via state-dependant choices using the daily energy budget rule (Stephens 1981), or, more recently, via cognitive mechanisms such that the mean energetic rewards provided by the constant and variable options may be perceived to be unequally rewarding (Kacelnik & Abreu 1998; Shapiro 2000). Where variance is in amount, under the energy-budget rule an animal on a positive energy budget is predicted to choose the constant option, as there is a chance of a run of bad luck and starvation on the variable options, while an animal on a negative energy budget is expected to choose the variable option as there is a chance of a run of good luck and

avoiding starvation (Stephens 1981). However, although animals on negative energy budgets do tend to choose the variable option more often than when on a positive energy budget, this choice is usually partial and often not in excess of 50% of the choices made (Kacelnik & Bateson 1996). The energy-budget rule does not, therefore, explain all of the observed variation in risk sensitivity (Kacelnik & Bateson 1996). Even elaborations of Stephens' (1981) model to allow for more dynamic choice patterns across the foraging day and incorporation of thresholds other than those maximising the probability of survival (e.g. maximising the possibility of reproduction), none of these more complex models fully explain the general pattern of risk proneness for delay and risk aversion for amount (Houston & McNamara 1990; Hurly 2003; McNamara et al. 1991). The energy-budget rule also predicts, at least for an animal on a positive energy budget, that low variability should be preferred to higher variability as it provides a lower risk of starvation. This does fit with experimental data but these data can also be explained by several other models (Stephens 1981).

Models of risk-sensitivity based on cognitive mechanisms (i.e. an animal's assessment/ mental representation of reward averages) do, however, predict partial preferences between the constant and variable options and general risk aversion for amount and proneness for delay (Couvillon et al. 1991; Kacelnik & Abreu 1998; Shapiro 2000). These models are based either on the principle of Weber's law or on animal learning processes.

The models based on Weber's law or on discrimination learning predict that animals will either underestimate the true value of variable rewards or, on average, find them less rewarding (in the case of amounts) than the constant option (Couvillon

et al. 1991; Kacelnik & Abreu 1998). Both of these models predict greater risk aversion to higher variability in reward value. Models based on reinforcement learning, where animals learn by trial and error, have also been used to predict risk aversion to variation in reward amount via exponentially heavier weighting in animals' memories of more recent rewards (Niv et al. 2002). In these models the animal is assumed to update its mental representation of reward value rapidly rather than calculating longer-term mean rewards (Niv et al. 2002). However, none of these models ever predict risk proneness for variation in amount and they make no predictions about how animals would behave on different energy budgets.

One major similarity among all these approaches is that they require the subjects to acquire and remember information about the different foraging options. They are also all based on animals attempting to optimise their net rate of intake. However, the energy-budget rule, models based on Weber's law, and discrimination learning put little emphasis on the possibility that there might be other currencies that animals may trade off against energy gain, such as time and information. The model based on reinforcement learning, however, does consider trade offs between exploitation and exploration in determining optimal foraging decisions although this is not discussed directly in association with risk-sensitive behaviour (Niv et al. 2002). It is possible that in situations where variability in the variable option is sufficiently low, that animals' may trade off the perceived cost of foraging from the variable option with the possible benefits of learning about it.

Risk-sensitivity experiments are designed so that the subjects cannot learn to predict the pattern of rewards in the variable option and gain above mean reward. All they are expected to learn about is the variance and mean of the rewards (Kacelnik &

Bateson 1996). However, there is substantial evidence that animals will work for information (e.g. Inglis et al. 1997; Talling et al. 2002). In the wild, animals face a degree of uncertainty about the true nature of resources, an uncertainty that will be higher for unpredictable resources and for those resources that are harder to learn about. Thus, animals must regularly choose between exploiting available knowledge or exploring by foraging on less well understood resources for further information (Niv et al. 2002). Indeed, animals will sometimes choose not to feed from a freely available food source but instead to work for rewards from a resource that cannot be visually assessed, thus learning about that resource from the rewards obtained, a behaviour known as contrafreeloading (Inglis 2000; Osborne 1977b; Woodworth 1958). The importance of information gathering to decision making (the information primacy hypothesis) has also proved useful in modelling several other foraging behaviours such as latent learning and responses to changes in food availability (Inglis et al. 2001). However, as making decisions and learning takes time and making accurate assessments about which resources can be exploited best takes longer (Chittka et al. 2009), there is a trade off between decision accuracy and decision speed.

In some situations errors in choice may be more costly than the length of time required to make a better decision (Chittka et al. 2009). Therefore, where animals find it harder to make an acceptably accurate decision they would be expected to take longer over the decision making process. It is possible that where variability is very high animals find it harder than when variability is lower to assess the reward's mean and compare it to that of a constant option. In this case they would be expected to take longer to assess information as it is acquired during each foraging bout. If birds

cannot assess the resources accurately enough to be certain that the means are in fact identical and learning about the resource is time consuming, then they may avoid it becoming risk averse.

If information gathering and learning do play a significant role in determining risk sensitivity then regardless of an animal's energy budget, we would expect behaviour to depend on the discriminability of constant and variable rewards. It is important to note that in the context of risk sensitivity two sorts of discrimination are necessary, discrimination among all the rewards available and discrimination between the mean reward of the variable option and that of the constant option. In this context the means are the same but animals may not be able to be sure of this. Where variability in the variable reward is low discriminating the variable options from the constant reward may be hard because they differ so little from each other while comparing the means of the variable and constant options may be relatively easy in comparison to the situation in which variability in the variable reward is high and the variable options are readily discriminated from the constant reward.

Where discrimination among all rewards, rather than between reward means, is harder (variability is low), Weber's law and discrimination learning would lead us to expect an animal to show weaker risk aversion to variability in amount but never to the extent of being risk prone. Whereas, if animals forage from the variable option to gather information, when variability is sufficiently low they may indeed become risk prone as the possible benefits of gathering information about the variable resource may be perceived to out-weigh the possible cost of foraging from it.

In a similar manner, animals would be expected to show stronger risk aversion when the amount/value of a type of information is easier to quantify. In

addition, if animals do attempt to gather information whilst foraging, they would be expected to take longer for each bout in order to assess foraging options more fully when variability is high and comparison of the means of the constant and variable options is more difficult.

Here we investigate the possible role of information seeking in risk-sensitive choices using two levels of variability in two sorts of information (volume and concentration). The natural food sources of rufous hummingbirds *Selasphorus rufus* (flower nectar) varies considerably in concentration and volume, within and between plants (Ornelas et al. 2007) so it seems plausible that these birds can respond to variance in reward for increasing foraging efficiency. Indeed, rufous hummingbirds generally avoid variance in reward volume (Biernaskie et al. 2002; Hurly & Oseen 1999).

Birds were expected to be more risk averse to higher levels of variability in the variable option and when rewards were variable in concentration rather than in volume as it would be harder for them to tell if the mean reward of the variable option was indeed the same as the constant reward combined. Foraging bouts were expected to be longer when variability was higher and assessing the mean of the variable reward relative to that of the constant reward is harder. Where variability is low and discriminating the rewards in the variable option from the reward in the constant option is hard, it is possible that the birds may increase foraging on the variable option in order to increase the information available to them in order to assess it. Assessing volumes is expected to be harder than assessing concentrations that can be told apart by one lick, rather than needing to drink the entire volume. Therefore, birds are expected to be more risk averse to variation in concentration, as

even slight variation will allow birds to discriminate rewards in the variable option from that in the constant option. However, as both high variability and concentration are expected to be easier to learn about than low variability and volume, animals would be expected to reach a stable decision faster when foraging from these. If reinforcement learning plays a significant role in decision making, we would expect choices of reward types to be strongly affected by recent experiences of rewards in the variable option such that birds should prefer the constant option when they have recently experienced poor rewards.

Methods

Subjects were thirteen male rufous hummingbirds who had been defending territories for several weeks in the Westcastle River Valley, Alberta, Canada (+49° 20' 56.41", -114° 24' 35.08"). Each male's territory contained a 14% sucrose *ad libitum* feeder that he defended and from which he obtained almost all of his nectar. To allow individual identification birds were marked with waterproof, colored, non-toxic ink on the upper breast.

Subjects were trained to feed from arrays of 20 wells each 10 mm deep x 3.5 mm in diameter drilled in to a rectangular plexiglas plate (28 x 21.5 x 1.2 cm) arranged in a hexagonal pattern such that neighboring wells were 5.2 cm apart. The plate was attached to a stake that held it approximately 60cm from the ground. Surrounding each well was a paper reinforcement that were yellow while birds were being trained to use the plate but in experiments different colours were associated

with the variability of the sucrose solution in the well. The colours used were green, pink, blue and orange.

We conducted two experiments, one in which sucrose concentration was variable and one in which sucrose volume was variable. In each experiment there were two reward types: a constant reward, which was 30 μ l of 30% sucrose solution; and a variable reward, in which either the concentration or the volume of that flower color varied unpredictably. There were ten wells of each reward type. We used two levels of variability (high and low) for both experiments, with each variable option offering a mean reward equal to that the constant reward.

In the high variability (easy discrimination) treatment in the Volume Experiment, the variable option consisted of five wells of 10 μ l of 30% sucrose and five wells contained 50 μ l of 30% sucrose. In the low variability (harder discrimination) treatment, the variable option consisted of five wells of 25 μ l of 30% sucrose and five wells of 35 μ l of 30% sucrose.

In the high variability treatment in the Concentration Experiment, the variable option consisted of five wells of 30 μ l of 10% sucrose and five wells of 30 μ l of 50% sucrose. In the low variability treatment, the variable option consisted of five wells of 30 μ l of 25% sucrose and five wells of 30 μ l of 35% sucrose.

The constant option in all four treatments contained ten wells of 30 μ l of 30% sucrose. Each bird experienced four flower colors in the experiment: one for the constant option in the low variability experiment, one for the constant option in the high variability experiment, and one each for both of the variable options. No birds experienced the same color/variability-well type combination. Seven birds were tested in the Concentration Experiment and eight birds were tested in Volume

Experiment. Two birds were used in both the Concentration and Volume Experiments as not enough naïve birds were available to avoid this. One did the concentration experiment first and one the volume experiment first. At least four days between these experiments. As hummingbirds current experience tend to affect their foraging decisions more strongly than past-experiences (chapter 4) four days was considered sufficient to minimize any effects of previous testing on results.

Artificial feeders were removed during testing. Birds obtained virtually all of their daily energy requirements from either the feeder or test arrays and were only seen to visit natural flowers on one or two occasions.

Once a bird had learnt to feed from the board testing began. Half the birds were presented with a choice between the constant and high variability options first and half were presented with a choice between the constant and low variability option first. During choice tests the positions on the board of the ten variable wells and ten constant wells were determined at random. To prevent birds from learning the locations of the most rewarding wells, we rotated the board by ninety degrees between each feeding bout and moved it at least 15cm. In addition, we changed the pattern of constant and variable wells on the board every four bouts. Each well type was marked with the appropriate color. The sequence of each well visited and the volume consumed from each was recorded for each feeding bout as was the bout duration. The volume of sucrose consumed from each well was measured by extracting the volume of any residue using a micro-capillary-tube and then subtracting that volume from the initial volume in the well. We recorded visits to 150 wells in each treatment. If treatments ended at the end of a day the next treatment was started the following morning. Where treatments ended during the day the next

treatment was started after a break of around 20 minutes while apparatus for the new treatment was set up. Birds were supplied with their feeder between treatments. Experiments were stopped during periods of heavy rain to prevent rain getting in to the wells on the boards and changing concentrations and volumes of rewards. On average each experiment took 3.13 ± 0.16 days per bird.

We used mean values for each level of variability for each bird. Data were tested for normality using a Shapiro Wilk test. The Mauchly-criterion test was used to test for sphericity. If variances were non-homogeneous, data were transformed.

Results

Birds tested in both the concentration and volume experiment

To determine if birds that had been used in both experiments made different decisions when they encountered the second experiment we compared their choices between variable and constant over the first ten wells they fed from in the second experiment, to the choice made by birds that only did that experiment. The bird that did the concentration experiment first did not choose a differently between constant and variable wells in the first ten wells visited in the volume experiment than birds that were only involved in the volume experiment (50% vs. mean 47.1 % constant wells; t-test: $t_6 = 0.51$, $p = 0.63$). The bird that did the volume experiment first did not choose a differently between constant and variable wells in the first ten wells visited in the concentration experiment than birds that were only involved in the concentration experiment (55% vs. mean 44.0 % constant wells; t-test: $t_6 = 1.22$, $p = 0.28$).

Variability in concentration experiment

Choices to constant: number of wells visited

To examine birds over all preferences in both the high and low variability treatment, we calculated the proportion of all constant wells visited for both the high and low variability treatments for each bird. When variability was higher, birds drank from significantly more constant wells than when the variability was lower (Mean high variability $75.17 \pm 3.79\%$ vs. Mean low variability $46.31 \pm 4.49\%$: Paired t-test: $t_6 = 6.47$, $p < 0.01$; Figure 1). Birds drank from constant wells on significantly more than 50% of occasions when the variability was higher, i.e. were risk averse (mean = 75.17% ; t-test: $t_6 = 6.65$, $p < 0.01$). However, the percent of choices to the constant option did not differ significantly from 50% when the variability was relatively low (mean = 46.31% ; t-test: $t_6 = 0.82$, $p = 0.44$).

To check that birds could not locate the better wells in the variable treatment without visiting them, we tested whether they visited more of the higher concentration wells than they visited the lower concentration wells in the variable options of each treatment. They did not (low variability treatment; Paired t-test: $t_6 = 1.09$, $p = 0.32$; high variability treatment; Paired t-test: $t_6 = 0.37$, $p = 0.73$).

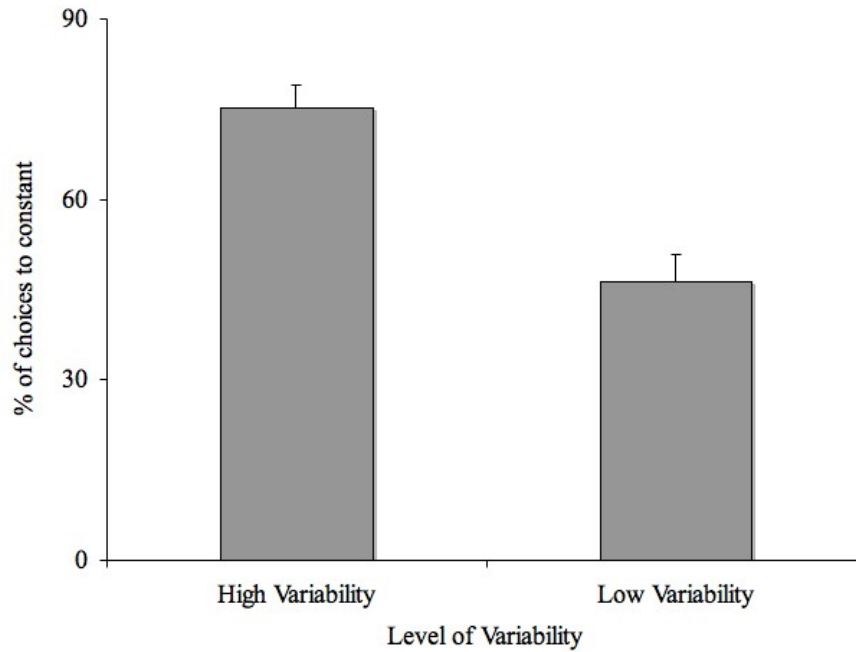


Figure 1: Concentration Experiment: The mean percentage of constant wells visited for the high and low variability treatments, the constant option being identical in each case. Data are means and standard errors.

Choices to constant: sucrose volume consumed

As birds could have visited many more wells in either the variable or constant option than they actually chose to drink from, we investigated whether their choice between variable and constant in terms of volume drunk were similar to their choices in terms of the number of wells visited. We calculated the percent of the total volume drunk from constant wells in both the high and low variability treatments for each bird. Similarly to the number of wells visited, when variability was higher, birds drank significantly more from constant wells than when the variability was lower (Mean high variability 78.95 ± 3.35 % vs. Mean low variability 47.84 ± 4.89 %: Paired t-test: $t_6 = 7.31$, $p < 0.01$). Birds drank more from constant wells on significantly

more than fifty percent of occasions in the higher variability treatment i.e. were risk averse (mean= 78.95; t-test: $t_6 = 8.64$, $p < 0.01$). However, the proportion drunk from the constant option did not differ significantly from 50% in the lower variability treatment (mean= 47.84; t-test: $t_6 = 0.44$, $p = 0.67$).

As birds had no knowledge of the options in either treatment when they first encountered them, they were expected to change their choice about the proportion of constant to variable wells they visited as they learnt about them. To determine whether preferences changed with increasing experience, we calculated the percentage of wells visited that were constant for each sequential block of ten wells (blocks 1-15) for each bird.

As birds initially had no knowledge of the treatments they were initially expected to show no preference between them. The choices made to constant during visit to the first ten wells in the both treatments did not differ significantly from 50% (high variability treatment; mean= 52.14%, t-test: $t_6 = 0.26$, $p = 0.80$: low variability treatment; mean= 45.57%, t-test: $t_6 = 0.57$, $p = 0.60$). We then used these data in a mixed model with birds as a random factor, for the higher and lower concentration variability treatments separately. Where variability was relatively high, birds increasingly preferred the constant option across the day (ANOVA: Adjusted $R^2 = 0.31$, $F_{1,97} = 10.69$, $p < 0.01$; Figure 2) but where variability was relatively low the preference for constant tended to decrease with increasing experience (ANOVA: Adjusted $R^2 = 0.33$, $F_{1,97} = 3.39$, $p = 0.07$; data were arrhenius transformed; Figure 2).

We looked at the pattern of change in preference with increasing experience in slightly more detail for the high variability treatment as choice seem to change

rapidly over the first 30 wells and then stabilize. The majority of increase in risk aversion when variability was relatively high occurred during visits to the first 30 wells. During visits to wells one to ten birds were significantly less risk averse than during visit to wells 11-30 (Mean wells 1-10 = 52.14 ± 8.20 ; mean wells 11-30 = 72.14 ± 6.06 ; Paired t-test: $t_6 = 2.67$, $p = 0.04$). They were not, however, significantly more risk averse during visits to the last 20 wells (wells 131 to 150; mean = 80.64 ± 7.59) than during visit to wells 11-30 (Paired t-test: $t_6 = 1.42$, $p = 0.21$). We did not repeat this analysis for the low variability treatment as the choices made by birds to constant, in wells 140-150 of the low variability treatment were still similar to 50% (mean = 39.57%, t-test: $t_6 = 0.92$, $p = 0.40$).

As birds' earlier experiences of the treatments tended to be earlier in the day, we tested whether time of day rather than increasing experience might explain the change in birds' choices with increasing experience. There was no relationship between the hour of the day at which observations were made and birds' preference for the constant option either in the high concentration variability treatment (ANOVA: Adjusted $R^2 = 0.24$, $F_{1,97} = 0.54$, $p = 0.46$), or in the low concentration variability treatment (ANOVA: Adjusted $R^2 = 0.30$, $F_{1,97} = 0.01$, $p = 0.76$).

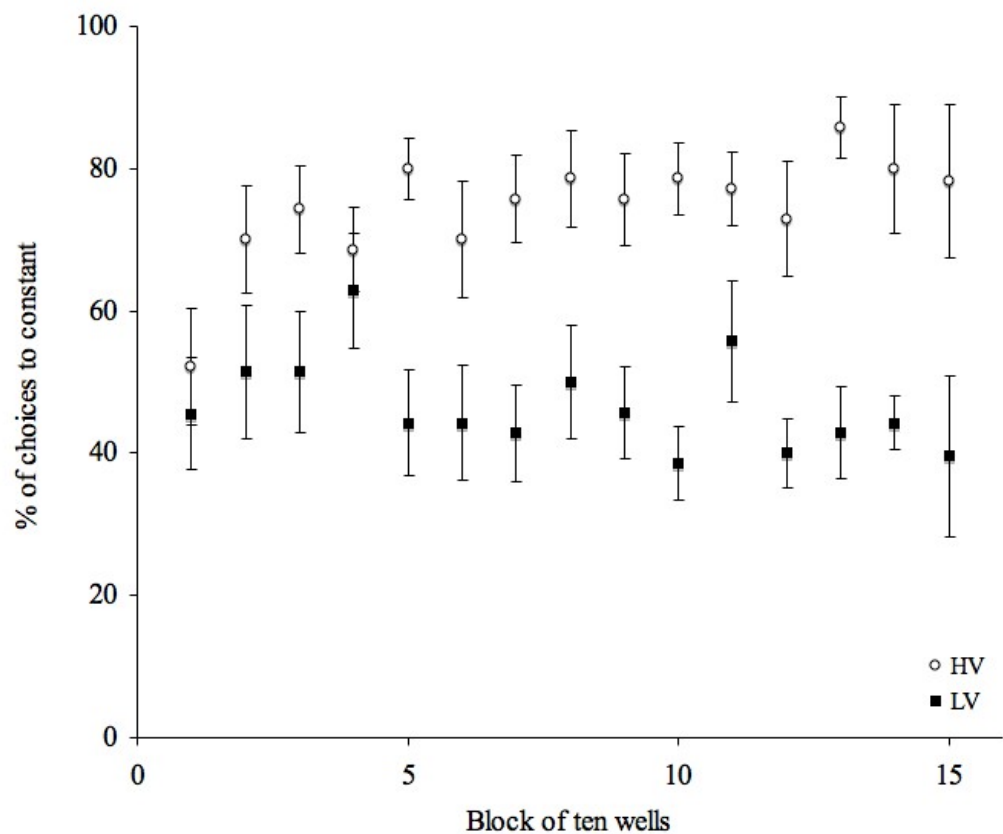


Figure 2: The percent of choices that were for the constant option where the high and low variability treatments (HV and LV) for successive blocks of ten wells visited. Data are means and standard errors.

To determine whether birds allocated less time to individual foraging bouts with increasing experience we used a mixed model with bird as a random factor. Data were the durations of each bout for each bird and treatment. Treatments were modelled separately. As not all birds made more than 35 foraging bouts only bouts one to 35 were included. Birds did not change their bout lengths with increasing experience on the low concentration variability treatment (ANOVA: Adjusted $R^2 = 0.45$, $F_{1,231} = 0.36$, $p = 0.55$; data were Log transformed) but tended to decrease bout length with increasing experience on the high variability treatment (ANOVA:

Adjusted $R^2 = 0.46$, $F_{1,234} = 5.11$, $p = 0.02$; Figure 3). Birds fed for similar lengths of time during each bout for both the high and low variability treatments (Paired t-test, mean bout duration for each bird and treatment: means= 9.14 vs. 9.01 sec; $t_6 = 0.16$, $p = 0.87$).

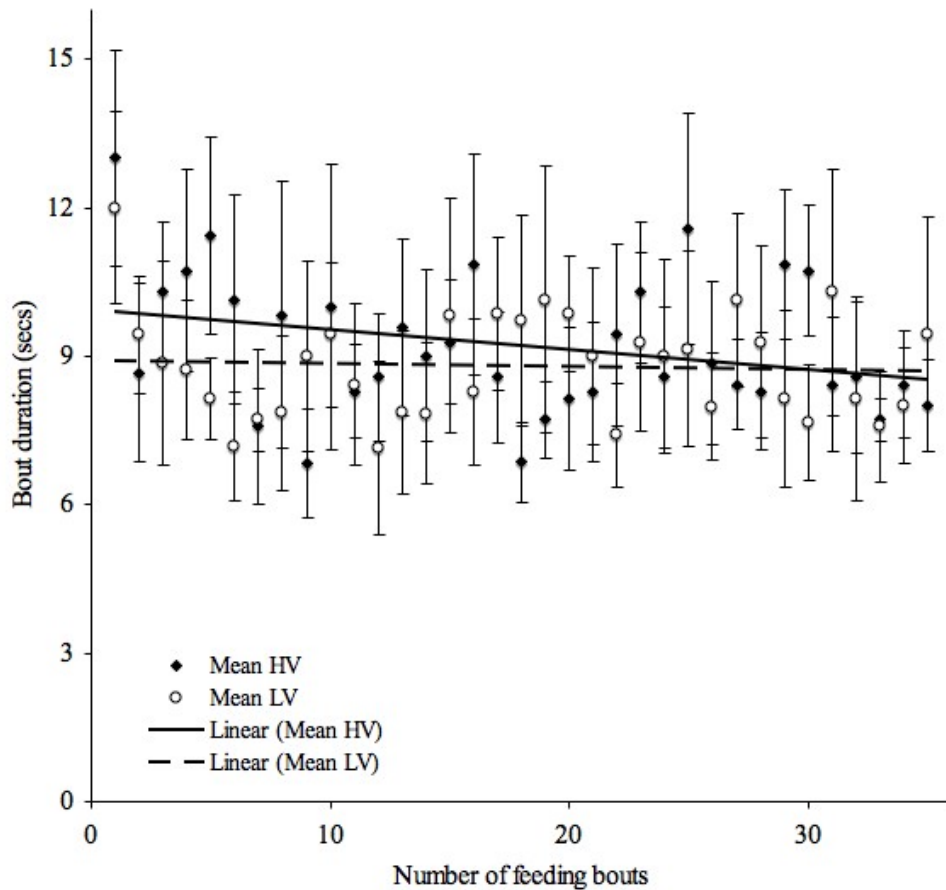


Figure 3: The durations of feeding bouts with increasing experience (number of bouts since treatments beginning) for both high and low concentration variability treatments. Data are means and standard errors for each bout and treatment. HV= high variability treatment, LV= low variability treatment.

We tested whether time of day rather than increasing experience might explain changes in bout-length. It did not. The hour of the day at which bouts one to

35 occurred had had no significant effect on bout length either in the low variability treatment (Adjusted $R^2 = 0.45$, $F_{1, 233} = 1.20$, $p = 0.27$; data were log transformed) or the high variability treatment (Adjusted $R^2 = 0.40$, $F_{1, 236} = 0.61$, $p = 0.44$).

As it take birds longer to drink more, we tested whether or not the change in bout lengths with increasing experience might be due to birds changing decreasing their meal size. The total volume drunk during each bout did not change with increasing experience, either in the low variability treatment (Adjusted $R^2 = 0.31$, $F_{1, 221} = 0.12$, $p = 0.73$; data were log transformed) or the high variability treatment (Adjusted $R^2 = 0.23$, $F_{1, 227} = 0.25$, $p = 0.62$; data were Arrhenius transformed).

We tested whether visiting more wells per-bout might affect bout-lengths. We used a mixed model with bird as a random effect, data were bout lengths and the number of wells visited for each bird for each bout and treatment. Across both treatments feeding-bout durations were longer if more wells were visited (ANOVA: Adjusted $R^2 = 0.57$, $F_{1, 476} = 210.14$, $p < 0.01$). We then looked to see if the number of wells visited per-bout might explain the change in bout-lengths with increasing experience. We used a mixed model with bird as a random factor, data were the bout number and the number of wells visited in that bout. The number of wells visited per bout did not change with increasing experience (only bouts one to 35 were included as in analysis of bout length) in either treatment (low variability treatment, Adjusted $R^2 = 0.28$, $F_{1, 237} < 0.01$, $p = 0.95$; high variability treatment, Adjusted $R^2 = 0.18$, $F_{1, 237} = 1.16$, $p = 0.28$). Birds visited a similar number of wells per bout during both the high and low variability treatments (Paired t-test, means wells per bout for each bird and treatment: means= 3.50 vs. 3.42 wells/bout; $t_6 = 0.31$, $p = 0.76$). The mean

number of wells visited during each bout did not differ between high and low variability treatments ($F_{1,6} = 0.11$, $p = 0.75$).

Sucrose left in wells

To investigate whether birds were avoiding drinking poorer concentrations in favour of more fully emptying higher concentration wells, we calculated the average volume left behind in wells of each of the three different concentrations in each treatment after birds had drunk for them. We examined these data in a repeated-measures ANOVA. There was a significant effect of treatment (ANOVA: $F_{1,6} = 16.22$, $p < 0.01$), well concentration (ANOVA: $F_{2,12} = 36.61$, $p < 0.01$) and a significant interaction between the treatment and the well concentration (ANOVA: $F_{1,6} = 37.76$, $p < 0.01$). We examined these relationships in more detail using post hoc paired t-tests. Alpha was set at 0.01 for five comparisons using Bonferroni correction for multiple comparisons. Birds left a similar volume in the constant 30% sucrose wells in both the high and low concentration variability treatments (means = 5.46 vs. 5.41 μl ; $t_6 = 0.05$, $p = 0.96$; Figure 4). In both treatments birds tended to leave more of the lower concentration in the variable option than in wells of the constant option although this was not quite significant for the low variability treatment after Bonferroni correction (High variability 10% sucrose (mean = 17.39 μl) vs. 30% sucrose: $t_6 = 6.96$, $p < 0.01$; low variability treatment 25% sucrose (mean = 7.72 μl) vs. 30% sucrose: $t_6 = 3.03$, $p = 0.02$). In both treatments, birds left a similar amount in the high concentration wells of the variable option compared to that they left in the constant option wells (High variability 50% (mean = 4.99 μl) sucrose vs. 30% sucrose: $t_6 = 0.51$, $p = 0.63$, low variability treatment 35% (mean = 5.34 μl)

sucrose vs. 30% sucrose: $t_6 = 0.10$, $p = 0.92$). Birds completely drained at least 18.04 ± 5.85 % of 10% wells and between 41.33 ± 5.17 to 51.61 ± 4.71 of all other concentrations.

On average, birds re-visited only 0.14 ± 0.12 % of wells within a bout. There was no difference between treatments in the number of wells re-visited within bouts (Paired t-test: $t_6 = 0.60$, $p = 0.28$).

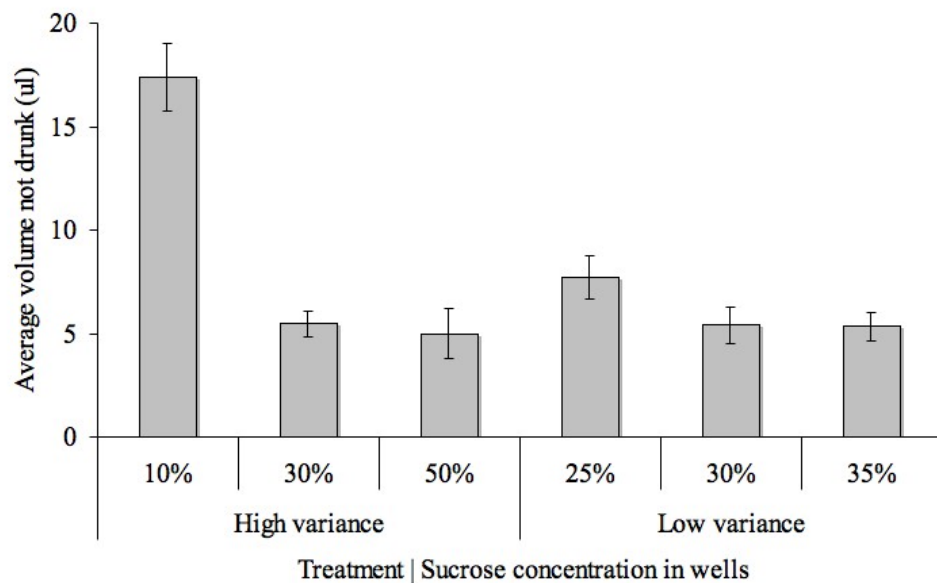


Figure 4: *The average volume birds left in wells containing different concentrations after drinking from them. Data are means and standard errors for both the high concentration variability and low concentration variability treatments.*

Energy intake

To determine whether birds obtained similar energetic rewards from both the high and low concentration variability treatments, we calculated the total energy in joules consumed by birds from each treatment and the total duration over which observations were recorded to give us the average energy intake rate. Birds foraging

from the high variability treatment took a similar number of joules per minute as when foraging from the low variability treatment (paired t-test: $t_6 = 1.07$, $p = 0.16$). Although inter-bout intervals tended to be longer (six of seven birds) during the high concentration variability treatment (mean 14.47 ± 1.25 mins) than they were during the low variability treatment (mean 13.11 ± 1.07 mins), this difference was not significant (paired t-test: $t_6 = 1.76$, $p = 0.13$). The amount of energy available to birds per minute remained similar with increasing experience in both treatments (ANOVA: low variability, Adjusted $R^2 = 0.06$, $F_{1, 219} = 1.05$, $p = 0.31$; data were log transformed; high variability, Adjusted $R^2 = 0.15$, $F_{1, 227} = 0.06$, $p = 0.81$; data were square-root transformed).

Within each bout birds would often visit wells of several different concentrations and drink different volumes of these. We tested whether on average they were drinking the mean concentration available of 30%. We calculated the mean concentration drunk by each bird for both the high concentration variability treatment and the low concentration variability treatment. On the low variability treatment the mean sucrose concentration drunk did not differ significantly from 30% (mean= 30.21%; t-test: $t_6 = 2.06$, $p = 0.09$). On the high variability treatment the average concentration drunk was significantly greater than 30% (mean=31.44%; t-test: $t_6 = 4.14$, $p < 0.01$). This was also significantly greater than the mean concentration drunk in the low variability treatment (Paired-t-test: $t_6 = 3.72$, $p < 0.01$).

As hummingbirds have optimal meal volumes that differ with sucrose concentration (smaller for higher concentrations), we looked to see if birds adjusted their bout volumes according to the mean concentration consumed (DeBenedictis et

al. 1978). We excluded bouts where only constant wells were visited as those bouts did not require birds to estimate the mean concentration of the bout. We calculated the mean concentration of the sucrose consumed in every bout and the total volume of every bout for each bird for treatment separately. Data were analysed using a mixed model with bird as a random factor.

Birds tended to drink less during a feeding bout when the mean concentration drunk during the feeding bout was higher in the high variability treatment (ANOVA: Adjusted $R^2 = 0.15$, $F_{1,168} = 6.07$, $p = 0.01$, data were Arrhenius transformed; figure 5) but they did not adjust their feeding-bout volumes in the low variability treatment (ANOVA: Adjusted $R^2 = 0.24$, $F_{1,276} = 0.04$, $p = 0.85$, data were log transformed). We then calculated the total energy consumed in each bout to see if this affected the following inter-bout interval as predicted by models of hummingbird foraging. Across both treatments inter-bout intervals were significantly longer following bouts during which birds had consumed more energy (ANOVA: Adjusted $R^2 = 0.42$, $F_{1,571} = 175.33$, $p < 0.01$; data were log transformed).

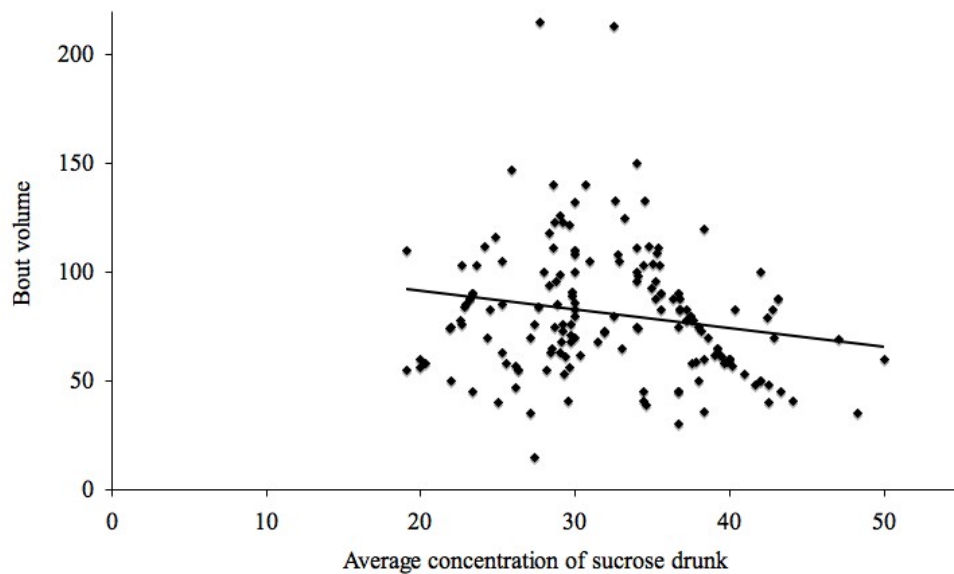


Figure 5: *The total volume in μl of each feeding bout plotted against the average concentration of the sucrose consumed during that feeding bout. The trend is plotted as a black line. Data are for the high concentration variability treatment only.*

Well to well and bout to bout choices

To investigate how birds' previous experience during foraging affected their future choices as would be expected from rapid reinforcement learning predictions, we examined the data both at the well-by-well scale and bout-by-bout. Firstly, we calculated the number of wells of each concentration visited immediately before visits to constant or variable wells for each bird. In the high concentration variability treatment birds were no more likely to visit a constant well after feeding from a 10% well than after feeding from a 50% well (Paired t-test: $t_6 = 0.70$, $p = 0.25$) nor were they more likely to visit a constant well than a variable well after feeding from a 25%

well in the low concentration variability treatment than after feeding from a 35% well (Paired t-test: $t_6 = 1.25$, $p = 0.13$). Secondly, we calculated the percentage of wells visits in each bout that were constant and used a mixed model to determine if this was affected by the mean concentration drunk during the previous bout. Birds did not change their decisions about what sort of wells to visit depending on the mean sucrose concentration consumed in the previous bout in the low variability treatment (ANOVA: Adjusted $R^2 = 0.17$, $F_{1, 303} = 1.36$, $p = 0.25$) or in the high variability treatment (ANOVA: Adjusted $R^2 = 0.17$, $F_{1, 291} < 0.01$, $p = 0.99$).

Variability in Volume experiment

Choices to constant: number of wells visited

To examine birds over all preferences in both the high and low variability treatment, we calculated the proportion of all constant wells visited for both the high and low variability treatments for each bird. When variability was relatively high, birds drank from significantly more constant wells than they did when the variability was lower (Mean high variability $58.20 \pm 2.49\%$ vs. Mean low variability $52.34 \pm 2.26\%$: Paired t-test: $t_6 = 2.89$, $p = 0.02$; Figure 6). Birds drank from constant wells on significantly more than 50% of occasions when the variability was higher i.e. were risk averse (mean = 58.20% ; t-test: $t_7 = 3.30$, $p = 0.01$). However, the proportion of choices to the constant option did not differ significantly from 50% when the variability was lower (mean = 52.34% ; t-test: $t_7 = 1.04$, $p = 0.33$). Compared to the concentration experiment, birds' preference for constant was weaker in the high

variability treatment (means= 58.19% vs. 75.16 %; t-test: $t_7 = 3.84$, $p < 0.01$), but similar in the low variability treatment (means= 52.34% vs. 46.30%; t-test: $t_7 = 1.25$, $p = 0.12$).

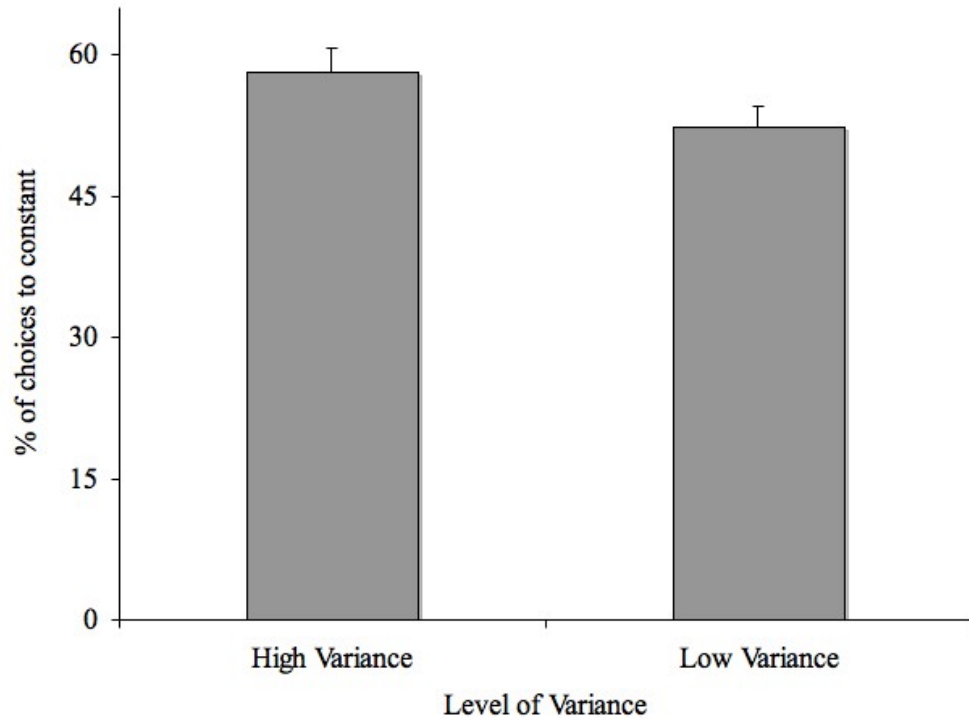


Figure 6: *The mean percentage of wells visited that were the constant option for two levels of variability in volume in the variable option, the constant option being identical in each case. Data are means and standard errors.*

Choices to constant: sucrose volume consumed

As birds can visit more wells than they actually drink from, we tested whether their choices between variable and constant wells in terms of volume drunk were similar to those in terms of the number of wells visited. Similarly to the proportion of wells visited, when variability was higher, birds drank from

significantly more constant wells than they did when the variability was lower (Mean high variability 60.04 ± 2.49 % vs. Mean low variability 50.78 ± 2.63 %: Paired t-test: $t_7 = 3.13$, $p < 0.01$). Birds drank from constant wells on significantly more than 50% of occasions when the variability was higher i.e. were risk averse (mean= 60.04% ; t-test: $t_7 = 4.52$, $p < 0.01$). However, the proportion of the volume drunk from the constant option did not differ significantly from 50% when the variability was lower (mean= 50.78% ; t-test: $t_7 = 0.44$, $p = 0.68$).

To check that birds could not locate the better wells in the variable treatment without visiting them, we tested whether they visited more of the higher concentration wells than they visited the lower concentration wells in the variable options of each treatment. Birds did not visit more of the higher volume wells than the lower volume wells in the variable option in either the treatment (Paired t-test: low variability: means= 37.00 vs. 35.37 ; $t_7 = 0.65$ $p = 0.27$. High variability: means= 32.12 vs. 30 ; $t_7 = 0.99$, $p = 0.18$).

As birds had no knowledge of the options in either treatment when they first encountered them, they were expected to change their choice about the proportion of constant to variable wells they visited as they learnt about them. To determine whether preferences changed with increasing experience, we calculated the percent of wells visited that were constant for each sequential block of ten wells (blocks 1-15) for each bird and then used these data in a mixed model with birds as a random factor for the higher and lower concentration variability treatments separately. Birds did not change their preference for constant with increasing experience in the high volume variability treatment (ANOVA: Adjusted $R^2 = 0.13$, $F_{1, 111} = 1.26$, $p = 0.27$) but where variability was lower, their preference for constant decreased significantly

with increasing experience (ANOVA: Adjusted $R^2 = 0.16$, $F_{1,111} = 6.25$, $p = 0.01$; Figure 7).

As birds earlier experiences of the treatments tended to be earlier in the day, we tested whether time of day rather than increasing experience might explain the change in birds' choices with increasing experience. The hour of day at which observations were taken did not affect the birds' preference for constant in either treatment (ANOVA: high variability, Adjusted $R^2 = 0.15$, $F_{1,111} = 3.19$, $p = 0.08$; low variability, Adjusted $R^2 = 0.11$, $F_{1,111} = 0.13$, $p = 0.72$).

As the decrease in risk aversion when variability was lower appeared to be gradual, therefore, we looked in more detail at preferences during visit to the first ten wells visited and the last ten wells visited (wells 141-150) in the lower volume variability treatment. The choices made to constant during visit to the first ten wells where variability was lower did not differ significantly from fifty percent (mean=47.50%, t-test: $t_7 = 0.51$, $p = 0.63$). However, by visits to wells 141 to 150, birds chose significantly less than 50% constant wells, were risk prone (mean=37.5%, t-test: $t_7 = 2.76$, $p = 0.03$).

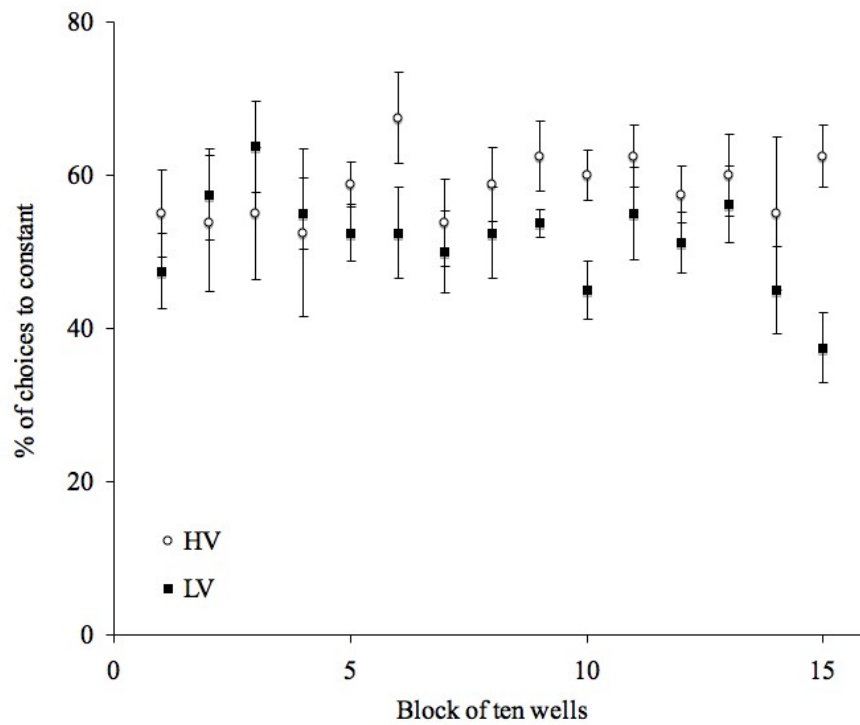


Figure 7: *The percent of choices that were for the constant option where the variable option had either higher variability (HV) or lower variability (LV) for successive blocks of ten wells visited. Variability was in volume. Data are means and standard errors.*

To investigate whether birds altered the time they allocated to individual foraging bouts with increasing experience we used a mixed model with bird as a random factor. Data were the durations of each bout for each bird and treatment. Treatments were modelled separately. One bird was excluded due to errors in measurements of its bout lengths. We used bouts one to 35 for the remaining birds. Birds did not change their bout lengths with increasing experience on the low variability treatment (ANOVA: Adjusted $R^2 = 0.44$, $F_{1,235} = 0.67$, $p = 0.41$; data were log transformed) but decreased bout length with increasing experience on the high variability treatment (ANOVA: Adjusted $R^2 = 0.48$, $F_{1,228} = 5.23$, $p = 0.02$;

Figure 8; data were log transformed). Birds fed for similar lengths of time during each bout for both treatments (Paired t-test: mean bout duration for each bird and treatment: means= 8.18 vs. 7.45, $t_6 = 0.71$, $p = 0.25$).

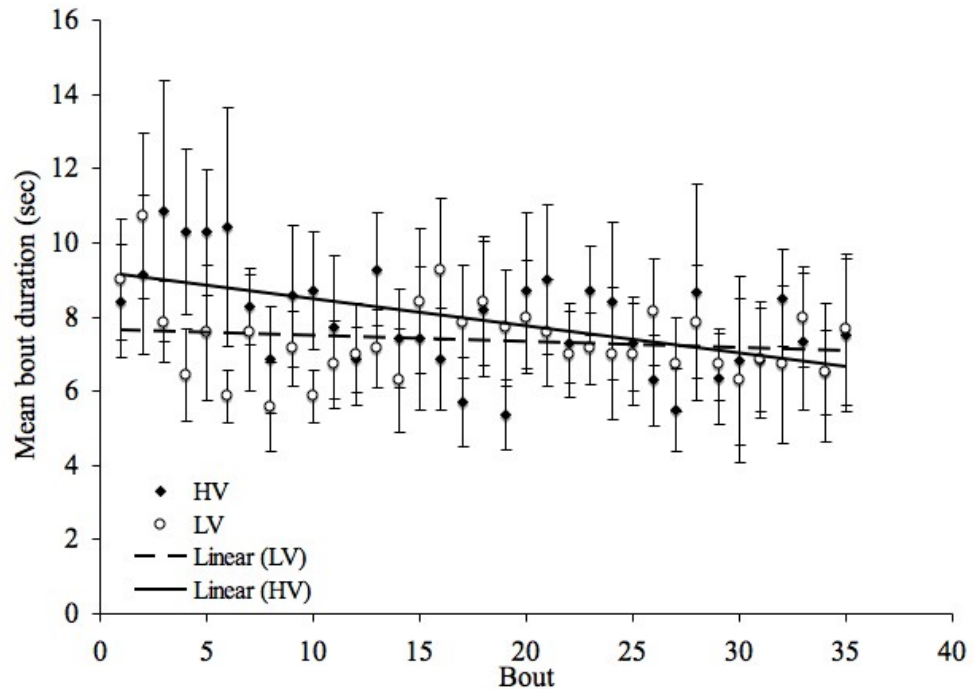


Figure 8: The durations of feeding bouts with increasing experience (number of bouts since treatments beginning) for both high and low volume variability treatments. Data are means and standard errors for each bout and treatment. HV= high variability treatment, LV= low variability treatment.

We tested whether the time of day rather than increasing experience might explain changes in bout length. The hour of the day at which bouts one to 35 occurred had had no significant effect on bout length either treatment (low variability: Adjusted $R^2 = 0.44$, $F_{1,238} = 2.33$, $p = 0.13$; data were log transformed; high variability: Adjusted $R^2 = 0.46$, $F_{1,228} = 0.59$, $p = 0.44$; data were log transformed).

As birds take longer to drink more we tested whether birds drank more with increasing experience. The total volume drunk during each bout increased with increasing experience on the low variability treatment (Adjusted $R^2 = 0.50$, $F_{1,249} = 5.73$, $p = 0.02$; data were Log transformed) but not in the high variability treatment (Adjusted $R^2 = 0.46$, $F_{1,242} = 1.87$, $p = 0.17$; data were Arrhenius transformed).

Visiting more wells per bout can also increase bout-length. We used a mixed model with bird as a random effect to test if the number of wells visited affected feeding bout duration, where the data were means for each bird for each bout and treatment. Across both treatments feeding-bout durations were longer if more wells were visited (ANOVA: Adjusted $R^2 = 0.64$, $F_{1,476} = 403.08$, $p < 0.01$). Examining each treatment separately we found that the number of wells visited per bout increased with increasing experience (only bouts one to 35 were included as in analysis of bout length) in the low variability treatment (ANOVA: Adjusted $R^2 = 0.30$, $F_{1,235} = 8.79$, $p < 0.01$; data were square root transformed) but not the high variability treatment (ANOVA: Adjusted $R^2 = 0.38$, $F_{1,228} = 0.05$, $p = 0.82$). On average, birds visited a similar number of wells per bout during both treatments (Paired t-test: means wells per bout for each bird and treatment: means = 3.65 vs. 3.28, $t_6 = 1.19$, $p = 0.14$).

Sucrose left in wells

To investigate whether birds were avoided lower volume wells in favour of more fully emptying fuller wells, we calculated the average volume left behind in wells of each of the three different volumes in each treatment after birds had drunk for them. We examined this data in a repeated measure ANOVA. There was no

significant effect of treatment (ANOVA: $F_{1,7} = 1.07$, $p = 0.34$). However, the amount left in wells varied significantly with well volume (ANOVA: $F_{1.1, 7.7} = 36.23$, $p < 0.01$) and there was a significant interaction between the treatment and the well concentration (ANOVA: $F_{2,6} = 46.17$, $p < 0.01$). We examined these relationships in more detail using post hoc paired t-tests. Alpha was set at 0.01 for five comparisons using Bonferroni correction for multiple comparisons. Birds left a similar volume in the constant 30 μ l sucrose wells in both treatments (means: high variability= 5.10 μ l vs. low variability= 6.25 μ l; t-test: $t_7 = 1.98$, $p = 0.09$; Figure 9). In both treatments birds tended to leave less of the lower volume in the variable option than in wells in the constant option (High variability 10 μ l sucrose (mean=0.71 μ l) vs. 30 μ l sucrose: $t_7 = 7.39$, $p < 0.01$, low variability treatment 25 μ l (mean= 4.01 μ l) sucrose vs. 30 μ l sucrose: $t_7 = 3.71$, $p < 0.01$). Birds left more sucrose in the higher volume wells in the variable option compared to wells in the constant options in the high volume variability treatment (50 μ l (mean=12.85 μ l) sucrose vs. 30 μ l sucrose: $t_7 = 7.43$, $p < 0.01$), but not in the low volume variability treatment (35 μ l sucrose (mean=6.74 μ l) vs. 30 μ l sucrose: $t_7 = 0.69$, $p = 0.51$). On average birds re-visited only $2.36 \pm 0.89\%$ of typically 3-4 wells visited within each bout. There was no difference between treatments in the number of wells re-visited within bouts (Paired t-test: $t_7 = 0.82$, $p = 0.22$). Birds completely drained between $35.83 \pm 5.81\%$ and $83.79 \pm 3.41\%$ of well of all volumes.

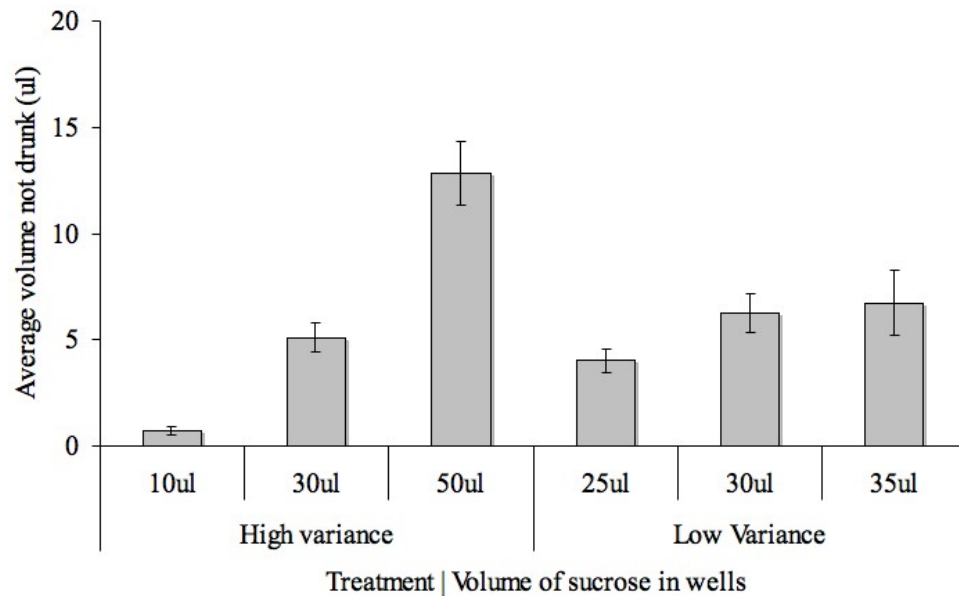


Figure 9: The average volume birds left in wells containing different volumes after drinking from them. Data are means and standard errors for both the high volume variability and low volume variability treatments.

Energy intake

We looked birds' energy intake rates to test whether they obtained more energy from either treatment. Birds foraging from the high variability treatment had a similar number of joules available to them per minute as when foraging from the low variability treatment (43.67 vs. 44.59 joule/min; paired t-test: $t_7 = 0.17$, $p = 0.43$). The amount of energy available to birds per minute remained similar with increasing experience, in both treatments (ANOVA: low variability, Adjusted $R^2 = 0.24$, $F_{1,242} = 1.86$, $p=0.17$; data were square root transformed; high variability, Adjusted $R^2 = 0.11$, $F_{1,238} = 0.01$, $p=0.93$; data were square root transformed). Data were the energy consumed per bout divided by the duration of the subsequent inter-bout interval, bird was included as a random factor. Inter-bout intervals were similar

during the high volume variability treatment (mean 14.08 ± 1.82 mins) to during the low variability treatment (mean 13.52 ± 1.84 mins; paired t-test: $t_6 = 0.91$, $p = 0.39$). The average volume drunk per bout did not differ between the high and low volume variability treatments (Paired-t-test: $t_7 = 0.13$, $p = 0.90$).

Well by well and bout by bout choices

To investigate how birds' previous experience of well volumes during foraging affected their future choices as would be expected from predictions of rapid reinforcement learning, we examined the data both at the well-by-well scale and bout-by-bout. Firstly, we calculated the number of wells of each volume visited immediately before visits to variable or constant wells for each bird. Birds were no more likely to visit a constant well after drinking from a $10\mu\text{l}$ well than a $50\mu\text{l}$ well (means= 18.25% vs. 17.62%; Paired-t-test: $t_7 = 0.65$, $p = 0.27$) however, they were more likely to visit constant wells after visiting a $25\mu\text{l}$ well than a $35\mu\text{l}$ well (means= 23.25% vs. 17.75%; Paired-t-test: $t_7 = 2.88$, $p=0.01$). Secondly we calculated the percent of wells in each bout that were constant and used a mixed model to determine if this was affected by the mean volume of wells visited during the previous bout. Birds did not change their decisions about what sort of wells to visit depending on the mean volume of wells in the previous bout in the low variability treatment (ANOVA: Adjusted $R^2 = 0.05$, $F_{1, 333} = 1.14$, $p=0.29$) or in the high variability treatment (ANOVA: Adjusted $R^2 = 0.08$, $F_{1, 308} = 1.32$, $p=0.25$).

Discussion

In both in the Concentration Experiment and the Volume Experiment birds were more risk averse, with regard to both the number of wells of each type (constant or variable) visited and to the volume drunk from each well type, in the high variability treatments than they were in the low variability treatments. Furthermore, they were more risk averse when variability was in concentration than when the variability occurred in volume. In general, therefore, the birds' overall preferences were consistent with the predictions from all of the relevant models: the energy-budget rule, Weber's laws based models, discrimination learning based models and information gathering.

However, although overall birds were risk indifferent on the low variability treatments, they tended to or became increasingly risk prone with increasing experience. Increasing risk proneness is not predicted by models based on Weber's law, on discrimination learning or by the energy-budget-rule for animals on a positive energy budget (Kacelnik & Abreu 1998; Shapiro 2000; Stephens 1981). Slight risk proneness for animals on positive energy budgets may, however, be due to information seeking behaviour as observed in other contexts such as contrafreeloading.

Birds were only predicted to forage for information if there was little or no cost of doing so. That there may well have been at least time costs associated with foraging from the high variability treatments is supported by a significant decrease in bout-lengths with increasing experience on these treatments but not with the low

variability treatments. This change in bout lengths may have resulted from the time taken to make an acceptably accurate decision when foraging from an increasingly lower proportion of variable wells at least in the concentration experiment (Chittka et al. 2009). On the high variability treatments birds initially visited about equal numbers of variable and constant wells. At this stage in the treatment their bout-lengths were longer than later in the treatment or in the low variability treatments, despite birds not visiting more wells per bout or drinking more per bout. This suggests that they may have been allocating more time to resource assessment. Indeed, where animals cannot accurately estimate whether a constant or variable option is better then they will choose the constant option even if in reality the variable option is slightly better (Shafir et al. 2008). This behaviour is referred to as the certainty affect. However, when animals are more certain of the relative payoffs to the two options they will chose the variable option if it is better (Shafir et al. 2008).

To determine whether variable or constant wells were better on average, birds would either need to compare both options in the variable option to the constant option and then average the difference (an average of zero would indicate similarity to the constant reward) or average between the two rewards in the variable option and compare that average to the constant option. Either way, where rewards in the variable option do not differ much from the constant option, then any errors will be comparatively small relative to instances when variation is higher. In such situations birds can be more certain that their comparison of the two rewards if fairly accurate and there is little or no cost to foraging on the variable option. Here, Weber's law may play a role although not in the context of how accurately animals can assess

actual amounts of food as described in previous models, but rather in how accurately animals can assess the size of differences between rewards (Figure 10). In both contexts smaller amounts/ differences would be expected to be assessed more accurately (Kacelnik & Abreu 1998).

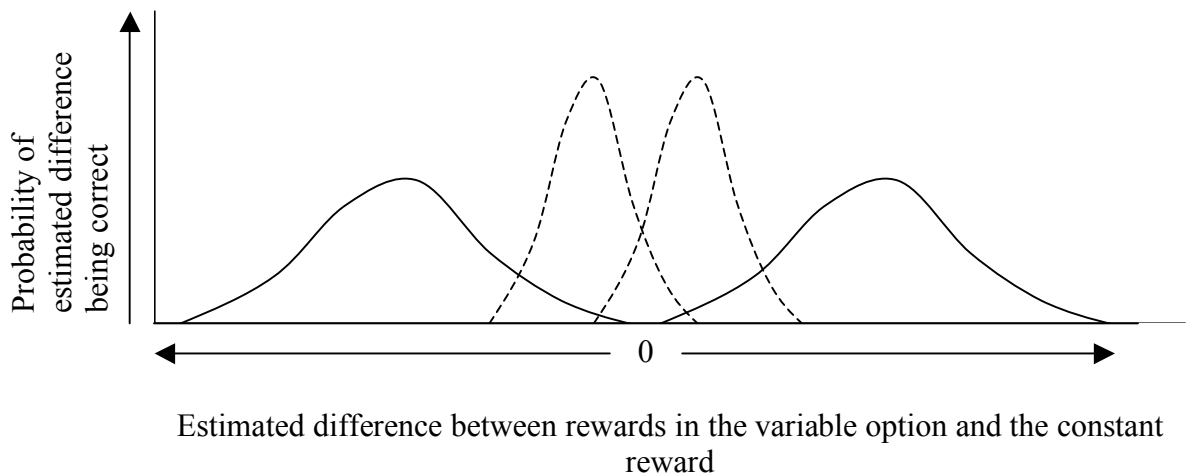


Figure 10: *How Weber's law may affect animals' perceptual accuracy of differences between reward values. Possible perception of two rewards in a higher variability option compared to the constant option, are shown in the solid lines; possible perception of two rewards in a lower variability option compared to the same constant option are represented by dashed lines. As the accuracy of perception of smaller differences is expected to be better, then birds can be more certain when variability is low that the constant and variable options provide about the same mean reward.*

It is possible that birds are not as able to assess variation in volume as accurately as they can assess concentrations, which they can tell apart in the range that we used by as little as 1% (Blem et al. 2000). Therefore, greater differences in μl volume would be necessary than in % sucrose concentration before any difference between rewards would be noticeable. Thus, the differences between a constant reward and rewards in a variable option that varies in volume will be fewer

perceivable units apart than would be the constant reward and rewards in an option that varies in concentration alone. The animals should be more confident that an option that varies in volume is not significantly different from the constant option than when variability is in concentration and should therefore be less risk averse, as we observed.

It is conceivable that for much lower volumes than we used here that hummingbirds' ability to discriminate among them is much better, again in accordance with Weber's law. This may explain why, unlike in this experiment, hummingbirds have been found to be more risk averse to variation in volume than in concentration where volumes used were only 0 to 3 μ l (Lara 2008). This greater aversion to variation in volume may also have been due to the inclusion of zero rewards in their volume but not in their concentration experiment, as zero rewards tend to disproportionately increase risk aversion (Shapiro 2000) and should be relatively easy to measure. Furthermore, accurate assessment of the concentration of the solution in wells may be more important and mistakes more costly as solution concentration is the major determinant of the amount a hummingbird should drink per bout, a trade off between intake and the flight costs of carrying the meal (DeBenedictis et al. 1978). Thus, because a variable resource is expected to be harder to assess accurately than is a constant one, birds should typically be more risk averse to variation in concentration than volume.

There may also be additional costs to foraging on highly variable resources, which may be slight but sufficient to prevent animals from gambling that their estimate of the mean value of the variable reward compared to the constant reward is too low, in which case foraging from the variable option would be better. Where

variability is in concentration, birds feeding from a mixture of concentrations should alter how much they drink towards the optimal amount of the mean concentration drunk, which will be less when the mean meal concentration is higher (DeBenedictis et al. 1978). As post-ingestive feedback (probably the most accurate sort of information for assessing meal quality), is not immediately available, much of the decision about how much to drink must be based on taste (Yearsley et al. 2006). Therefore, decisions about how much to drink in a bout may be less accurate when a bird constantly encounters different concentrations than when it always drinks the same concentration and can use post-ingestive feedback to inform its decision. Birds would, therefore, be expected to avoid highly variable resources. However, where variation in food quality is very low any errors about how much to drink may be so small that they have no negative impact. Indeed, our birds certainly made some attempt to adjust their bout volume to the mean concentration they had consumed in a bout when variation was high but did not seem to do this when variation was lower. When variation is in volume there be other costs to foraging on the highly variable option due to the handling times and energy intake rates associated with different meal volumes. Intake rates increase with increasing reward volumes but do so more rapidly when volumes are lower, thus the mean intake rate to highly variable rewards may fall below that of the constant reward (Montgomerie 1984).

There was no evidence that rapid reinforcement learning explained birds' foraging decisions. From models of reinforcement learning we would expect to find some relationship between birds' recent experience and their subsequent choice (Niv et al. 2002). Visits to low and high reward wells in the variable options in three out of four treatments did not affect birds' choices about whether to visit a variable or

constant well next. Similarly, in neither experiment was there any evidence that the mean concentration or volume achieved in a bout affected the ratio of constant to variable wells chosen in the next bout.

In conclusion, it seems likely that trade-offs between exploitation and exploration may well play a significant role in risk-sensitive behaviour, particularly where risk proneness is observed. Highly variable resources appear to be avoided possibly as the accuracy with which they can be compared to the constant option is lower than when variability is lower, increasing the risk of making poor decisions. In addition, there may be hidden costs of foraging from highly variable resources both in time and energy that can make constant options more rewarding. We feel the inclusion of information gathering in to future models of risk sensitivity may greatly enhance our understanding of risk-sensitive choice.

Chapter 6 Identifying wildflowers visited by rufous hummingbirds on their breeding grounds

Abstract

Experiments designed to investigate foraging behaviour attempt at least to some extent to mimic animals' natural foraging conditions. Studies of risk sensitive foraging behaviour look at how animals choose between more or less variable resources with equal means. Hummingbirds are often used in such experiments yet our knowledge of the levels of variability they encounter naturally is poor. By investigating the species of flowers visited by hummingbirds in the wild and examining the variation in nectar rewards, we may be able to design more naturalistic experiments. The species of wild flowers visited by free-living hummingbirds were investigated by observing birds and analysing of pollen collected from their bills and artificial hummingbird feeders. Variation in nectar rewards was investigated by collecting nectar and analysing its concentration and volume. We identified twenty species of flower that were visited by hummingbirds and found significant variation in nectar production between species. However, we encounter problems with pollen identification and evaporation of nectar from bagged flowers. Additional data will be required before we have a full picture of the different species visited and the true variation in the nectar rewards they offer.

Introduction

The study of foraging behaviour allows us not only to better understand and predict foraging but also to investigate a number of aspects of animal cognition and decision making (Healy et al. 2009; Stephens et al. 2007). Understanding the types and variability of animals' natural diets allows experiments to be designed so that they test animals' abilities within the range of conditions they have evolved to deal with (Healy et al. 2009; Kacelnik & Bateson 1996; Shettleworth 2001; Winter & Stich 2005). For example, hummingbirds are often used as a model species for investigating animal decision-making and cognition with the concentrations of sucrose used in experiments kept similar to the range of concentrations experienced by hummingbirds foraging from floral nectar (Ornelas et al. 2007). Much research into animal cognition and risk-sensitive foraging is conducted using hummingbirds as subjects at the University of Lethbridge field station in the Westcastle River Valley, in the Rocky Mountains, Alberta, Canada: Lat 49.349297, Lon: 114.410849 (e.g. Healy & Hurly 1998; Henderson et al. 2006; Hurly & Healy 1996; Hurly & Healy 2002; Hurly & Oseen 1999). There are only a few typical/known hummingbird food plants at the site and we know very little about which of the remaining hundreds of natural flowers available to them that hummingbirds sample or about the variability of nectar rewards within or between these species. This knowledge would be particularly useful when it comes to designing experiments to test birds' responses to variability in nectar rewards.

Compared to many food types nectar is fairly simple and its energy content is easily calculated. Hummingbirds can taste the differences between solutions differing by as little as 1%, although this varies with concentration, so they are easily able to choose between flowers by the concentration of their nectar reward (Blem et al. 2000). However, when it comes to investigating how and why hummingbirds may respond to variability in the nectar rewards they encounter the picture is more complicated. The field of risk sensitivity, which hummingbirds have been used as subjects in for a number of studies (e.g. Hurly 2003; Hurly & Oseen 1999; Lara 2008; Waser & McRobert 1998), deals with the decisions animals make when choosing among resources that differ not in their mean reward but in the variability of the resources about that mean (Kacelnik & Bateson 1996). Information about the range and type of variability that hummingbirds encounter naturally would give us a better idea of how to design and interpret experiments to investigate their foraging decisions when feeding from variable resources. Indeed, there is growing evidence that hummingbirds tend to avoid plants with highly variable nectar rewards at least where variation is in volume (Kearse et al. 2008; Lara 2008). This is consistent with general risk aversion of hummingbirds in risk sensitivity experiments (Hurly 2003; Hurly & Oseen 1999; Lara 2008; Waser & McRobert 1998). However, these data are responses only to variability in plant nectar within a single plant species and they tell us little about differences in floral characteristics and nectar among plant species or how this variability may affect hummingbirds' sampling frequencies among multiple plant species. Such among species differences probably represent the majority of variability among food resources that hummingbirds encounter.

There may be tens or hundreds of flower species and hundreds of plants of each species and many flowers on each plant within a hummingbirds feeding range. Not every plant of the same species, or even every flower on each plant, will necessarily provide the same nectar reward (e.g. Herrera et al. 2006; McDade & Weeks 2004; Pleasants 1983). Visiting and remembering information about every single flower head may not be possible and would be energetically expensive, so being able to recognise and remember information about the most profitable plant species would be highly beneficial.

There are certain plant characteristics that make them particularly suited to hummingbird pollination. They are typically: tubular (to restrict access by other pollinators and increase handling time); red (to increase visibility to birds against a green background and to make them harder for bees to find); scentless (as birds forage predominantly by sight); downward facing (as this increases handling time and therefore pollen deposition, prevents dilution by rain and allows flowers to remain open in the rain); thick tissue (to reduce damage) and, lastly, contain large volumes of dilute, sucrose-predominated nectar compared to bee flowers (Aizen 2003; Grant 1966; Grant & Grant 1968; Proctor et al. 1996; Rodriguez-Girones & Santamaria 2004). There are exceptions, for example, upward facing hummingbird flowers such as Paintbrush, which contain fine hairs to preclude rain, so facing downwards for this purpose is not always necessary (Corbet 1990). While many hummingbird flowers provide variable nectar rewards this variation in nectar rewards among flowers (volume and concentration) may be a result of evaporation as well as difference among the original nectars produced (Corbet 2003; Nicolson 2002).

In British Columbia, which is the adjacent province to Alberta and the field site, the plant species associated with hummingbird pollination are: Columbine, *Aquilegia formosa*; Paintbrush, *Castilleja spp*; Sky rocket, *Gilia aggregata* or *Ipomopsis aggregata*; Orange Honeysuckle, *Lonicera ciliosa*; Fuschia-Flowered Gooseberry, *Ribes lobbii*, and Cooley's Hedge Nettle, *Stachys cooleyae*. In addition: Orange Jewelweed, *Impatiens capensis*; Western Jewelweed, *Impatiens noli-tangere*, Colombia Lilly, *Lilium columbianum*; Limber Honeysuckle, *Lonicera dioica*; Wild Bergamot, *Monarda fistulosa*; Red-flowering current, *Ribes sanguineum*; Twinberry, *Lonicera involucrate*; Arbutus, *Arbutus menziesii*; Nuttall's Larkspur, *Delphinium nuttallianum*; Fireweed, *Epilobium angustifolium*; Purple-loosestrife, *Lythrum salicaria*; Willow, *Salix spp* and Salmonberry, *Rubus spectabilis* are also often visited by hummingbirds (Grant & Grant 1968; Pojar 1975). However, hummingbirds are highly opportunistic foragers and will also visit flower species that are not typically hummingbird pollinated and do not have characteristics typical of hummingbird flowers (Faegri & Der Pijl 1979; Grant & Grant 1968; Pojar 1975). Besides flowers, hummingbirds will also often feed on sap in tree holes made by woodpeckers and sapsuckers and will base feeding territories around such resources, particularly in early spring in more northern regions where flowers are scarce early in the year (Miller & Nero 1983; Sutherland et al. 1982).

Very little is known about what species other than typical/known hummingbird flowers rufous hummingbirds will sample, or how regularly, at our field site on their breeding grounds in the Rocky Mountains. Additionally, very little is known about variation in the nectar rewards of known hummingbird plants in the region. In this study I aimed to investigate the range of species visited by rufous

hummingbirds in the Westcastle River Valley, Alberta and compare techniques for identifying those species, including direct observation and pollen analysis. I expected hummingbirds to virtually ignore non-profitable species and to focus on a few highly profitable species most suited to hummingbird pollination/feeding.

The hummingbirds at Westcastle were expected to visit those plant species associated with hummingbirds in British Columbia that also occur in Westcastle. These species include: Columbine, *Aquilegia flavescens* (rather than *formosa*); Paintbrush, *Castilleja spp*; Gooseberry, *Ribes oxycanthoides* (rather than *lobbii*); Twinberry, *Lonicera involucrate*; Nuttall's Larkspur, *Delphinium nuttallianum*; Honeysuckle, *Lonicera utahensis* (rather than *ciliosa* or *dioica*), Raspberry, *Rubus idacus* (similar in form to Salmonberry, *Rubus spectabilis*) and Willow, *Salix spp*. There are also a number of *Penstemon* species. Some species of *Penstemon* are adapted for hummingbird pollination in other areas and species of typically bee pollinated *Penstemon* may also be visited occasionally by hummingbirds (e.g. Kimball 2008; Ornelas & Lara 2009; Wilson & Jordan 2009).

A preliminary study examining pollen on hummingbirds bills in the Westcastle Valley in 2007 found pollen from: Paintbrush, *Castilleja spp*; Red-clover, *Trifolium pratense*; Blue-eyed grass, *Sisyrinchium montanum*; Bull thistle, *Cirsium vulgare*; Bunchberry, *Cornus canadensis*; Harebell, *Campanula rotundifolia*; Purple geranium, *Geranium viscosissimum* and Pine/ conifers (Bowdrey 2008). However, the accuracy of identification of pollen was highly uncertain as only a limited photographic library of pollen from plants was available to compare with the pollen found on birds' bills. One outcome of that study was that it seemed that slides of

pollen taken from plants, in addition to photographs of pollen grains, should increase the certainty with which pollen taken from hummingbirds' bills can be identified.

Methods

Flowers available

In 2008, to investigate the different species of flowers available to Rufous hummingbirds and their abundance across the breeding season I walked transects through hummingbirds' territories and recorded what species were flowering and the number of flowers open. Transects were twenty meters long and placed to include as many different vegetation types within birds' territories as possible. For example, one might start a couple of meters into the woodland edge, cross through an area of low shrubs into a fertile meadow, cross a patch of bog and then turn up hill over a drier more gravel based, bank. In this way, I hoped to include as many species as possible in the transects. The same transect was walked at each of eleven locations on every day of monitoring. Every three paces along the transect, I recorded the number of plants of each species that were in flower within 1.5 meters to the sides and front of where I was standing (six of my feet lengths). Transects were placed in territories well spread out along the valley, again to increase the chances of including as many species as possible (Figure 1). Data were collected between the 4th of June and the 5th of July and transects were walked about every 4th day giving a total of seven monitoring sessions for each transect.

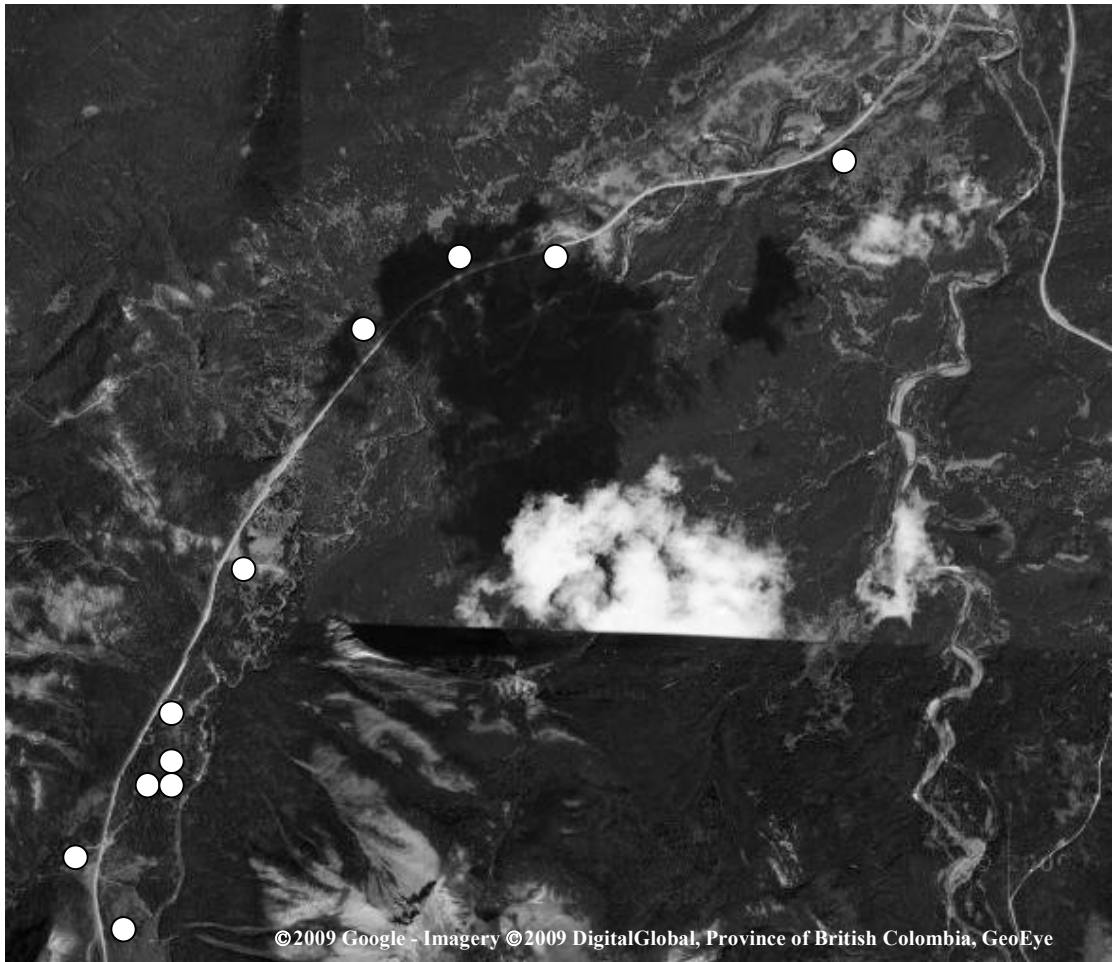


Figure 1: The Westcastle River valley, Alberta, Canada: Lat 49.349297, Lon: 114.410849. Locations of flower monitoring transects are marked with white dots. The width of the image represents approximately 7km.

Flowers visited by hummingbirds

I investigated which flower species hummingbirds were visiting using opportunistic observational data of what species they probed (stuck their bills into) collected by myself and other field workers, pollen collected from hummingbirds' bills and pollen collected from their feeders. Observational data were collected each year between the end of May and early July in 2007 to 2009.

Pollen from bills was collected by luring birds into a mesh cage with a hummingbird feeder, catching them and swabbing their bills with about 2mm² of a sticky gel containing a red stain, held in some sterilized forceps. The gel was temporarily stored in small plastic bags before being transferred to a microscope slide. The gel was composed of: distilled water 175cc, glycerine 150cc and gelatin 50g, it was stained with crystalline basic fuchsin stain, no phenol was added (see: Kearns & Inouye 1993). The gel was kept cool with an ice pack as it had a tendency to melt and become unusable on warmer days.

Pollen from feeders was collected by swabbing the area surrounding and inside the opening to the sucrose with cotton wool. I also attempted to increase the collection of pollen from feeders by placing small strips of cotton wool above the openings into the sucrose so the birds' bills had to brush the wool while they drank. Pollen was removed from the wool using the same gel that was used to swab bills.

Pollen slides from both bills and feeders were compared to pollen collected from local flowering plants (the pollen library) using a 400× light microscope. Pollen collected from plants to create the pollen library was collected and prepared in the same way as pollen from birds' bills. All pollen library samples were collected between the 28th of May and the 7th of July 2008.

Nectar rewards

I investigated the nectar rewards (production rather than standing crop) provided by the different species by bagging flower heads to exclude nectivores. As day and night production rates can vary, flowers were bagged for twenty-four hours and subsequently collecting nectar using micro-capillary tubes. Flowers were bagged

in nylon meshing. I measured the sucrose equivalent nectar sugar concentration (% mass) using a refractometer. Where nectar volumes were too small to be measured with the refractometer, an equal volume of water was added onto the refractometer and the reading doubled. Nectar volume was measured using the length of the nectar column in the micro-capillary tube. Nectar collected in the field was transported back to the lab and stored in a fridge to reduce the risk of evaporation and was measured within an hour and a half of collection. I chose six species on which hummingbirds had either been seen feeding from on multiple occasions or that are known hummingbird food plants: Columbine (*Aquilegia flavescens*), Gromwell (*Lithospermum incisum*), Lark's spur (*Delphinium bicolor*), Paintbrush (*Castilleja species*), Purple geranium (*Geranium viscosissimum*) and Twinberry (*Lonicera involucrate*). In addition, I collected nectar from: Raspberry (*Rubus idacus*) as a related species Salmonberry (*Rubus spectabilis*) and with a similar floral morphology, which is one of their main food plants in British Columbia. Shrubby penstemon (*Penstemon fruticosus*) as many *Penstemon* species are hummingbird pollinated, and White geranium (*Geranium richardsonii*).

Results

Flowers available

I identified ninety-eight species of plants, shrubs and trees, however, it should be noted that some species in the valley were certainly missed, meaning the actual number of species available to birds was in excess of this number. In addition, coniferous trees and willows were only identified to the genus level. The majority of

trees were coniferous, but there were also large stands of Black poplar, Aspen and Alder. I collected pollen samples from many but not all of the species on the species list (Appendix 1). The number of species in flower along the monitoring transects increased from around twenty-five in early June to over forty in early July (Figure 2).

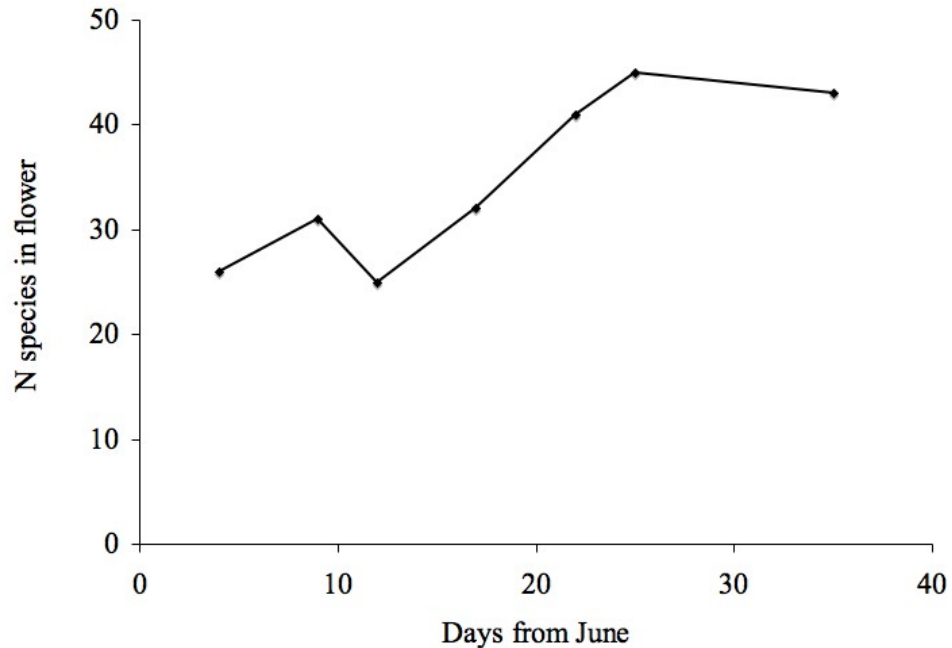


Figure 2: Changes over the summer in the number of plant species in flower along monitoring transects in the Westcastle River Valley, Alberta, Canada. Data are the number of days from the beginning of June onwards. The dip in the graph around day 11 corresponds to a period of snowfall.

Of the ninety-eight species identified only ten had tubular flowers: Paintbrush, *Castilleja species*; Columbine, *Aquilegia flavescens*; Gromwell, *Lithospermum incisum*; Larkspur, *Delphinium bicolor*; Sticky current, *Ribes viscosissimum*; Twinberry, *Lonicera involucrate*; Utah honeysuckle, *Lonicera utahensis* and three species of penstemon, *Penstemon Albertinus*, *P. fruticosus* and *P.*

confertus. Of these only Paintbrush was reddish in colour (dark orange). The commonest colour was white-cream (thirty-five species; Figure 3).

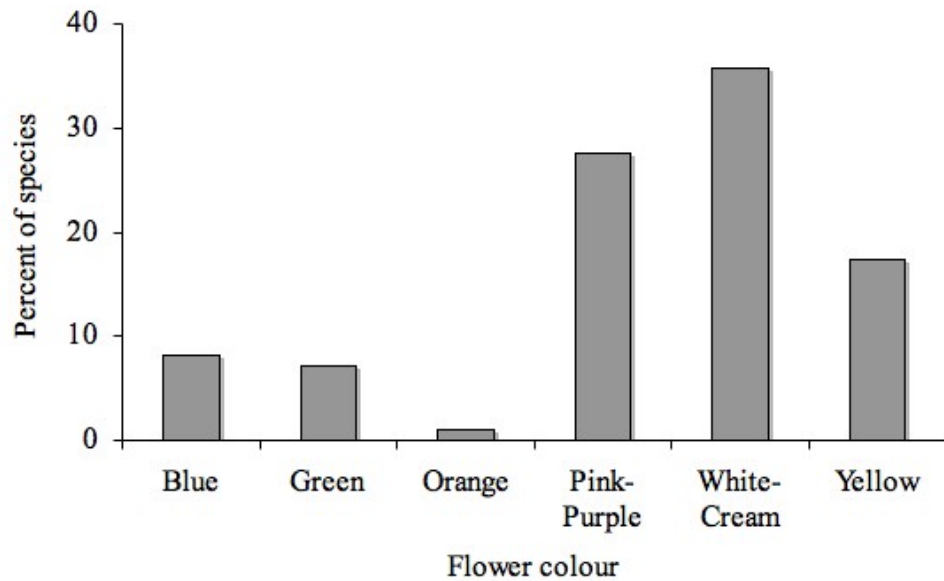


Figure 3: The percentage of plant species with flowers of different colours.

Flowers visited by hummingbirds

During fieldwork between 2006 and 2009 hummingbirds were observed probing the following six species on multiple occasions: Twinberry (*Lonicera involucrate*), Purple geranium (*Geranium viscosissimum*), Gromwell (*Lithospermum incisum*), Utah honeysuckle (*Lonicera utahensis*), Willow (*Salix spp*), Gooseberry (*Ribes oxyacanthoides*) and Bull thistle (*Cirsium vulgare*). They were also seen probing, but on only one occasion, the following seven species: Stickseed (*Hackelia floribunda*), Dandelion (*Taraxacum spp*), Clematis (*Clematis columbiana*), Mahonia (*Mahonia repens*), Saskatoon (*Amelanchier alnifolia*), Rose (*Rosa woodsii*) and Groundsell (*Senecio integerrimus*).

Analysis of pollen samples was hampered by deficiencies in the pollen library and insufficient magnification to see all pollen grain surface features in enough detail to separate species with very similar grains or identify grains with very few defining features (for pollen photos see appendix 1). Thus, I cannot be 100% certain of my species assignments for some pollen grains, however, some species were, very easy to identify (e.g. Pine and Twinberry) and I am sure of those assignments. Those grains that did not appear to come from any of the plants in the pollen library or that I was very unsure about remained unclassified (for examples see Appendix 2). I examined thirty-eight slides of pollen taken from birds' bills between the 28th of May and the 20th of June 2008 (Mean 2nd of June) and 26 slides of pollen collected from feeders between the 28th of May and the 7th of July 2008 (Mean 28th June). I identified pollen from five plant species across all 64 slides: Twinberry (*Lonicera involucrate*), Paintbrush (*Castilleja spp*), Clematis (*Clematis columbiana*), Prarie Smoke (*Geum triflorum*) and Pine (Any coniferous species). However, there were pollen grain types of a nearly equivalent number that I could not identify (Figure 4).

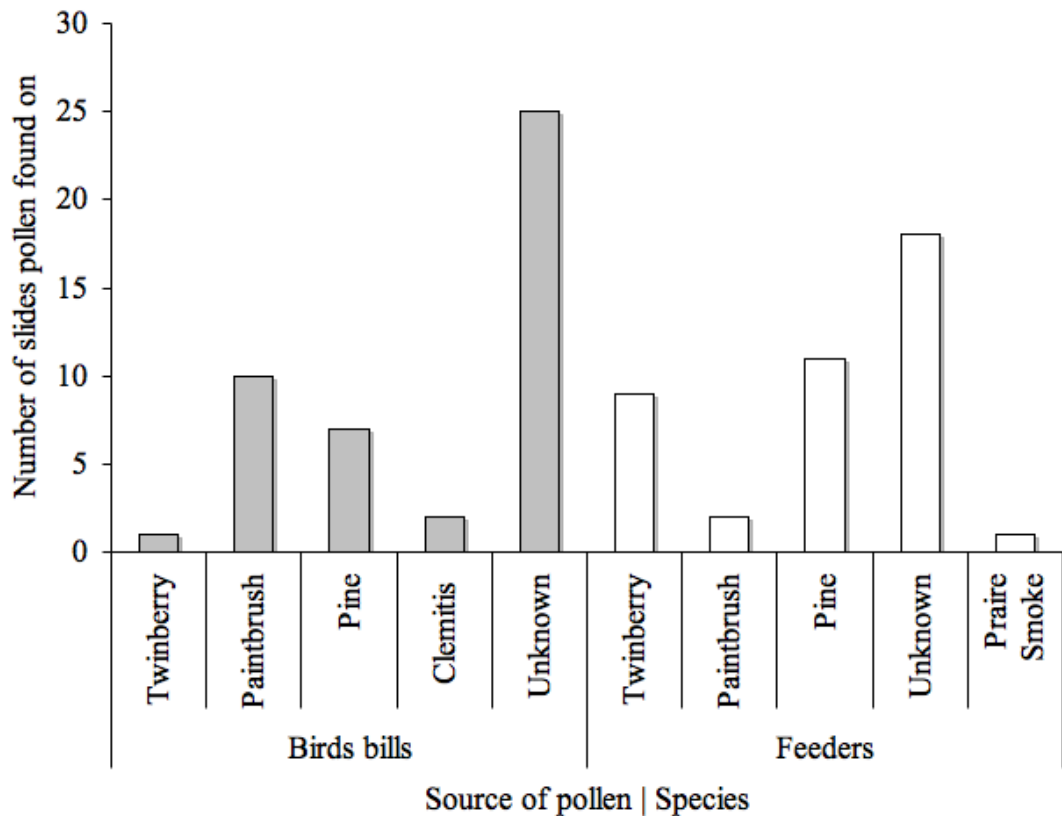


Figure 4: The number of slides that pollen from different species of flower was found on. Pollen slides were made up from pollen collected from either hummingbirds' bills ($n=38$) or from their sucrose feeders ($n=26$).

As the identity of many pollen grains was uncertain we plotted the flowering times for all plant species birds were seen probing and all those that pollen was found either on bills or feeders. This would allow us to check that those species were actually in flower when the pollen was collected and to show over what time period we would expect to find each species in the pollen data. For each day of flower monitoring, we calculated the percentage of the total number of flowers recorded for each species over the season that were in flower that day (Figure 5). We had no data for Pine, Willow or Utah Honeysuckle. However, Willow was already in flower at

our arrival on the study site on the 27th May. Although paintbrush did not appear in our monitoring transects on birds territories until mid to late June we did find patches of it in flower before then. On the 9th of June we recoded one patch consisting of thirty-four plants bearing a total of ninety-one inflorescences. As Twinberry was only coming into flower on the 4th of June its flowering period may explain the absence of Twinberry pollen from bills but not from feeders as the feeder samples were collected later in the season. As Gooseberry was in flower early in the season and was frequently visited by hummingbirds, it seems likely that pollen from gooseberry may be among the unidentified pollen taken from bills and feeders. It is surprising that we did not find any Purple geranium pollen on feeders as it was in peak flower and frequently visited about the time the feeder samples were collected. It is also surprising that not to find Gromwell pollen as it is another frequently visited species and was in flower all season.

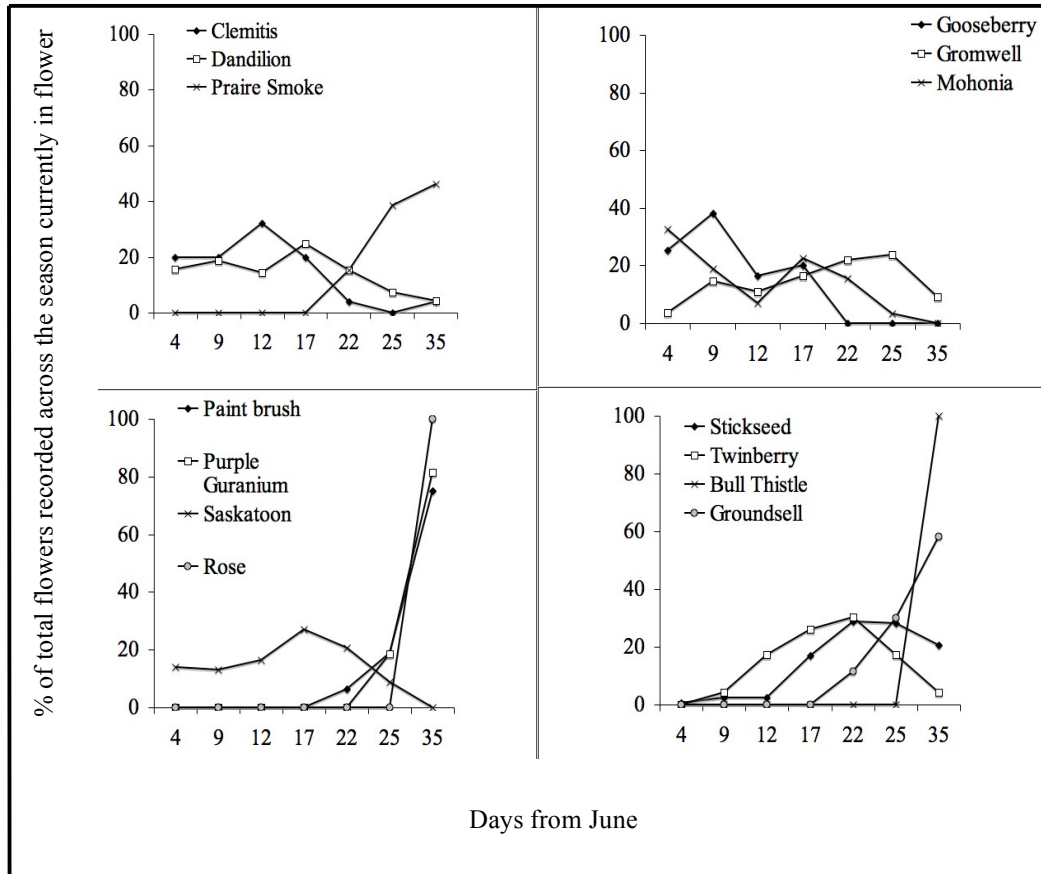


Figure 5: Flowering phenologies for plant species hummingbirds have been seen probing or whose pollen was found on hummingbirds' bills or on their sucrose feeders. Data are for the percent of the total number of flowers recorded across the season (for each species) that were open on each day of monitoring. Monitoring started at the beginning of June. Data not available for Pine, Utah Honeysuckle, or Willow.

Nectar Rewards

On average the flower species monitored had unexpectedly high sugar concentrations in comparison to data on hummingbird pollinated species from other studies on average around $26.1 \pm 13.4\%$ (Nicolson 2002; Ornelas et al. 2007): Columbine (44.15 ± 1.43 , N=33), Gromwell (62.44 ± 1.27 , N=145), Lark's spur (67.64 ± 3.08 , N=11), Paintbrush (41.25 ± 2.08 , N=20), Purple geranium (77.23 ± 2.42 , N=12), Twinberry (42.81 ± 1.34 , N=141), Raspberry (84.00 ± 3.05 , N=3), Shrubby penstemon (63.00 ± 3.41 , N=12), White geranium (77.50 ± 7.76 , N=4), data are means and standard errors for N flowers of each species. The mean volume of nectar in flowers ranged from a mean of $4.46 \pm 0.47 \mu\text{l}$ for Columbine to $0.62 \pm 0.16 \mu\text{l}$ for White geranium. The sample sizes for Raspberry and white geranium were very low as it was almost impossible to exclude ants from the flowers and wilting of flowers in the bags respectively. In order to estimate the likely profitability of these species to hummingbirds, I calculated the mean sucrose reward per flower in milligrams for each species (Figure 6). This is considered to be generally sufficient to compare sugar production between plants when evaporation makes comparisons of concentrations unreliable (Corbet 2003). There was significant variation among species in the mass of sucrose provided per flower (ANOVA $F_{1,8} = 8.79$, $p < 0.01$ data are means for each plant of each species). There was no significant variation in nectar reward among plants of the same species (ANOVA $F_{1,10} = 0.54$, $p = 0.85$).

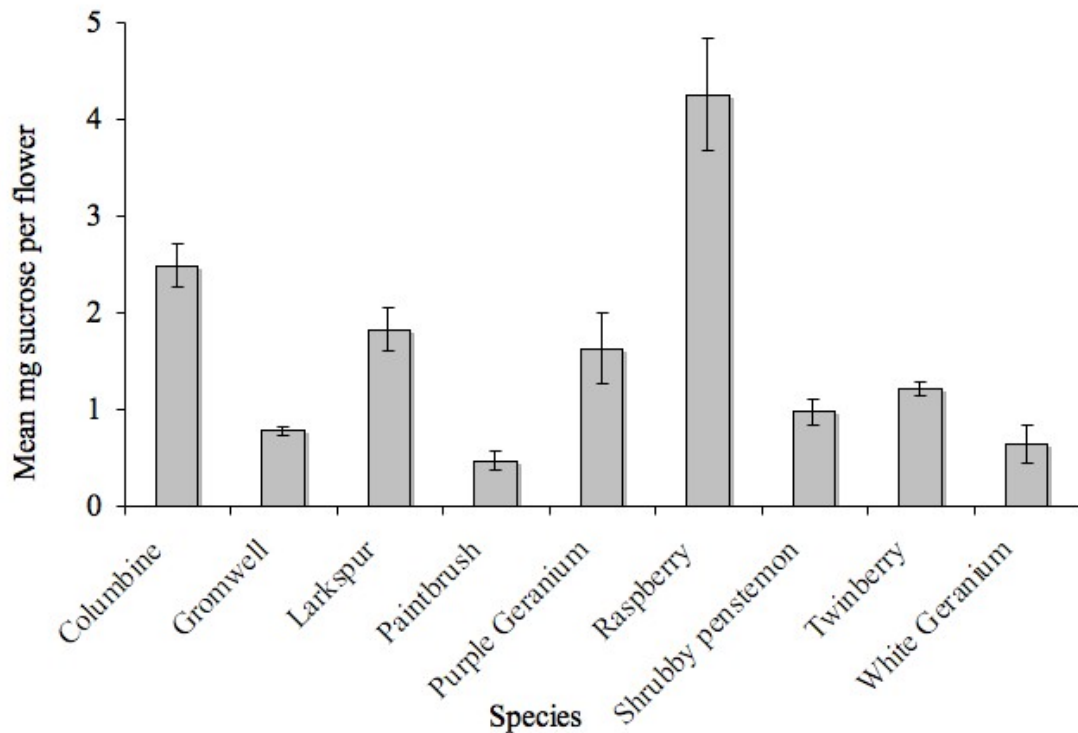


Figure 6: The mean mg of sucrose produced in 24 hours by species of wildflowers.

I used the Tukey-Kramer HSD test to test which plant species differed significantly from one another in the mass of sucrose in their flowers (Table 1). Raspberry produces significantly more sucrose per flower than any other species followed by Columbine and Larkspur. Paintbrush produced the least sucrose per flower followed by White Geranium and Gromwell. Purple Geranium and White Geranium did not differ significantly in the mass of sugar provided in each flower (Table 1, Figure 6).

Table 1: Differences in sugar production among plant species. Species that do not share a letter produce significantly different amounts of sugar. Magnitude of sugar production is $A > B > C > D$.

Species	Groups of similarity			
Raspberry	A			
Columbine	A	B		
Larkspur	A	B	C	
Purple Geranium		B	C	
Twinberry			C	D
Shrubby Penstemon			C	D
Gromwell			C	D
White Geranium			C	D
Paintbrush				D
q* (quantile) = 3.23, $\alpha = 0.05$				

For the two species for which I had the most data (Twinberry and Gromwell), I looked for any relationship between nectar concentration and nectar volume using REML in the statistical programme JMP. I included the plant the samples had come from as a random factor. There was a weak but significant relationship between nectar volume and nectar concentration such that nectar was more concentrated when the volume was lower (Twinberry: adjusted $R^2 = 0.03$, $F_{1,120} = 10.97$, $p < 0.01$ Figure 7, Gromwell: adjusted $R^2 = 0.11$, $F_{1,142} = 9.43$, $p < 0.01$, Figure 8). However, in the Gromwell dataset, when one outlying point was excluded this relationship was no longer significant (adjusted $R^2 = 0.06$, $F_{1,134} = 3.00$, $p = 0.09$).

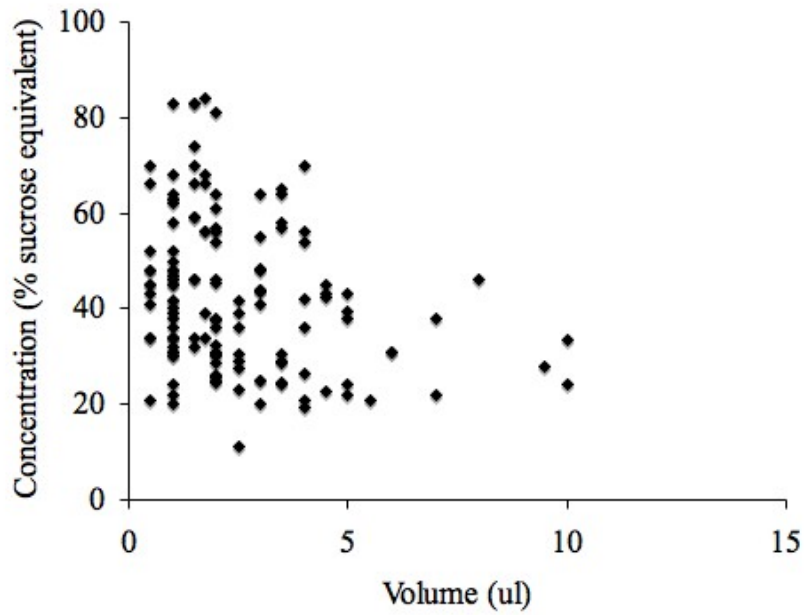


Figure 7: The relationship between nectar sugar concentration and nectar volume for Twinberry. Nectar concentration tends to be higher when the volume is lower.

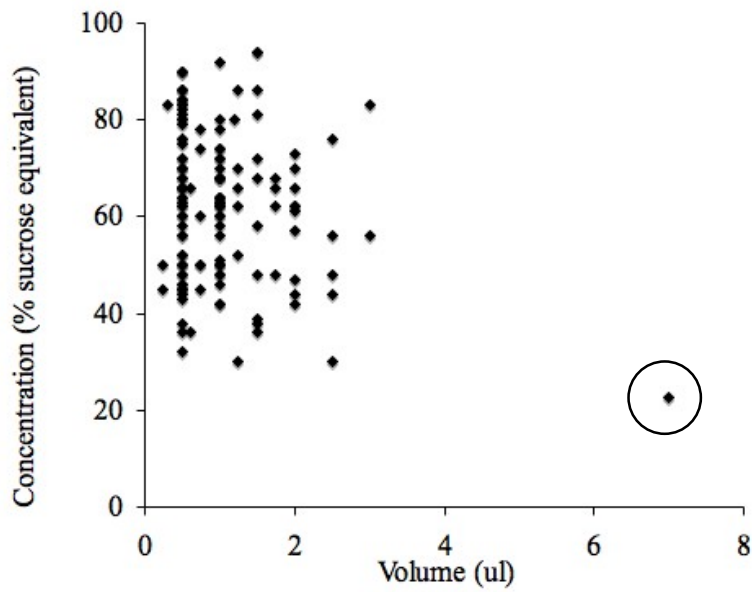


Figure 8: The relationship between nectar sugar concentration and volume for Gromwell. There is no significant relationship once the outlying data point (in black circle) is removed.

Including findings from the preliminary study in 2007, there are twenty species that hummingbirds visit or may visit (excluding conifers as these are wind pollinated; Table 2). These species do not all share similar floral traits in shape or colour. I used a contingency table to compare the frequency of flower colours of flowers visited by hummingbirds to the frequency of flower colours in the local flora. The frequency of flower colours visited by hummingbirds was similar to the frequency of flower colours in the local flora ($\chi^2_5 = 4.19$, $p = 0.52$). As hummingbird flowers are typically tubular I investigated whether the nineteen flower species I identified as hummingbird visited were more frequently tubular than expected. They were not ($\chi^2_1 = 1.20$, $p = 0.27$, p corrected for 1df using Yates' correction). However, when I just looked at the flowers that hummingbirds visit frequently (more than one record), tubular flowers were significantly more common than in the local flora (four of seven hummingbird flowers vs. ten of the ninety-eight species found in the valley, $\chi^2_1 = 4.89$, $p = 0.03$, p corrected for 1df using Yates' correction).

Table 2: The floral characteristics of flowers visited by hummingbirds in the Westcastle River Valley. Data are from this study (Observation and Pollen) and from a preliminary study by Bowdrey 2008 (Pollen-Bowdrey 2008). Visitation by hummingbirds was classed as often if birds were seen probing the species on multiple occasions or if there are multiple records in the pollen analysis (excluding wind pollinated species) or infrequent if there was only a single record.

Common name	Latin name	Colour	Tubular	Orientation	Source	Estimated visitation by Hummingbirds
Blue-eyed grass	<i>Sisyrinchium montanum</i>	Blue	No	Up to horizontal	(Pollen - Bowdrey 2008)	Infrequent
Bull thistle	<i>Cirsium vulgare</i>	White-purple	No	Up	Observation & (Pollen-Bowdrey 2008)	Infrequent
Bunchberry	<i>Cornus canadensis</i>	White	No	Up	(Pollen-Bowdrey 2008)	Infrequent
Clematis	<i>Clematis columbiana</i>	Lilac	No	Any	Observation	Infrequent
Dandelion	<i>Taraxacum spp</i>	Yellow	No	Up	Observation	Infrequent
Gooseberry	<i>Ribes oxycanthoides</i>	Green	No	Horizontal to down	Observation	Often
Gromwell	<i>Lithospermum incisum</i>	Yellow	Yes	Horizontal to down	Observational	Often
Groundsell	<i>Senecio integerrimus</i>	Yellow	No	Up	Observation	Infrequent
Harebell	<i>Campanula rotundifolia</i>	Blue	No (bell shaped)	Down	(Pollen-Bowdrey 2008)	Infrequent
Mahonia	<i>Mahonia repens</i>	Yellow	No	Any	Observation	Infrequent
Paintbrush	<i>Castilleja spp</i>	Orange, Pink, Red	Yes	Up	Pollen & (Pollen - Bowdrey 2008)	Often
Pine/ conifers	<i>Pinus spp</i>	Cream	No	Any	Pollen & (Pollen - Bowdrey 2008)	Never
Prarie Smoke	<i>Geum triflorum</i>	Pink-white	No	Down	Pollen	Infrequent
Purple geranium	<i>Geranium viscosissimum</i>	Pink-purple	No	Up	Observation & (Pollen-Bowdrey 2008)	Often

Common name	Latin name	Colour	Tubular	Orientation	Source	Estimated visitation by Hummingbirds
Red-clova	<i>Trifolium pratens</i>	Pink-red	No	Up to horizontal	(Pollen-Bowdrey 2008)	Infrequent
Rose	<i>Rosa woodsii</i>	Pink	No	Any	Observation	Infrequent
Saskatoon	<i>Amelanchier alnifolia</i>	White	No	Up	Observation	Infrequent
Stickseed	<i>Hackelia floribunda</i>	Blue	No	Up	Observation	Infrequent
Twinberry	<i>Lonicera involucrate</i>	Yellow	Yes	Horizontal to down	Observation & Pollen	Often
Utah honeysuckle	<i>Lonicera utahensis</i>	White	Yes	Any	Observation	Often
Willow	<i>Salix spp</i>	White-yellow	No	Any	Observation	Often

Discussion

Observational data are clearly the most reliable measure of the flower species hummingbirds visit. However, these data are very time consuming to collect and in this data set did not include Paintbrush, an important hummingbird food species in North America (Grant & Grant 1968; Pojar 1975). In this respect the pollen data were useful in confirming that hummingbirds did, indeed, visit Paintbrush as well as several species on which they were observed to forage. The large number of unidentified pollen grains found on the pollen slides suggests that hummingbirds may well be visiting more species than they were observed to and may be contaminated with air borne pollen. It is probable that pollen from Gooseberry, which they have been seen to visit on several occasions, and Columbine that I am fairly confident they visit based on their use of this species elsewhere, are among the unidentified grains. However, grains from some species are clearly incidental (not due to birds visiting flowers) such as, wind-pollinated species like pine. However,

the degree of uncertainty in pollen identification due both to inadequacies in the pollen library and insufficient magnification, casts some doubt on the accuracy of these results. Based on my experience of identifying pollen under 400× light microscopes as used in this study I believe that this method of identifying pollen grains is sufficient for most species given a sufficiently large pollen library and experience. However, for species with less distinct or very similar grains a higher magnification may be necessary.

There may be additional problems associated with using pollen analysis to identify the flower species the birds visit. Hummingbirds were seen to visit several species on multiple occasions, species that had fairly distinct pollen grains and were in flower at the time of pollen collection but were not found in the pollen samples e.g. Gromwell and Purple Geranium. This suggests that the pollen of some species may not be equally deposited on, or adhere as well to, hummingbirds as pollen from other species and so may be absent from or disproportionately rare in the pollen records. At the other extreme some species such as Paintbrush appear to deposit large very large amounts of pollen on hummingbirds (the whole bill and head may be white with it). In this respect I feel there is very little point in reading much about the frequency of visitation into the number of grains of pollen from a species found on a slide. The number of slides on which the pollen is found will give a better estimate but will still be biased depending on deposition and retention of pollen.

Further problems arise over the certainty of the origin of the pollen, as in some instances it could arrive on birds and their feeders accidentally. For example, bees and wasps also occasionally visit feeders and may leave pollen there and as Hummingbirds fly through the undergrowth they may knock pollen onto themselves

from species that they do not feed from. This problem may be particularly hard to assess when looking at potentially very important nectar sources like willow.

Hummingbirds have been seen probing willow flowers of several occasions. The willow flowers/catkins produce large quantities of nectar (on average 2.2 to 4.6 l per catkin, data are from a similar latitude as this study (Maine, USA): Heinrich 1975).

In early spring when few other nectar sources are available Willow is probably a vital and very attractive source of nectar for rufous hummingbirds (Dalby 1999).

Indeed, Willow is frequently visited early in the season (mid-May) by Ruby

Throated Hummingbirds (*Archilochus colubris*) in Manitoba, Canada (Sealy 1989).


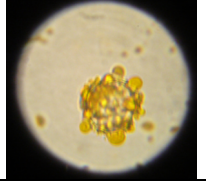
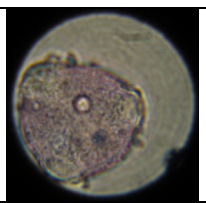
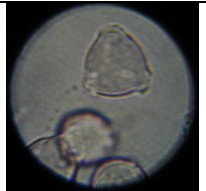
However, Willow produces very large quantities of pollen and is partially wind pollinated, which results in willow pollen being present in the air and on vegetation (Dalby 1999). This loose pollen could be picked up easily by a bird perching in, or flying through, a willow bush, which they do frequently as many territories border willow scrub. For plants like Willow, observational data will be vital to discovering how often these resources are visited as the probability of willow pollen in pollen samples coming from accidental contamination is very high.


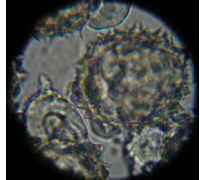
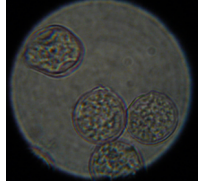

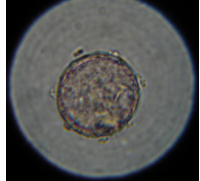
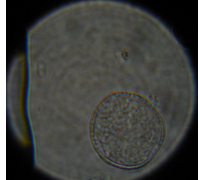
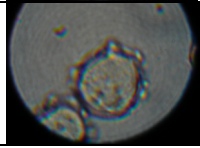
This study has shown there is a long way to go before it is clear which plant species the hummingbirds sample in the Westcastle Valley, and how often. However, it is probable that hummingbird visitation rates reflect plants' nectar rewards. It is surprising that the plants that appear to be most frequently used by hummingbirds in the Westcastle valley are not those that appear to produce the most sugar. Indeed, Paintbrush, Gromwell and Twinberry were among the lowest yielding species. As yet the nectar data are available for only a few species. Furthermore, it is possible that the methodologies I used led to inaccuracies in these data. One concern was that

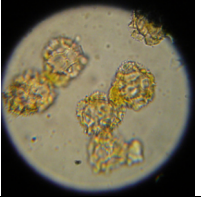

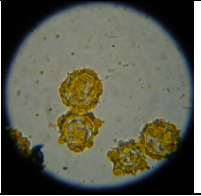

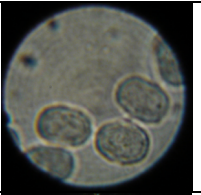
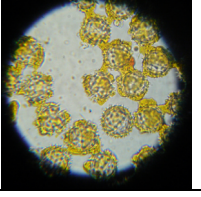
I found unexpectedly high sugar concentrations for hummingbird flowers (Nicolson 2002; Ornelas et al. 2007). The nectar concentrations I found were between 40-77% and yet the usual mean concentration of hummingbird-pollinated species is around $23.5 \pm 6.9\%$, for insect pollinated plants $26.1 \pm 13.4\%$ (Ornelas et al. 2007). The unexpectedly high concentrations may be due to evaporation caused by elevated temperatures in the bags used to exclude nectivores or evaporation from the microcapillary tubes between collection and measurement (Corbet 2003). Future studies should probably take all measurements in the field at the time of nectar collection. Although nylon netting is recommended for bagging flowers (Corbet 2003), it did cause wilting at least in White Geranium. I suggest bagging flowers either for shorter periods e.g. overnight or for measuring standing crops rather than nectar production. Measurement of the standing crop does not require bagging but would require more intensive sampling (Corbet 2003). The high concentrations that I recorded mean that the nectar I collected would have been very viscous (Roberts 1995). This may have resulted in incomplete emptying of the flowers and thus underestimating their sugar production.



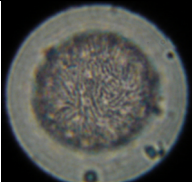



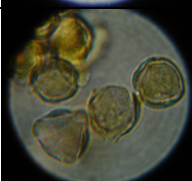
Although the flower species most frequently probed by hummingbirds did not produce the most sugar, they were, as expected of hummingbird-pollinated flowers, more likely to be tubular than the flowers in the local flora. However, hummingbirds clearly visited many other species and it is not yet clear how their floral characteristics relate to hummingbird visitation rates.

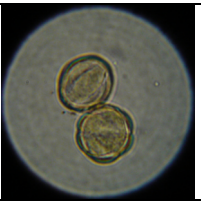


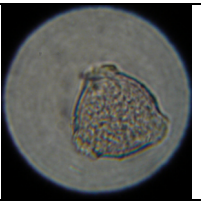
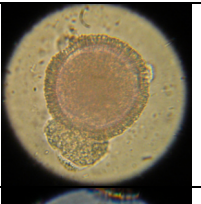
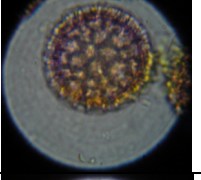

Appendix 1: A list of plant species and photos of their pollen (where collected and photographed) from the Westcastle River Valley. Additional information is provided on flower colour and whether or not flowers are tubular as is typical of hummingbird flowers.

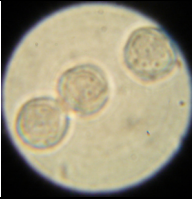
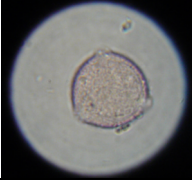
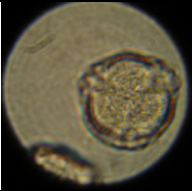

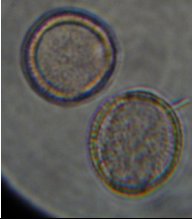

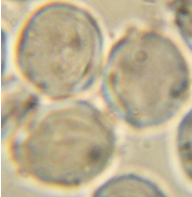
COMMON NAME (<i>Latin name</i>)	Colour/ Tubular (Y/N)	Pollen Collected (Y/N)	Pollen photo (not to scale)
ALDER LEAVED BUCKTHORNE: <i>Rhamnus alnifolia</i>	Green N	N	
ALUM ROOT: <i>Heuchera cylindrica</i>	Cream N	Y	
ANENOME: <i>Anenome sp</i>	Pink-white N	N	
ARNICA: <i>Arinca sp</i>	Yellow N	Y	
ASPEN: <i>Populus tremuloides</i>	Green N	N	
BANE BERRY: <i>Actaea rubra</i>	White N	N	
BLACK COTTONWOOD: <i>Populus balsamifera ssp trichocarpa</i>	Green N	N	
BLACK HAWTHORN: <i>Crataegus douglasii</i>	White N	Y	
BLUE EYED GRASS: <i>Sisyrinchium montanum</i>	Blue N	N	
BLUE EYED MARY: <i>Collinsia parviflora</i>	Blue N	N	
BLUE PENSTEMON: <i>Penstemon Albertinus</i>	Blue N	Y	
BRISTLY BLACKCURRENT: <i>Ribes lacustre</i>	Green-pink N	N	



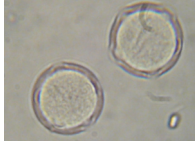
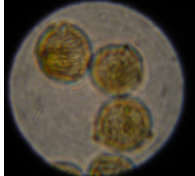

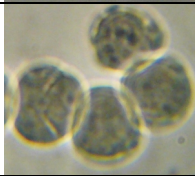
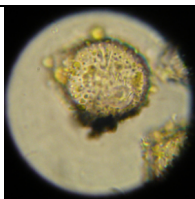
COMMON NAME (<i>Latin name</i>)	Colour/ Tubular (Y/N)	Pollen Collected (Y/N)	Pollen photo (not to scale)
BUGLOSS VIPER'S or BLUEWEED: <i>Echium vulgare</i>	Purple N	Y	
BULL THISTLE: <i>Cirsium vulgare</i>	White-pink N	Y	
BUNCHBERRY: <i>Cornus Canadensis</i>	White N	Y	
CANADA VIOLET: <i>Viola Canadensis</i>	White N	Y	
CHICKWEED: <i>Cerastium sp</i>	White N	Y	
CHOKER CHERRY: <i>Prunus virginiana</i>	White N	N	
CINQUEFOIL WHITE: <i>Potentilla arguta?</i>	White N	N	
CINQUEFOIL YELLOW: <i>Potentilla recta?</i>	Yellow N	N	
CLEMATIS: <i>Clematis columbiana</i>	Purple N	Y	
COLOMBINE: <i>Aquilegia flavescens</i>	Yellow Y	Y	
CORALROOT: <i>Corallorhiza striata</i>	Pink-brown N	Y no grains found on slide	
COW PARSNIP: <i>Heracleum maximum</i>	White N	N	


COMMON NAME (<i>Latin name</i>)	Colour/ Tubular (Y/N)	Pollen Collected (Y/N)	Pollen photo (not to scale)
CUT LEAVED DAISY: <i>Erigeron compositus</i>	White-pink N	N	
DANDILION: <i>Taraxacum sp</i>	Yellow N	Y	
DESERT PARSLEY: <i>Lomatium triternatum</i>	Yellow N	Y	
DESERT PARSNIP: <i>Lomatium dissectum</i>	Yellow N	N	
DOGWOOD: <i>Cornus stolonifera</i>	White N	N	
DUTCH CLOVA: <i>Trifolium repens</i>	White N	N	
ERIOGONIUM: <i>Eriogonum androsaceum</i>	Cream N	N	
FALSE SOLOMONS'S SEAL: <i>Maiantheumum racemosum</i>	White N	N	
FIR TREES: <i>Abies spp</i>	Cream N	Like pine	
GAILLARDIA: <i>Gaillardia aristata</i>	Yellow N	Y	
GEUM: <i>Geum triflorum</i>	Pink N	Like prairie smoke	
GLACIER LILLY: <i>Erythronium grandiflorum</i>	Yellow N	Y	
GOOSEBERRY: <i>Ribes oxycanthoides</i>	Green N	N	
GROMWELL: <i>Lithospermum incisum</i>	Yellow N	Y	
GROUNDSELL: <i>Senecio integerrimus</i>	Yellow N	Y	

COMMON NAME (<i>Latin name</i>)	Colour/ Tubular (Y/N)	Pollen Collected (Y/N)	Pollen photo (not to scale)
GROUSEBERRY: <i>Vaccinium scoparium</i>	Pink N	Y	
HAREBELL: <i>Campanula rotundifolia</i>	Blue N	Y	
HOUND'S TOUNGE: <i>Cynoglossum officinale</i>	Blue-purple N	N	
JACOB'S LADDER: <i>Polemonium pulcherrimum</i>	Purple N	Y	
LARK'S SPUR: <i>Delphinium bicolor</i>	Blue Y	Y	
LOCOWEED_REFLEX: <i>Oxytropis deflexa</i>	Purple N	N	
LUPIN: <i>Lupinus pusillus?</i>	Blue N	N	
MAHONIA: <i>Mahonia repens</i>	Yellow N	Y	
MARIPOSA LILLY: <i>Calochortus apiculatus</i>	White N	Y	
MEDDOW BUTTER CUP: <i>Ranunculus acris</i>	Yellow N	Y	
MEDDOW RUE: <i>Thalictrum occidentale</i>	Green N	N	
MOUNTAIN ALDER: <i>alnus tenuifolia</i>	Green N	N	

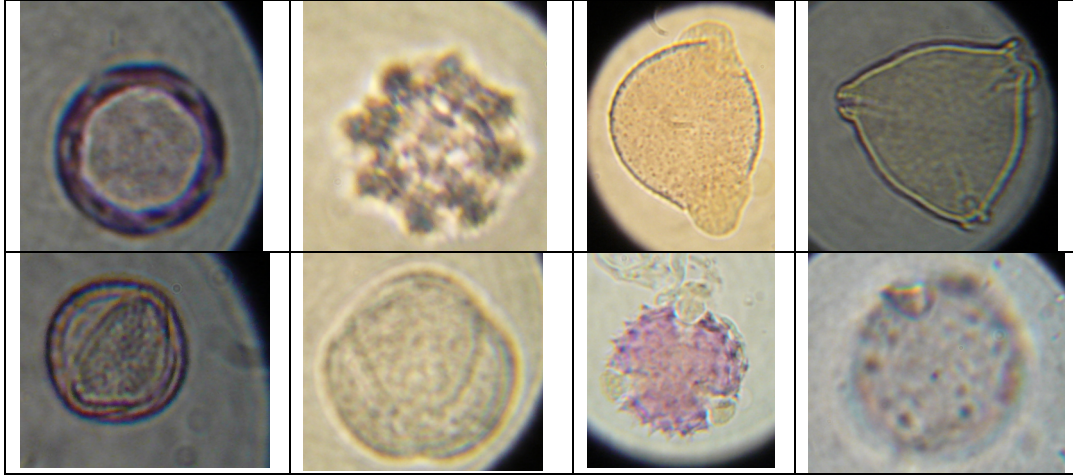
COMMON NAME (<i>Latin name</i>)	Colour/ Tubular (Y/N)	Pollen Collected (Y/N)	Pollen photo (not to scale)
NORTHERN BEDSTRAW: <i>Rubiaceae</i>	White N	Y	
OX-EYE DAISY: <i>Leucanthemum vulgare</i>	White N	N	
PAINT BRUSH: <i>Castilleja species</i>	Orange, Pink, Red N	Y	
PINE TREES: <i>Pinus spp</i>	Cream N	Y	
PINK PUSSY TOES: <i>Antennaria rosea</i>	Pink N	N	
Prarie smoke: <i>Geum triflorum</i>	White-pink N	Y	
PURPLE AVENS: <i>Geum rivale</i>	Pink N	Like prairie smoke	
PURPLE GERANIUM: <i>Geranium viscosissimum</i>	Pink-purple N	Y	
PURPLE LEAVED WILLOWHERB: <i>Epilobium ciliatum</i>	Pink N	Y	
PURPLE VETCH: <i>Vicia americana</i>	Purple N	Y	

COMMON NAME (<i>Latin name</i>)	Colour/ Tubular (Y/N)	Pollen Collected (Y/N)	Pollen photo (not to scale)
RASPBERRY: <i>Rubus idacus</i>	White N	Y	
RED CLOVA: <i>Trifolium pratense</i>	Pink-red N	Y	
RED ELDER: <i>Sambucus racemosa</i>	White N	N	
ROCK CRESS: <i>Arabis drummondii</i>	Purple N	N	
ROSE: <i>Rosa woodsii</i>	Pink N	Y	
SASKATOON: <i>Amelanchier alnifolia</i>	White N	Y	
SELFHEAL: <i>Prunella vulgaris</i>	Purple N	Y	Slide contaminated with other pollen
SHEPHERDS PURSE: <i>Capsella bursa-pastoris</i>	White N	Y	
SHOOTING STAR: <i>Dodecatheon pauciflorum</i>	Pink N	Y	
SHOWY LOCOWEED: <i>Oxytropis splendens</i>	Purple N	Y	
SHOWY PUSSY TOES: <i>Antennaria pulcherrima</i>	White N	Y	

COMMON NAME (<i>Latin name</i>)	Colour/ Tubular (Y/N)	Pollen Collected (Y/N)	Pollen photo (not to scale)
SHRUBBY PENSTEMON: <i>Penstemon fruticosus</i>	Purple N	Y	
SHRUBBY POTENTILLA: <i>Dasiphora floribunda</i>	Yellow N	N	
SILKY PHACILIA: <i>Phacelia sericea</i>	Purple N	Y	
SNOWBERRY: <i>Symphoricarpos albus</i>	Pink N	N	
SPIKY BLACKCURRENT: <i>Ribes montigenum</i>	Green-pink N	Y	
SPRUCE TREES: <i>Picea spp</i>	Cream N	Like pine	
STAR FLOWERED SOLOMON'S SEAL: <i>Maianthemum stellatum</i>	White N	Y	All grains collapsed and featureless
STICKSEED: <i>Hackelia floribunda</i>	Blue N	Y	
STICKY CURRENT: <i>Ribes viscosissimum</i>	Cream Y	Y	
STRAWBERRY: <i>Fragaria virginiana</i>	White N	Y	
SWEET CISILY: <i>Osmorhiza spp</i>	Green N	Y	
THIMBLEBERRY: <i>Rubus parviflorus</i>	White N	Y	
TWIN FLOWER: <i>Disporum trachycarpum</i>	White N	N	
TWINBERRY: <i>Lonicera involucrate</i>	Yellow Y	Y	

COMMON NAME (<i>Latin name</i>)	Colour/ Tubular (Y/N)	Pollen Collected (Y/N)	Pollen photo (not to scale)
UTAH HONEYSUCKLE: <i>Lonicera utahensis</i>	White Y	N	
VALARIAN: <i>Valeriana dioica</i>	White N	N	
VIOLET: <i>Viola adunca</i>	Purple N	N	
WESTERN MINNIEBUSH: <i>Menziesia ferruginea</i>	Pink N	N	
WESTERN SPRING BEAUTY: <i>Claytonia lanceolata</i>	White N	N	
WHITE GERANIUM: <i>Geranium richardsonii</i>	White N	N	
WHITE VETCH: <i>Lathyrus ochroleucus</i>	White N	N	
WILLOW: <i>Salix sp</i>	White N	N	
YELLOW ANGELICA: <i>Angelica dawsonii</i>	Yellow N	N	
YELLOW PENSTEMON: <i>Penstemon confertus</i>	Cream N	Y	
YELLOW SWEETCLOVA: <i>Melilotus officinalis</i>	Yellow N	Y	Slide contaminated with other pollen grains
YELLOW VIOLET: <i>Viola orbiculata</i>	Yellow N	N	

Appendix 2: Photographs of pollen grains that could not be identified. Grains were found on slides made up from pollen collected either from hummingbirds' bills or from hummingbirds sucrose feeders.



**Chapter 7 Behaviour of rufous hummingbirds around
feeders in British Columbia**

Abstract

Animals' feeding behaviour may depend on a range of variables including resource quality, competition, the availability of alternative resources and genetic tendencies for, for example more aggressive/territorial behaviour. It may not be immediately obvious, when differences in behaviour are observed between locations, which of these variables may be responsible. Here we investigate anecdotal evidence that rufous hummingbirds breeding in British Columbia tend to feed together in much greater numbers than those birds breeding in Alberta. Specifically we were interested in whether population density differences between regions might explain differences in foraging behaviour or whether birds breeding in British Columbia might be less aggressive and more inclined to feed together. We used observational data of the number of birds visiting artificial feeding sites and of males' territorial behaviour from both regions. Differences in behaviour between the regions appear to be due to population density differences. Site providing more feeders had more birds visiting each feeder than site with fewer feeder.

Introduction

Many animal species exhibit flexible behavioural strategies due to variation in environmental and social conditions and genetics. Behaviour may vary within an individual, among individuals or among populations. For example, in the North East Pacific Ocean, two populations of killer whale with over-lapping ranges have distinct foraging strategies: one population is relatively sedentary and feeds mostly on fish while the other is more transient and specializes in feeding on marine mammals (Baird et al. 1992). The difference in foraging strategies between the two populations is thought to have arisen initially as different foraging strategies among individuals within the same population utilising different food sources in slightly different habitats and then by assortative mating (Baird et al. 1992).

Variation in foraging strategies may occur for several reasons, including, competition/population density, resource defensibility, aggression, genetic differences and diet (Baird et al. 1992; Dobbs et al. 2007; Inouye et al. 1991; Kim & Grant 2007). Increased competition results in closer proximity among foraging individuals or in larger group sizes. Under greater competition, less competitive individuals may be excluded from many resources and so benefit from alternative foraging strategies such as scrounging and diet diversification (Collias & Southwick 1952; Coolen et al. 2007; Svanback & Bolnick 2007). For example, at higher population densities, male Black-throated Blue Warblers (*Dendroica caerulescens*), hold smaller territories and increase their use of more energetic foraging strategies such as aerial attacks (Dobbs et al. 2007).

Aggression can act in a similar way to population density by further limiting the access of less competitive individuals to food (Lopez-Sepulcre & Kokko 2005). Aggression is partly under genetic control and can vary among populations (Dingemanse et al. 2007; Lema 2006). In populations where individuals are more aggressive resources are more likely to be dominated by one or a few individuals (assuming similar population densities) so that there may appear to be fewer individuals competing for those resources (Lopez-Sepulcre & Kokko 2005). Whether observed differences in, for example aggression, among regions in the number of individuals using a resource patch is due to differences in competition (population density) or to genetic differences may have implications for species conservation. For example, food provision may need to be more dispersed in more aggressive populations (e.g. Garcia de Leaniz et al. 2007; Meretsky & Mannan 1999; Romanov et al. 2009; Valeur 1988).

Rufous hummingbirds frequently use artificial feeders and are a species of particular conservation interest as their population is declining by about 3% a year (Sauer et al. 2008). This decline appears to be more pronounced west of the Rockies in British Columbia. Reports from ornithologists suggested that many rufous hummingbirds use each feeder in British Columbia (Figures 1) whereas, in Alberta, east of the Rockies, hummingbird feeders are typically defended and used almost exclusively by a territorial male. It is unclear whether more birds use feeders simultaneously in B.C. than in Alberta because there are more birds in B.C., because there more competition in B.C. due to enhanced quality of provisioning or because the B.C. birds are less aggressive than are the Albertan birds. Here I investigate the reported differences in foraging behaviour between the two populations of rufous

hummingbird with a view to evaluating this difference and assessing whether it is more likely to be due to differences in population density and food availability or genetics.



Figure 1: Rufous hummingbirds of both sexes using a feeder in British Columbia.

Data provided by the United States Geological Survey (USGS) North American Breeding Bird Survey show that B.C. does have a higher population density of rufous hummingbirds than does Alberta (Figure 2, Sauer et al. 2008). A high population density may increase competition to the point that defence of a feeder by a single individual becomes un-economical especially if it is in a location that is not readily defended (Carpenter 1987). It seems plausible that males would find it easier to defend a single feeder that is coupled with nearby perches than to defend a group of feeders that intruders can readily move between. In this case, the differences in behaviour seen in numbers of hummingbirds at feeders in B.C. and Alberta might simply be due to the density of feeders.

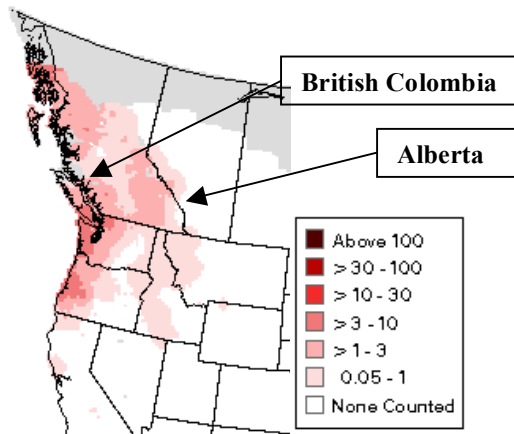


Figure 2: The map of the average number of rufous hummingbirds that were recorded in about 2.5 hours of bird-watching along roadsides by an experienced bird watcher. Predictions are based on counts along BBS routes between 1994 - 2003 (Sauer et al. 2008).

However, a second possibility is that, in addition to differences in population density, the rufous hummingbirds breeding in B.C. are also less aggressive and are more tolerant of other individuals around food sources than birds in Alberta. This would lead to very little feeder defence even at easily defended sites.

The aim of this study was then to investigate the number of birds using feeders in B.C. and to assess levels of aggression and defence of feeders.

Method

Sites

In April and May 2007, I surveyed ten sites across Vancouver Island to gather observational data on feeder use and territorial behaviour: two sites in Tahsis (T1 and T2), one site near Port Alberni (JT), two sites near Sturdies Bay on Galliano Island (BK1 and BK2), two sites near Fulford harbor on Saltsprings Island (MC and JS), two sites in Victoria (Victoria and WK), and one site near Genoa bay on Vancouver Island (CM), Figure 3). Observations in Alberta were carried out along the Westcastle River Valley (Lat: 49.349297, Lon: 114.410849) at site where hummingbird feeders have been traditionally placed each year, for over ten years, by researchers from Lethbridge University.

The field sites in B.C. were the private gardens of people who fed hummingbirds. The sites were selected opportunistically as I met or contacted people who were happy to let me observe their hummingbird feeders. As far as possible I made sure these sites were as widely distributed across Vancouver Island so to include sites that were as varied as possible.

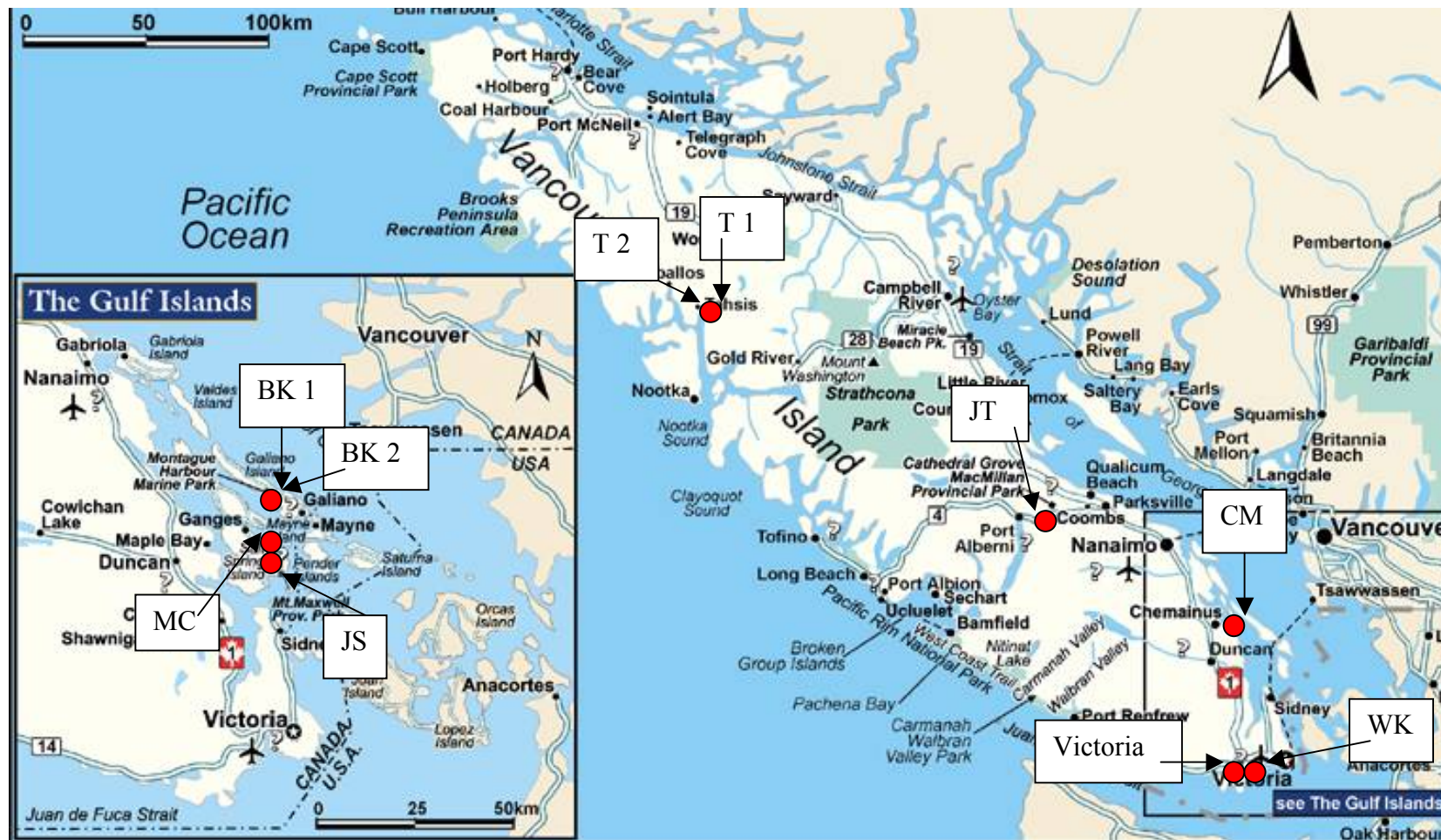


Figure 3: Field sites on Vancouver Island and the Gulf Islands, British Columbia, Canada.
 Map modified from: <http://www.ulyssesguides.com/loa/bc/vancgulf/maps/vancouver-island.jpg>

I used a variety of methods to collect sufficient data to enable comparisons among the sites (see below). This was necessary due to high variability in the number of hummingbirds using each site. Some sites were too busy for me to be able to use the most detailed survey method (continuous feeder activity). The methods used at each site are summarised in Table 1. For general site characteristics see Table 2. The time I spent at sites depended on what was convenient for the owners and the availability of alternative sites.

Table 1: *The methods used to record the behaviour of hummingbirds around feeders and on their territories at different field sites on Vancouver Island, British Columbia, Canada.*

Site ID	Census	Tally	Continuous feeder activity (CFA)	Feed times	Territorial Behaviour and territory sizes
CM	√	√	-	√	√
MC	√	-	√	-	√
JS	√	-	√	-	√
BK 1	√	√	-	√	√
BK 2	√	√	-	√	√
JT	√	-	√	-	-
T 1	√	√	-	-	-
T 2	-	-	-	-	√
WK	√	-	√	-	-
Victoria	No rufous - detailed results not reported				

Census

In order to assess how many birds were using feeders simultaneously, I recorded the number, sex and species of hummingbirds on each feeder for all feeders at the site simultaneously once every ten minutes. This method was used at all sites.

Continuous feeder activity (CFA)

To estimate the relative number of birds visiting each feeder at sites, I recorded for each hummingbird that fed from a the feeder, its species, sex, how long it spent at the feeder, and whether it left of its own accord or was chased off by another hummingbird. I observed each feeder for ten minutes. Observation periods of ten minutes per feeder were chosen as typical inter-bout intervals for hummingbirds feeding from 30% sucrose are around twelve to fifteen minutes therefore reducing the probability of counting the same bird twice within any ten minute period (Lopez-Calleja et al. 1997; Wolf & Hainsworth 1977). To avoid recording unrepresentative data for a site should a particular feeder be preferred I changed the feeder I was observing after every ten minute period. This method was used only at quieter sites as it was impossible to keep track of all the birds on a feeder when there were more than about three birds feeding once. If the site was too busy, I obtained comparable data using the two following methods (Tally and Feeding bout durations).

Tally

As above, I observed one feeder at a time for ten minutes. I counted the species and sex of all the hummingbirds that fed from that feeder. This method was used at busy sites where the equivalent data could not be obtained from the CFA method.

Feeding bout durations

Where feeding bout durations and reasons for leaving the feeder could not be obtained by using the CFA method, I recorded the feeding bout durations and reason for departure of a sample of hummingbirds that landed on the feeder being observed for the tally method. I waited until the bird I was recording a finished feeding then started recording the feed length of the next bird to arrive at the feeder.

Territorial behaviour

For each site, I observed only one male, whichever one held a territory closest to or encompassing the feeders, and recorded his behaviour continuously for one hour. I recorded the start and end times of each behaviour (Feeding, Perching, Chasing and Displaying) and for interactions with intruders, the behaviour of both the male and the intruder and the species and sex of the intruder. These data were recorded at all sites with territorial males. Courtship displays are known as 'J-flights' (Hurly et al. 2001), were also recorded.

Territory diameter

Only males hold territories during the breeding season. I was able to mark individual males at only one site (CM), so observations at other sites are based on the assumption that if a male defending an area passed out of sight the subsequent male to be spotted defending that same area was the same individual. To get an estimate of territory sizes at sites, I mapped the main perches of all territorial males adjacent to the feeding area. The main perch was the perch at which a male spent most time and was usually centrally located in his territory. A territorial male was defined as: A male using prominent perches within a restricted area, displaying to females and chasing intruders who enter that area. Territories usually shared borders, making adjacent territory holders' main perches approximately a territory width apart. Territories were only delimited after each territorial male and each of his neighbouring territorial males had been seen simultaneously, to ensure they really were different birds. Each male was then observed in order to locate his main and sub-perches and to establish where territory boundaries lay.

General

Observation sessions for each method were conducted for at least an hour and in most cases more than two hours. The majority of observations are of unmarked individuals. However, I colour marked five males and five females at the first site I visited (CM) under the supervision of a trained hummingbird bander Cam Finlay. Data were analysed in the statistical program JMP.

Results

The B.C. sites were highly variable in feeder provision and habitat (Table 2). Feeders at all but one site were used by rufous hummingbirds. At the site in Victoria the feeder was defended by a territorial male Anna's hummingbird (*Calypte anna*). Only this bird and an occasional Anna's female were observed to use the feeder despite its proximity to the WK site, which was used mainly by rufous females. There were very few Anna's hummingbirds that visited the feeders where rufous hummingbirds were present. Excluding Anna's from the data set did not significantly affect estimates of the number of birds using sites (Paired t-test, rufous feeding every ten minutes vs. rufous +Anna's feeding every ten minutes: $t_8 = 1.69$, $p = 0.12$). Therefore, only data for sites used by rufous are reported hereafter. All feeders at all sites contained sucrose solution of approximately 30% at the time of observation.

Table 2: *The relative hummingbird activity at field sites on Vancouver Island, the habitat around those site and the number of hummingbird feeders they provided. Sites are classed as 'Busy' if there were typically too many hummingbirds visiting it to record data for every bird to visit the feeder (CFA method) and 'Territorial male' if the feeder was defended and used almost exclusively by a territorial male.*

Site	Number of feeders	Activity	Surroundings	No Days observed
CM	7	Busy	Suburban	4
MC	1	Territorial male	Broken Woodland	4
JS	2	Busy	Broken Woodland	3
BK 1	5	Busy	Broken Woodland	2
BK 2	1	Territorial male	Broken Woodland	2
JT	2	Intermediate	Woodland edge	3
T 1	1	Quiet	Suburban	1
T 2	0 (flowers)	Territorial male	Natural scrubland	1
WK	1	Intermediate	Suburban	3

Visitation rate

The number of birds visiting each feeder every ten minutes was calculated from the tally and CFA data (not available for the first two days at CM). There were significant differences among sites (ANOVA; $F_{7,18} = 30.28$, $p < 0.01$; Figure 4) and significant differences among sites in the relative number of birds of each sex (Interaction sex*site, ANOVA; $F_{7,18} = 28.59$, $p < 0.01$). However, overall sites were typically visited by more females than males (ANOVA; $F_{1,18} = 79.34$, $p < 0.01$). Data were means for each day of observation at each site for both sexes. The most active sites were CM and BK 1, both of which were used predominantly by females. Sites very close together (BK1 and BK 2, and MC and JS) were not necessarily very similar: within each pair one feeder was undefended and very busy while the other was defended and used almost exclusively by a single territorial-male. Females predominated at both sites where there were no territorial males defending areas around, or close, to the feeders (JT and WK). There was no obvious effect of local habitat type. The quietest site was at T1. This feeder was in the centre of a small village and fairly exposed (15ft up on the wall of a building with no near by vegetation in which the birds could shelter). Most properties in the area had similar feeders, all of which I was informed had been used regularly earlier in the spring but less so since the salmonberry had flowered. JS was the only site being used predominantly by males. Although there was frequent aggression between males at this site, neither of the two feeders appeared to be at the centre of a male's territory. Rather, males appeared to be defending patches of salmonberry scrub adjoining the

garden. I concluded that, at best, these feeders were on the periphery of these territories (diameter approx 35m).

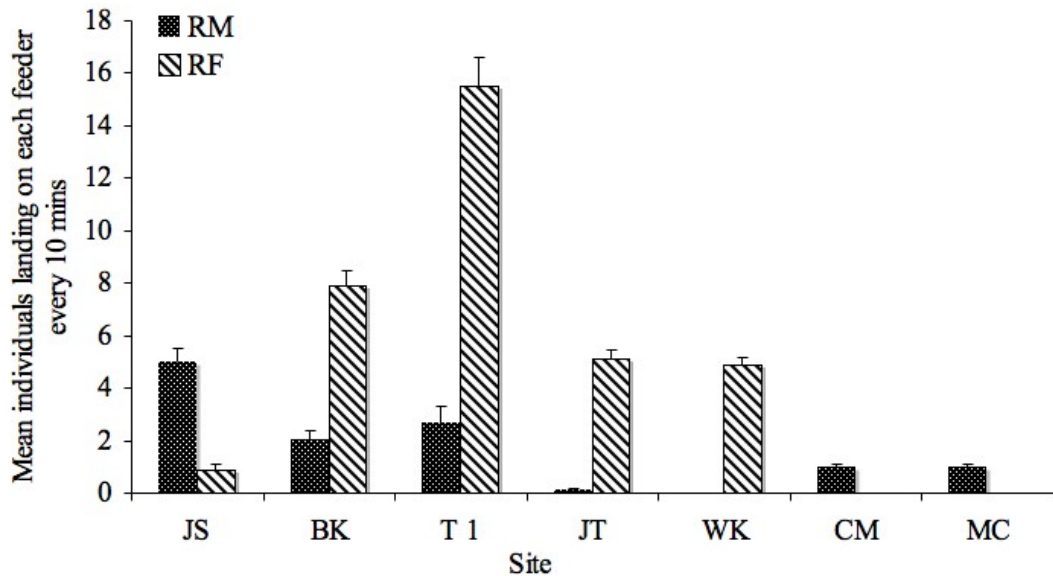


Figure 4: The mean number of birds visiting each feeder at a site every ten minutes.

Data are means and standard errors for each site. RF= rufous females, RM= rufous males.

Maximum number of birds using a feeder simultaneously

The most birds recorded using a feeder simultaneously were six at site CM and five at BK. However, as I was particularly interested in differences in the number of birds that would regularly use a feeder simultaneously, I took the maximum number of birds recorded on a feeder at each ten minute census for each site (the number of birds on the busiest feeder), and calculated a mean of this maximum for each day of observation. As for the number of birds visiting feeders every ten minutes, there was also considerable variation among sites in both the

maximum number of birds that would use a feeder simultaneously, although this variation was not quite significant (ANOVA; $F_{6,12} = 2.76$, $p = 0.06$; Figure 5).

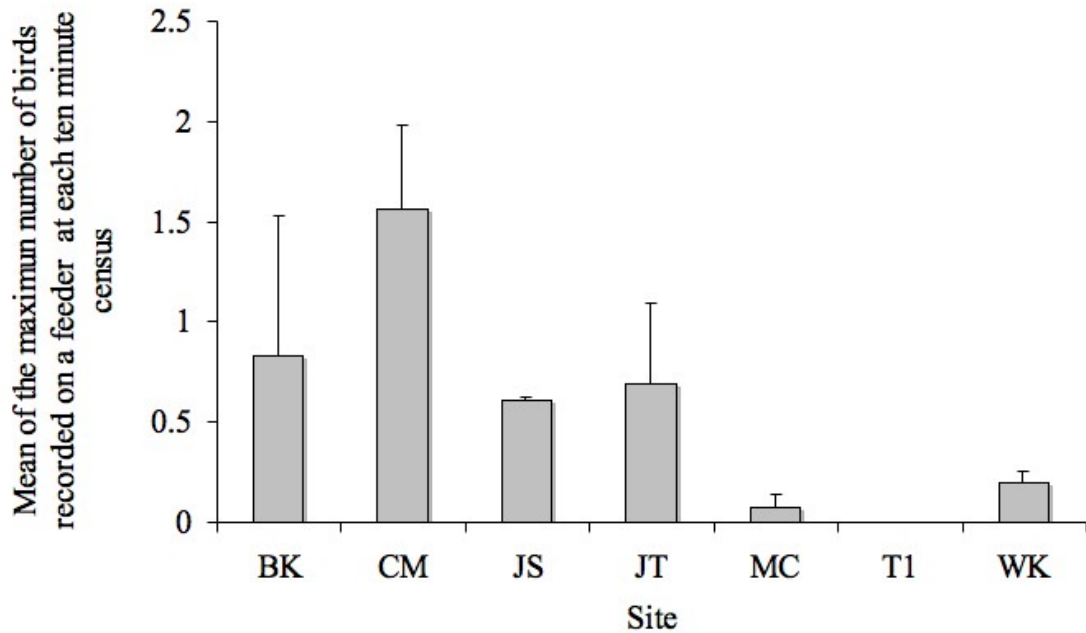


Figure 5: *The mean of the maximum number of rufous hummingbirds seen on a feeder simultaneously at every ten minute census at each site. Data are means and standard errors.*

As it appeared that the sites with the most birds visiting each feeder every ten minutes were the sites with the most feeders I investigate this relationship using a mixed model. Data were the mean number of birds visiting each feeder at each site and the number of feeders at the site. The number of feeders explained the majority of the variation between sites in the number of birds visiting each feeder such that more birds visited each feeder at sites with more feeders (Adjusted $R^2 = 0.93$, trend estimate = 3.45, $F_{1,6} = 89.37$, $p < 0.01$).

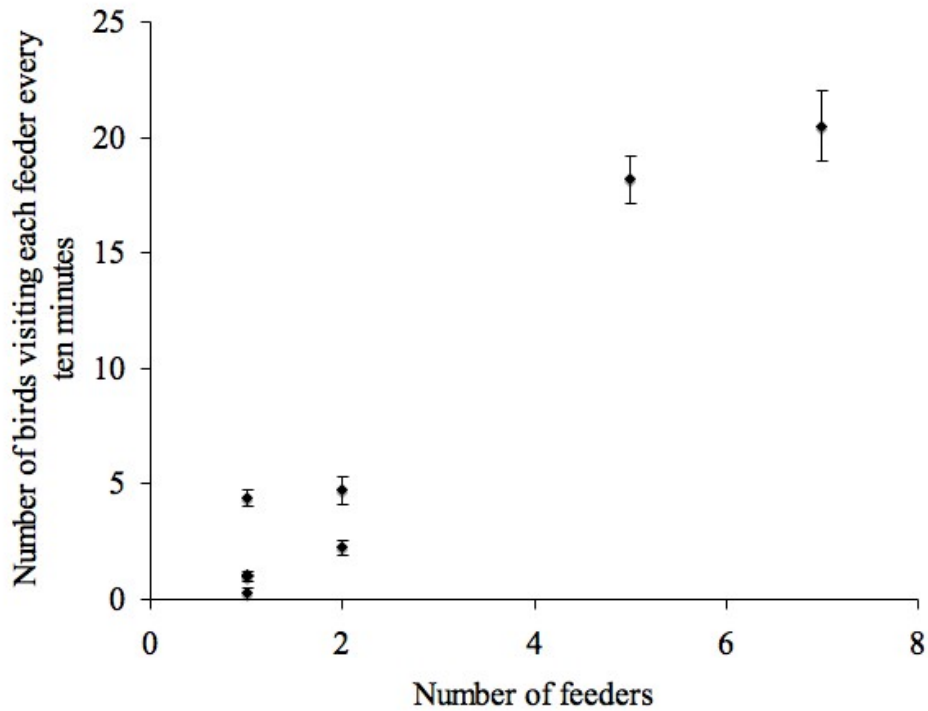


Figure 6: The mean number of birds visiting each feeder at each site and the number of feeders at those sites. More birds visit feeders at sites with more feeders. Data are a mean for each site and standard errors.

Sites JS and WK were visited on three consecutive days at the same time of day, I compared the air temperature at the time of data collection with the mean number of birds visiting each feeder within ten minutes, for each day for both sites. Site was included as a random factor. Significantly fewer birds visited each feeder when the air temperature was higher (Adjusted $R^2 = 0.72$, trend estimate = -0.57, $F_{1,3.3} = 9.19$, $p = 0.05$; Figure 7).

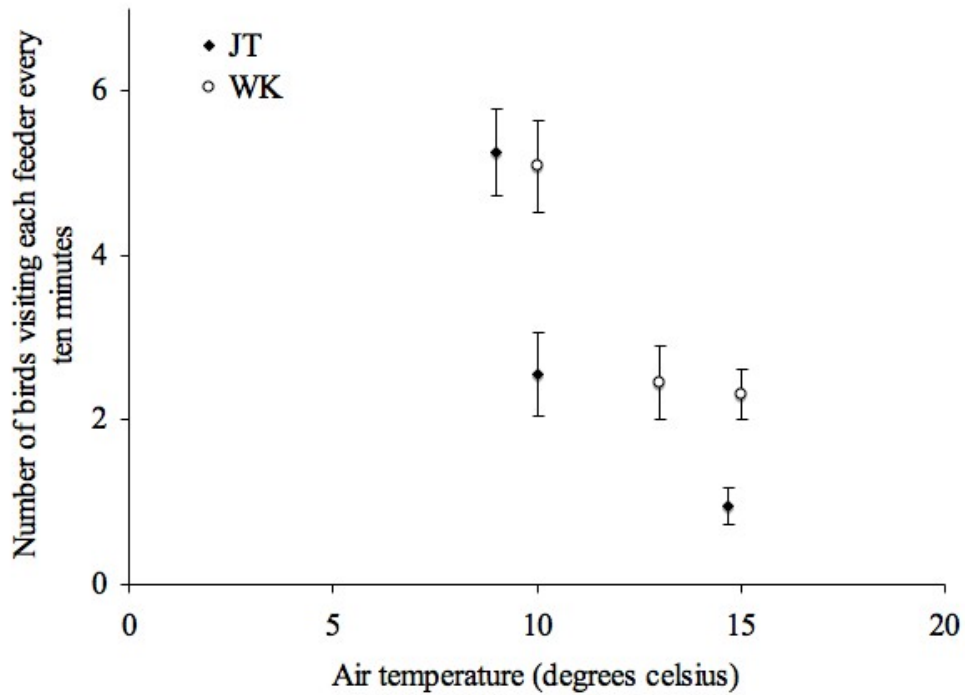


Figure 7: *The number of birds visiting each feeder at a site every ten minutes in relation to air temperature. Data are means and standard errors.*

I calculated the mean feeding bout durations for male and female rufous using data from the CFA and feeding bout duration methods for sites where both sexes were present. Rufous males' feeding bout durations were significantly shorter than those of rufous females (19.05 vs. 44.18 sec; Paired t-test: $t_3 = 5.88, p = 0.01$; Figure 8).

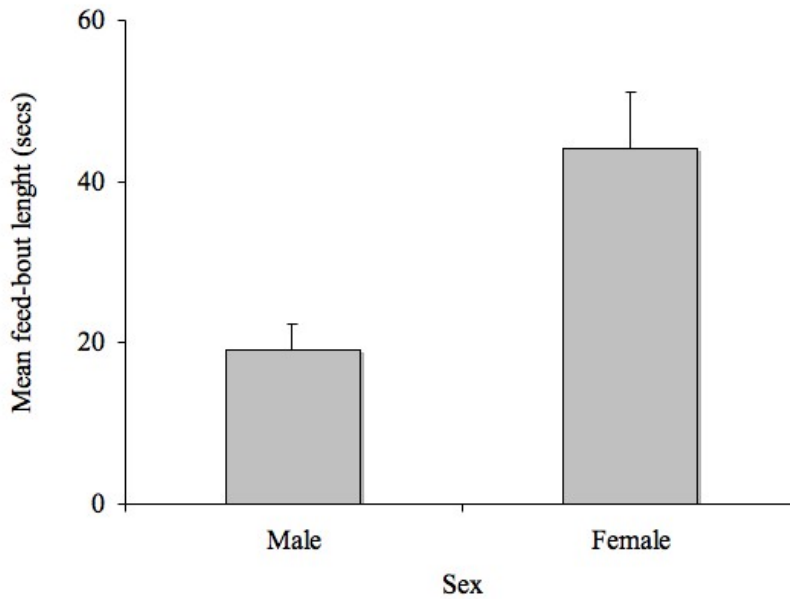


Figure 8: *The feeding bout durations for male and female rufous hummingbirds using artificial feeders. Data are means and standard errors.*

As there was such variation among sites in the number of birds using feeders, I tested whether the percentage of birds visiting each site that were chased away from the feeder was higher at busier sites. I excluded sites that were so heavily defended by a single male that no other birds could feed (MC and BK2). There was a tendency for a higher percentage of birds to be chased from feeders at busier sites, however, this trend was not quite significant and depended on one outlier (Adjusted = 0.83, trend estimate= 0.76, $F_{1,3} = 15.67$, $p = 0.06$; with outlier removed, Adjusted > 0.05, trend estimate= 0.11, $F_{1,2} < 0.05$, $p = 0.96$; Figure 9).

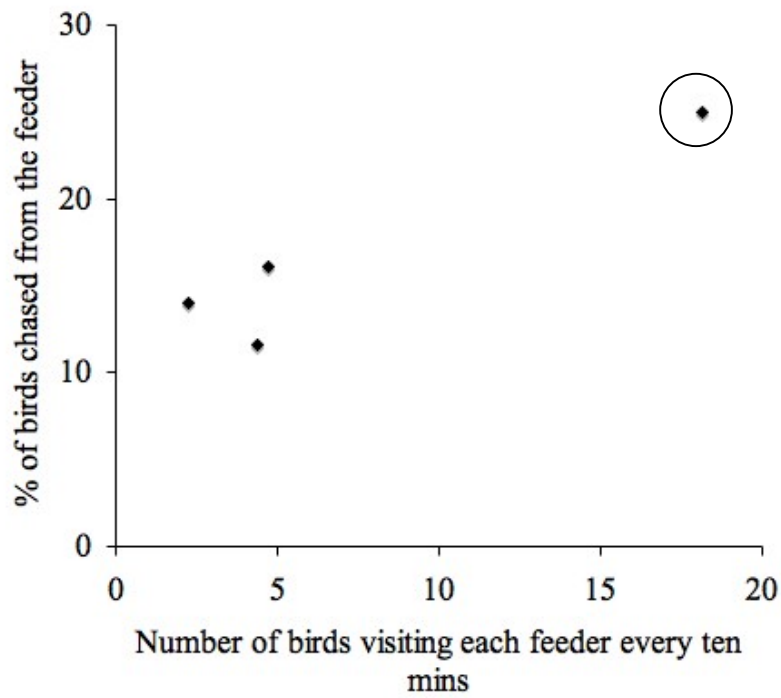


Figure 9: *The percentage of hummingbirds chased away from feeders in relation to the number of birds visiting feeders. Data are means. This relationship was not quite significant and depends heavily on the circled point.*

However, birds that were chased from feeders fed on average for less time than birds that were not chased (means= 26.28 vs. 31.43 secs; Paired t-test: $t_3 = 3.28$, $p = 0.05$; Figure 10).

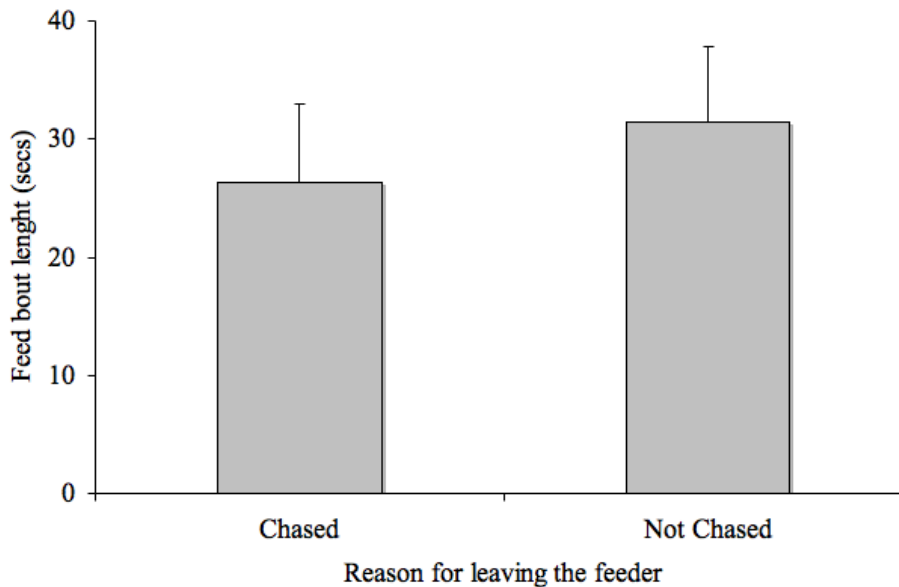


Figure 10: The feed bout lengths of hummingbirds that were chased away from the feeder by other hummingbirds and of hummingbirds that were not chased. Data are means and standard errors.

Territorial Behaviour

Male rufous hummingbirds were highly territorial and held territories around most of the sites visited, in a similar manner to that observed in Alberta. They spent $92.76 \pm 1.15\%$ of their time perching, $1.91 \pm 0.38\%$ displaying to females, $3.01 \pm 1.03\%$ chasing intruders and $2.32 \pm 0.25\%$ feeding. Data came from five sites in British Columbia (MC, JS, T2, BK2 and CM) and nine sites in Alberta. There was no significant difference in the percentage of time males in Alberta and British

Colombia spent in any activity (Perching: $F_{1,4.5} = 2.59$, $p = 0.18$; Displaying: $F_{1,6.4} = 0.29$, $p = 0.61$; Chasing: $F_{1,4.2} = 1.63$, $p = 0.27$ and Feeding: $F_{1,8.1} = 1.19$, $p = 0.31$). Data were compared using ANOVA Welch tests as variances were unequal.

As there was more competition at busier feeding sites. I tested whether this had any affect on males' territory size. Males holding territories adjacent to busier feeding sites tended to hold smaller territories (Adjusted $R^2 = 0.92$, trend estimate = -0.77, $F_{1,3} = 48.24$, $p = 0.01$; Figure 11).

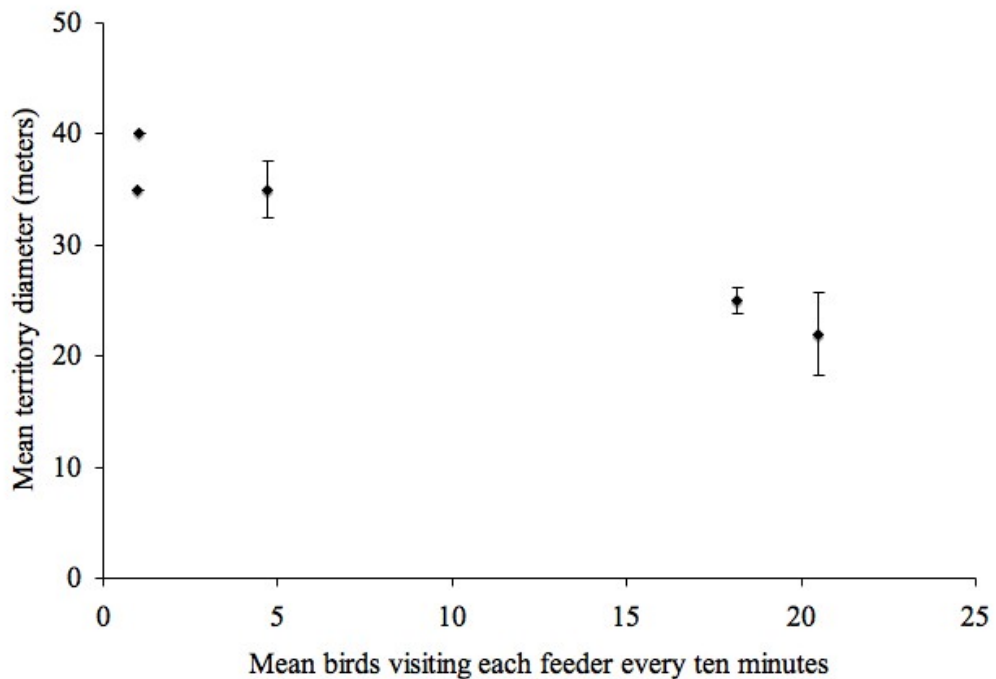


Figure 11: The average diameter of males' territories adjacent to feeding sites in relation to the number of birds visiting feeders at those sites. Data are means and standard errors.

I used the five colour-marked males at CM to investigate how far they might be travelling to use the feeders, if they were floating males or males with territories near by. Of the five colour-marked males two defended territories adjacent to the feeders

and two defended territories within 500m of the site, while the fifth was not seen again. On my return visit, three weeks later, all four males were still holding the same territories. In Alberta, some feeders were undefended by a male and were often not visited by a single hummingbird within an hour. These feeders were often within a few hundred yards of defended feeders and certainly within flying range of local birds.

Additional Notes

Estimates of the number of birds visiting a feeder at sites with multiple feeders may be slightly inflated as birds that were chased may have returned and fed twice at the same feeder within ten minutes. The probability of this happening would be directly proportional to the percentage of birds chased and the number of feeders available, which would mean that my estimates might be inflated by 5.00 to 11.56% (mean $7.90 \pm 1.37\%$). However, this error is fairly consistent among sites so comparison of their relative use should not be significantly affected.

In addition, females were occasionally noted to perch nearby after feeding and feed again after less than ten minutes following a defaecation (no detailed data collected). After this second feed they appeared to leave the site immediately. Several were observed flying at least several hundred meters over the sea towards nearby headlands (0.5km+) so were clearly travelling some distance to and from the feeders. Again errors in estimates of site activity due to this behaviour seems likely to be fairly consistent among sites.

On four separate occasions intruding males appeared to challenge territory holders by flying a horizontal figure of eight at about a foot above their head. The

length of this figure varied from about 1 to 4 meters and the flight was repeated up two to four times in immediate succession.

Hummingbirds were observed to probe a number of flower species in addition to feeding from the feeders: Flowering current (*Ribes sanguineum*), Salmonberry (*Rubus spectabilis*), Columbine (*Aquilegia spp*), Mahonia (*Mahonia repens*), Crane's bill (*Geranium spp*), Twinberry (*Lonicera involucrate*), Elephant's ear (*Bergenia cordifolia*), Camellia (*Camellia spp*), Rosemary (*Rosmarinus officinalis*), Huckleberry (*Vaccinium parvifolium*), Cherry (*Cerasus spp*), Rhododendron (*Rhododendron spp*), Lavender (*Lavandula spp*), Wallflower (*Erysimum spp*), Magnolia (*Magnolia spp*), Maple (*Acer macrophyllum*) and Willow (*Salix spp*). Hummingbird-visited flowers were in flower at the time of visits to all sites. It would be necessary to estimate the standing nectar crop of these species before it is possible to test whether flowers play a significant role in determining in the differences among sites in feeder use.

Discussion

Although many birds undoubtedly used the same feeder at some sites in British Columbia (up to five birds in some cases), there was no evidence that the behaviour of territorial males breeding in British Columbia was any different or that birds were any less defensive than those in Alberta. Males were observed defending territories against both females and males at six of the nine sites with rufous hummingbirds. The other three sites were visited only by females.

Wherever there was a single feeder surrounded by convenient perches only a single male was observed to feed there, except for the occasional intruder who was chased off (See MC and BK 2). However, the feeders in both these sites were close to feeders being used by multiple individuals, BK 2 was just the other side of the house from BK and JS was only about two hundred meters away from MC. Therefore, something other than the number of birds in the local area must influence how many birds choose to use a certain feeder. At sites with more active feeders such as BK and JS there were typically more feeders and despite territorial males frequently chasing individuals from the feeders, they did not manage to prevent many other individuals from feeding. This suggests some advantage to intruders of feeding in congregations and at sites where food is more widely distributed. The number of competitors and resource distribution within a patch and resource visibility can all significantly affect resource defensibility and therefore accessibility to intruding individuals (Chamberlain et al. 2007; Inouye et al. 1991; Johnson et al. 2004; Kim & Grant 2007; McKenzie et al. 2007; Meretsky & Mannan 1999). Thus, it is probable that feeding from sites with more feeders (a more distributed resource) reduced that intruders' risk of being excluded from that resource by more dominant individuals and that this risk was further reduced by multiple intruders feeding at once further reducing resource defensibility.

However, my results also indicate that there may be costs to feeding at busier sites as, excluding sites defended by single males, the chances of being chased off while feeding, which significantly reduces feeding bout duration, increased with the number of birds visiting each feeder. Increased aggression among individuals with increasing competitor number is fairly typical (e.g. Johnson et al. 2006; Johnson et

al. 2004; Vahl et al. 2005). However, as there were multiple feeders allowing birds to move between them and perhaps offsetting short feeding bouts at any one feeder.

The feeding bout durations of males were significantly less than those of females. This suggests that males drink less than females as time spent drinking is proportional to the amount drunk. Males may also feed more frequently than do females as inter-bout intervals are proportional to digestion time (Lopez-Calleja et al. 1997; Stromberg & Johnsen 1990b). From models of optimal foraging, hummingbirds with un-restricted access to food should not, and observations confirm they do not tend to, completely fill their crops presumably to reduce flight costs associated with larger meals (DeBenedictis et al. 1978) and it seems likely that this explains the males' smaller meal size. However, there are several circumstances when hummingbirds are expected to fill or nearly fill their crops: firstly, if they are travelling some distance to feed, over 15-30 seconds transit time (DeBenedictis et al. 1978); secondly, if they are intruders and cannot be sure of when they will be able to feed again (Carpenter et al. 1991); and thirdly, if they are feeding young (Hainsworth 1977). As females were breeding at the time of observation and were not often excluded from feeders it seems likely that transit times to nesting areas and the requirement to feed chicks may explain their greater meal sizes. Territories near busier sites were smaller, which may be because there was a ready supply of food that could support multiple males. Indeed, territory size in some species is often related to both food availability and competition (Adams 2001; Dobbs et al. 2007; Marshall & Cooper 2004). Or, it may be because females are more likely to pass through males' territories at busier sites. As male hummingbirds will defend breeding territories even in the absence of food, the main role for these territories is

likely to have been to enable the males to obtain exclusive access to females. The territories were positioned to increase the chances of encountering females rather than in relation to food abundance, although food abundance is an additional benefit (e.g. Armstrong 1987; Powers 1987). Thus, it seems likely that territories are smaller at busier sites predominantly due to competition for females.

Hummingbirds also changed their use of feeders in response to changes in air temperature: use of the feeders was greater when air temperature was cooler. When plentiful natural resources are available birds are less likely to travel to artificial feeders (Chamberlain et al. 2007; Inouye et al. 1991; McKenzie et al. 2007). However, on colder days the availability of natural resources decreased as plants produce poorer nectar (Michaud 1990) but the birds' energy demands are increased (Gass et al. 1999). Thus, travelling to feeders may become more beneficial on cooler days.

From the data I have collected, it now seems more likely that the observed differences in the number of birds around feeders between Alberta and British Columbia are due to difference in rufous hummingbird population density rather than to differences aggression. Variation in number of hummingbirds using feeders is correlated both with feeder number and air temperature. However, it remains unclear whether it is the number of feeders in an array that is the sole determinant of visitation rates or whether people simply put more feeders in parts of the garden favoured by hummingbirds.

**Chapter 8 Resource distribution affects the use of artificial
feeders by hummingbirds**

Abstract

Where resources are more spread out it is harder for a single individual to defend and monopolise those resources, individual foragers tend to be further apart and there are fewer aggressive interactions between them. However, the significance of resource distribution in the provision of supplementary food to garden birds has received little attention. Here we investigated whether providing more distributed resources would allow more small garden birds to feed at artificial provisioning sites. We manipulated the number of artificial feeder provided at hummingbird feeding sites and recorded the number of birds visiting these sites and interactions between individuals. Adding more feeders (increasing resource distribution) did not result in more birds visiting sites. However it did result in fewer birds visiting each feeder and in a lower proportion of birds being chased away by other individuals. Providing more distributed food resources may help reduce aggressive interactions and increase the benefits of supplementary food provision to wild birds.

Introduction

In the United Kingdom alone, 500,000 tonnes of birdseed, enough to support three million small birds for a year, are purchased annually (Robb et al. 2008; Toms 2003). Although such provisioning is assumed to have a large, positive impact on the survival of small birds, it can significantly alter behaviours such as mixed species flocking (less common when food is abundant, Kubota & Nakamura 2000), territoriality (can break down or be enhanced, Strain & Mumme 1998; Wilson 2001) and aggression (generally increased when resources are clumped, Johnson et al. 2004; Meretsky & Mannan 1999; Vahl & Kingma 2007). Aggression is affected both by the availability of food and the number of individuals competing for each food patch. In vultures, for example, aggression around feeding sites can be reduced by increasing the spatial dispersion of food (Meretsky & Mannan 1999). However, little is known about how the spatial distribution of such substantial additional food affects the behaviour and feeding success of small birds at garden feeding sites. In particular, there are very few data on the use of artificial feeding sites by nectivores such as hummingbirds, which are very popular targets of wild-bird feeding in North America.

The degree to which birds rely on supplementary food, and therefore compete for it, depends on the availability of natural food, its nutritional value and animals' energy demands. Hummingbirds are attracted to feeders more when there are fewer natural flowers and when the sucrose provided is more concentrated (Camfield 2006; Inouye et al. 1991) while Dark-eyed Juncos (*Junco hyemalis*) use supplementary food more as ambient temperatures fall (Rogers & Reed 2003). Although

supplementary feeding increases the number of individuals of the target species, there can also be downsides to such supplementation (Robb et al. 2008). For example, the more birds that use a food patch, the greater the risk of disease transmission and outbreaks (Brittingham & Temple 1986). Additionally, competitive exclusion, particularly of subordinate and younger individuals, can be high around such highly clumped resources (Meretsky & Mannan 1999; Robb et al. 2008).

Highly clumped resources are much easier to defend than those that are more dispersed (Kim & Grant 2007). Dominant individuals in pairs of Ruddy Turnstones (*Arenaria interpres*) presented with closely or widely spaced patches of clumped or dispersed food, completely excluded subordinate individuals when resources were clumped and close together but not when they were more widely spaced and more dispersed (Vahl & Kingma 2007). More dispersed food may not only make it more accessible to more individuals but may also reduce aggression around food patches and reduce disease transmission. In socially foraging species such as the House Sparrow (*Passer domesticus*) individuals will call to attract other individuals when they find dispersed but not clumped food resources (Elgar 1986). Furthermore, the frequency of aggression among sparrows and density of foraging individuals is lower when food patches are larger (Johnson et al. 2004). Similarly, the density of free-living Ruddy Turnstones (*Arenaria interpres*) was lower when the distance between food patches was increased although the total number of individuals feeding on the experimental plot was greater (Vahl et al. 2007). Understanding how resource distribution affects the number of birds using feeding sites and level of competition

may enable us to determine both what negative impacts e.g. increased aggression, are associated with supplemental feeding and how we might limit those impacts.

Rufous hummingbirds (*Selasphorus rufus*) are one of the most common and widespread hummingbird species in North America. Their use of feeders is of particular interest as it is thought to be one of many possible reasons for the decline in the populations of this species (Calder & Healy 2006). There is no evidence for this but it is considered that feeders may spread disease or allow local populations to increase above natural levels. Hummingbirds' use of feeders is known to be sensitive both to the availability of alternative food resources and the sucrose concentration of the food provided, as feeders are used less when there are more wildflowers and when they contain a lower concentration (Camfield 2006; Inouye et al. 1991).

This study was designed to determine whether the distribution of food at feeding sites (groups of feeders in peoples' gardens) affects the number of hummingbirds using the site, the number of individuals using each feeder within the site, or aggression around feeders. Increasing the number of feeders available at a feeding site was expected to increase the total number of individuals feeding at that site (birds that had previously excluded from the site by aggression were expected to move in). The amount of food available at sites was not limited as feeders were never empty but access was restricted by birds chasing others away despite there being enough feeding holes for four to six birds to feed simultaneously. The number of individuals around individual feeders was expected to be lower at the higher resource distribution as although the total number of birds was expected to be greater, birds would be dispersed among more feeders. Aggression among individuals was expected to be greater when more birds were using each feeder. As hummingbirds

use artificial feeders more when alternative resources such as wildflowers (more abundant in warmer weather, Michaud 1990) are scarce, and as costs of thermoregulation are greater when it is cooler, I expected more birds to visit sites on cooler days (Gass et al. 1999; Inouye et al. 1991; Welch & Suarez 2008). As only males are territorial in the breeding season and play no role in parental care we expect males and females may respond differently to changes in resource dispersion.

Methods

Sites and subjects

Observations were made at six sites in British Columbia, Canada: Point No Point (1 site), Victoria (1 site), and Port Alberni (2 sites) on Vancouver Island and Fulford (2 sites) on Salt Springs Island, between April 17TH and May 20TH 2008. These data were collected at the end of spring migration when birds were likely to be relatively sedentary and influxes of migratory individuals unlikely. The field sites were six private gardens containing one or two hummingbird feeders of various designs. All feeders contained 30% sucrose solution and had done so since the arrival of hummingbirds several weeks previously.

The main study subjects were Rufous hummingbirds (*Selasphorus rufus*) but Anna's hummingbirds (*Calypte anna*) were present at several sites and as I interested was in the number of hummingbirds using feeders were, therefore, included in observations.

Experimental design

I manipulated the number of feeders at sites and recorded the change in the number of birds visiting each feeder and in their behaviour. Each observation period lasted for two hours and was always taken at the same time each day for each site. There were two treatments: Low Distribution and High Distribution. The Low Distribution consisted of the original feeders present in the garden (one or two feeders). The High Distribution consisted of adding two 500ml, four-holed, hummingbird feeders to the one or two original feeders to give a total of three or four feeders. All feeders were hung in a line at the same height so all the feeders at the site were 70-100 cm apart.

At all sites, the order of treatments was one day of Low Distribution, two days of High Distribution, two days of Low Distribution, one day of High Distribution. Observations on the first day of the experiment provided a measure of the usual activity at the site. Immediately after observations were completed, the extra feeders were added. This gave the birds approximately 22 hours to respond to the new distribution before observations were made on Day Two. To allow further time for birds to respond to the manipulation the additional feeders were left in place until after observations on Day Three when they were removed. Observations were made similarly on Days Four and Five at the Low Distribution treatment. As this gave a total of three days of observations at the Low Distribution but only two days of observation at the High Distribution, the two additional feeders were returned after observations on day five and final observations at the High Distribution taken on Day Six. In addition to giving an equal total period of observations for each distribution, having three days of observation at each distribution also allowed a

better chance of detecting any effect of air temperature on the number of birds visiting sites.

To estimate the numbers of birds using each feeder I recorded data of three kinds: (1) the number and sex of birds at each feeder instantaneously, once every 10 minutes. A visit to a feeder was defined as any bird that drank from (inserted its bill into) the feeder. (2) the activity at a single/ different feeder every ten minutes for ten-minute periods. For every bird visiting the feeder I recorded the length of time it fed for (Feeding-bout duration), its sex, and the reason it left the feeder (either because it was chased or of its own accord). (3) the total mass of sucrose solution consumed during each two-hour observation period using an electronic balance. Occasionally, there was too much activity at a feeder to record durations of feeding bouts accurately as birds occasionally moved positions on the feeder. Where the duration of feeding bouts could not be recorded accurately in the field, they were measured later by analysing video footage taken during the first half hour of every observation period.

Results

I examined the total number of hummingbirds visiting each site within ten minutes. Data were means for each site and each treatment day. I used a mixed model in the statistics package JMP. The data were normalised using a square root transformation. As the data were repeated measures I tested for sphericity using Mauchly's sphericity test. The data just met the sphericity assumption (Chi Square₁₄ = 23.27, $p = 0.06$).

The mixed model included site as a random effect, the Resource level treatment (either High or Low), the number of days since the last change in the number of feeders in case birds took more than 24 hours to respond, the average air temperature and all two-way interactions. As only females provide parental care during the breeding season I expected the sexes feeding behaviour may also differ so data for males and females was analysed separately.

Resource level had no effect on either the number of females (Adjusted $R^2 = 0.88$, $F_{1,24} = 0.65$, $p = 0.42$) or the number of males (Adjusted $R^2 = 0.82$, $F_{1,24} = 0.86$, $p = 0.36$) visiting each site. There was no effect of the number of days since the last change in the number of feeders (Females: $F_{1,24} = 0.35$, $p = 0.55$; Males: $F_{1,24} < 0.01$, $p = 0.97$). Fewer birds visited sites when the air temperature was higher (Females, $F_{1,24} = 21.39$, $p < 0.01$; Males: $F_{1,24} = 17.33$, $p < 0.01$; Figure 1). There were no significant interactions between the resource level and days since change in resource level (Females: $F_{1,24} = 1.22$, $p = 0.28$; Males: $F_{1,24} = 1.85$, $p = 0.19$) or days since change in feeder number and air temperature (Females: $F_{1,25} = 0.04$, $p = 0.84$; Males, $F_{1,25} > 0.01$, $p = 0.97$). There was a significant interaction between resource level and air temperature such that the number of birds visiting sites decreased faster with increasing air temperature when there were fewer feeders (Females: $F_{1,24} = 10.05$, $p = 0.03$; Males: $F_{1,24} = 7.22$, $p = 0.01$). Although two data points at 3°C appeared to have a major effect on the relationship between the number of birds visiting sites and air temperature, when these points were excluded from the analysis, the effect of air temperature was still significant at or below $p = 0.01$.

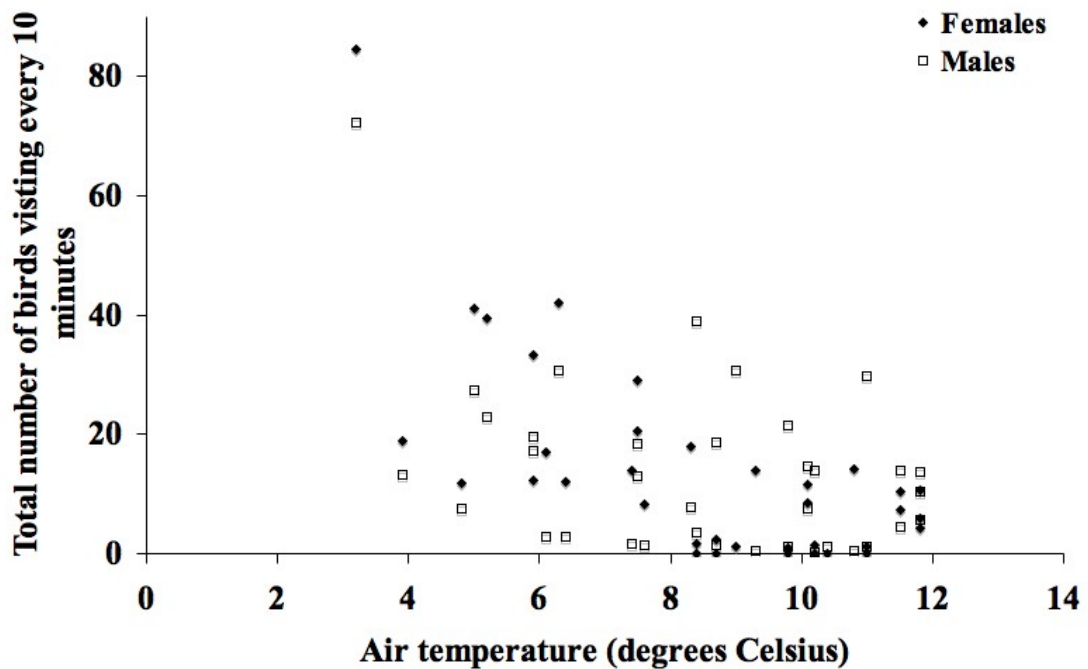


Figure 1: The total number of birds visiting each site every ten minutes against average air temperature. For both males and females. $N = 6$ sites.

As the data only just met sphericity assumptions and as Mauchly's sphericity test may underestimate deviations from sphericity particularly in small data sets (Boik 1981; Cornell et al. 1992), I examined the relationship between the number of birds visiting sites and air temperature using a more conservative approach. Data were analyzed using a repeated-measures Manova which adjusts P-values and degrees of freedom where the assumption of sphericity is not met. The data were the number of birds visiting each site for each treatment. I used the relative temperature on each of the three days of each treatment to further group the data by the warmest to coolest day. Again, fewer birds tended to visited sites on the warmer days (Females: $F_{2, 10} =$

5.29, $p = 0.03$; Males: $F_{1.1, 5.6} = 4.86$, $p = 0.07$). There was no effect of adding feeders (Females: $F_{1, 5} = 0.29$, $p = 0.61$; Males: $F_{1, 5} = 1.29$, $p = 0.31$). Using this more conservative approach, there was no longer a significant interaction between air temperature and treatment (Females: $F_{2, 10} = 0.81$, $p = 0.47$; Males: $F_{2, 10} = 1.24$, $p = 0.33$).

Sucrose consumption

If more birds feeding at an individual feeder is representative of a greater number of individuals visiting the volume of sucrose consumed might also be expected to be greater. I examined the total volume consumed per ten minutes at each site (Tvol) using a mixed model in the statistics package JMP. The data were transformed to meet the assumptions of normality using a square-root function. As the data were repeated measures I tested for sphericity using Mauchly's sphericity test. The data just met the sphericity assumption ($\text{Chi Square}_{14} = 22.70$, $p = 0.06$).

The model included site as a random effect, the resource level treatment and the total number of hummingbirds visiting the site every 10 minutes (a mean across all feeders) as well as all of the two-way interactions.

The resource level treatment did not influence the total amount of sucrose consumed (Adjusted $R^2 = 0.81$, $F_{1, 26} = 0.13$, $p = 0.72$). The more birds that visited each site the greater the volume of sucrose consumed ($F_{1, 32} = 14.24$, $p < 0.01$, Figure 2). As the data almost did not meet sphericity conditions I repeated the modeling procedure described above using only two points per site (a mean for each treatment) in the model so that violations of sphericity were not possible. This model (Adjusted

$R^2 = 0.99$) also showed increasing number of birds visiting sites led to increased sucrose consumption ($F_{1,9} = 58.90$, $p < 0.01$).

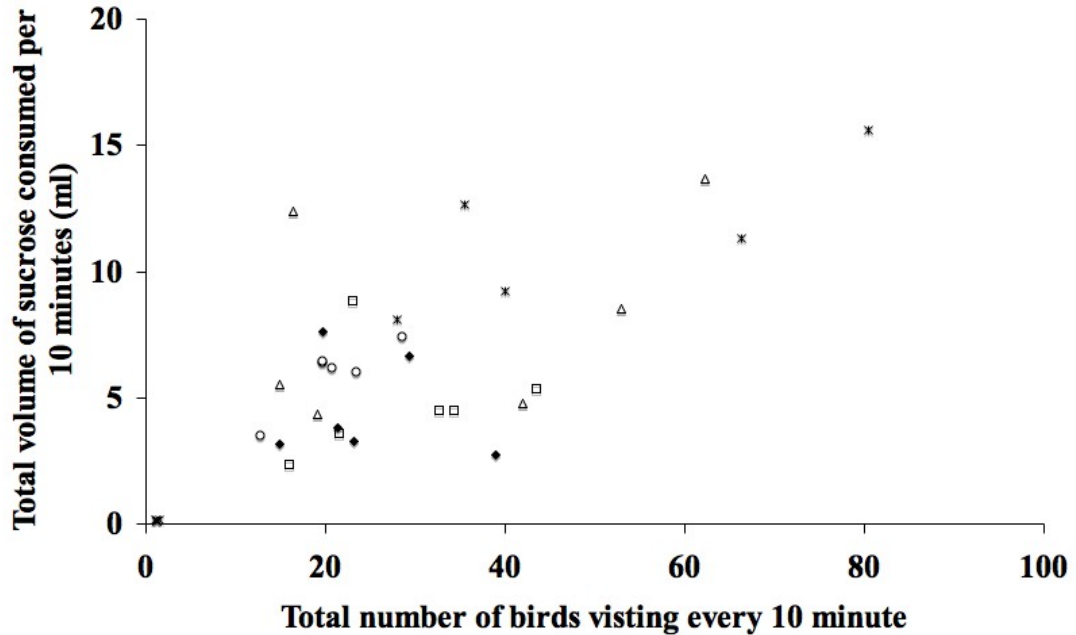


Figure 2: The total volume of sucrose consumed per ten minutes against the total number of birds visiting each site every ten minutes. Each of the six sites is represented by a different symbol. $N \text{ sites} = 6$.

I investigated the mean number of birds using each feeder every ten minutes to investigate the effect of the number of feeders provided on the aggregation of birds around individual feeders. I used a repeated-measures MANOVA, as the data did not meet the assumption of sphericity ($\text{Chi Square}_{14} = 25.95$, $p = 0.03$). As for the total number of birds visiting each site, the data for each site were grouped by treatment and by day. Days were ranked from the coldest to warmest day of the three days data were collected on. Using temperature categories in this way allowed us to test for

effects of both treatment air temperature. Fewer birds visited each feeder when the ambient temperature was higher ($F_{2,10} = 7.10$, $p = 0.01$; Figure 3). There was a non-significant tendency for fewer birds to visit individual feeders when there were more feeders ($F_{1,5} = 5.09$, $p = 0.07$).

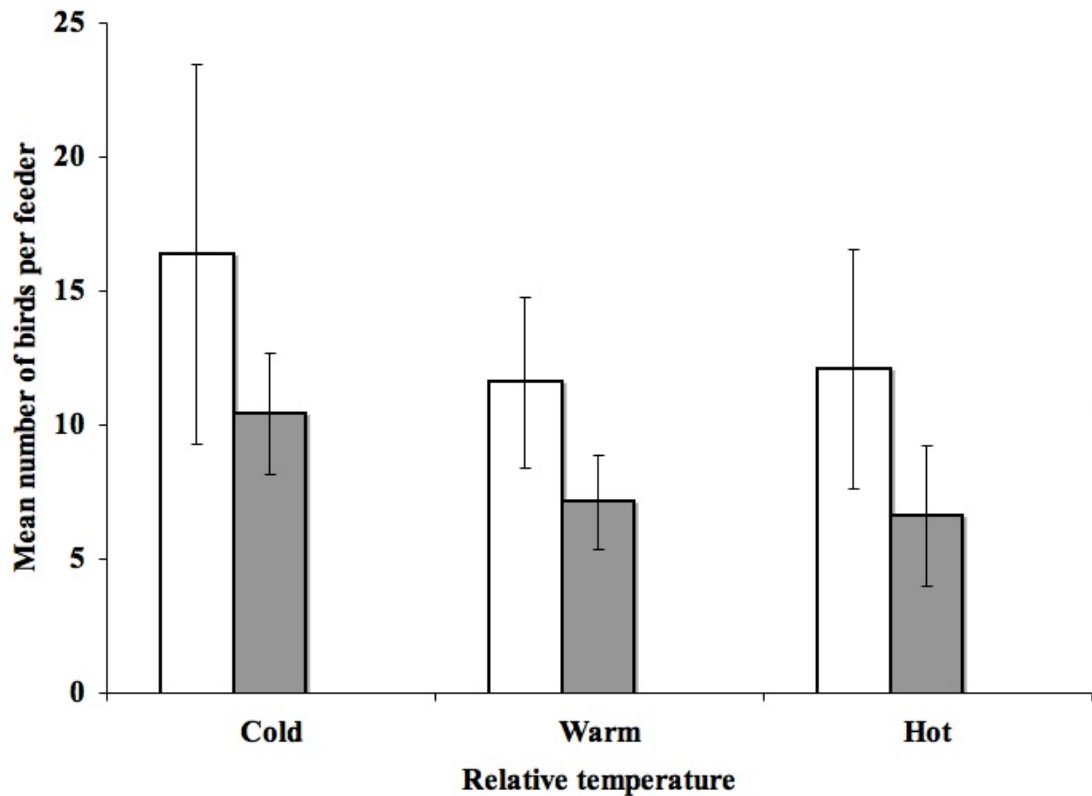


Figure 3: The mean number of birds visiting each individual feeder every ten minutes against mean temperature. The data were grouped by treatment: White = Low distribution (1 or 2 feeders), Grey= High distribution (3 or 4 feeders). The data are means and standard errors. N sites =6

In order to see if the number of birds around feeders affected the level of aggression among individuals, I tested whether the number of birds using an individual feeder

affected the percentage of birds chased off the feeder while feeding. I excluded one site that was defended so vigorously that only the defending male ever fed there so I had no measure of birds being chased from the feeders at that site. Again I analyzed the data for each sex separately as males are territorial during the breeding season and do not contribute to raising young whereas the females are not territorial and care for the young, such that the cost of aggression is likely to differ between the sexes.

Across days, the data did not meet the sphericity assumption ($\text{Chi Square}_{14} = 39.01, p < 0.01$) so I used the mean number of birds per feeder and percentage of birds chased off the feeders while feeding for each treatment and site. The data were analyzed in a linear mixed model with site as a random factor. The percentage of male hummingbirds chased off feeders while feeding was greater when there were more birds using each feeder (Adjusted $R^2 = 0.94, F_{1,5} = 17.14, p = 0.01$; Figure 4). The percent of female hummingbirds that were chased off feeders while feeding remained constant regardless of the number of birds using the feeder (Adjusted $R^2 = 0.97, F_{1,5} = 1.21, p = 0.33$).

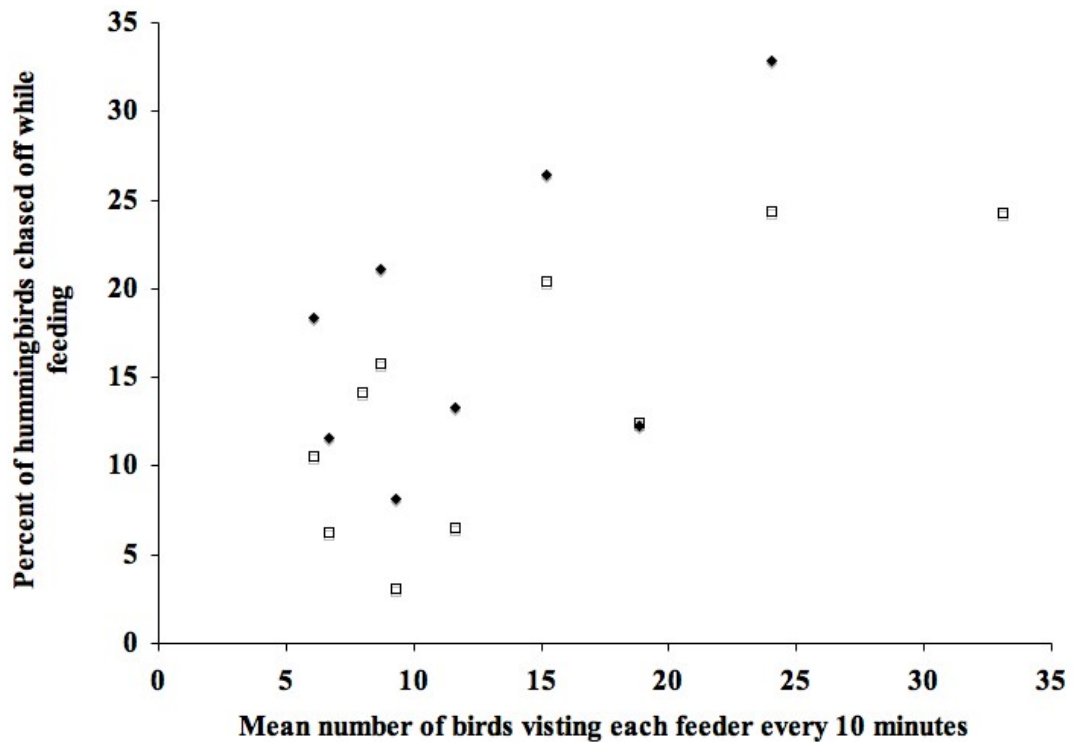


Figure 4: The percent of birds that were chased off the feeder while feeding against the mean number of birds landing at a feeder within every ten minutes. Females = black diamonds, Males = open squares. N sites = 5. Data are means for each treatment and site.

I used a MANOVA to test whether being chased while feeding significantly reduced the length of time birds fed for, for both for males and females. The data for each site were grouped by sex and whether or not birds were chased while feeding. Overall, males tended to feed for less time than did females ($F_{1,4} = 6.55$, $p = 0.06$) and birds that were chased off the feeder fed for significantly less time than birds that were not chased ($F_{1,4} = 118.53$, $p < 0.01$, Figure 5). However, there was a significant interaction between sex and whether or not an individual was chased such that being

chased reduced the feeding time of females more than that of males ($F_{1,4} = 15.72$, $p = 0.02$).

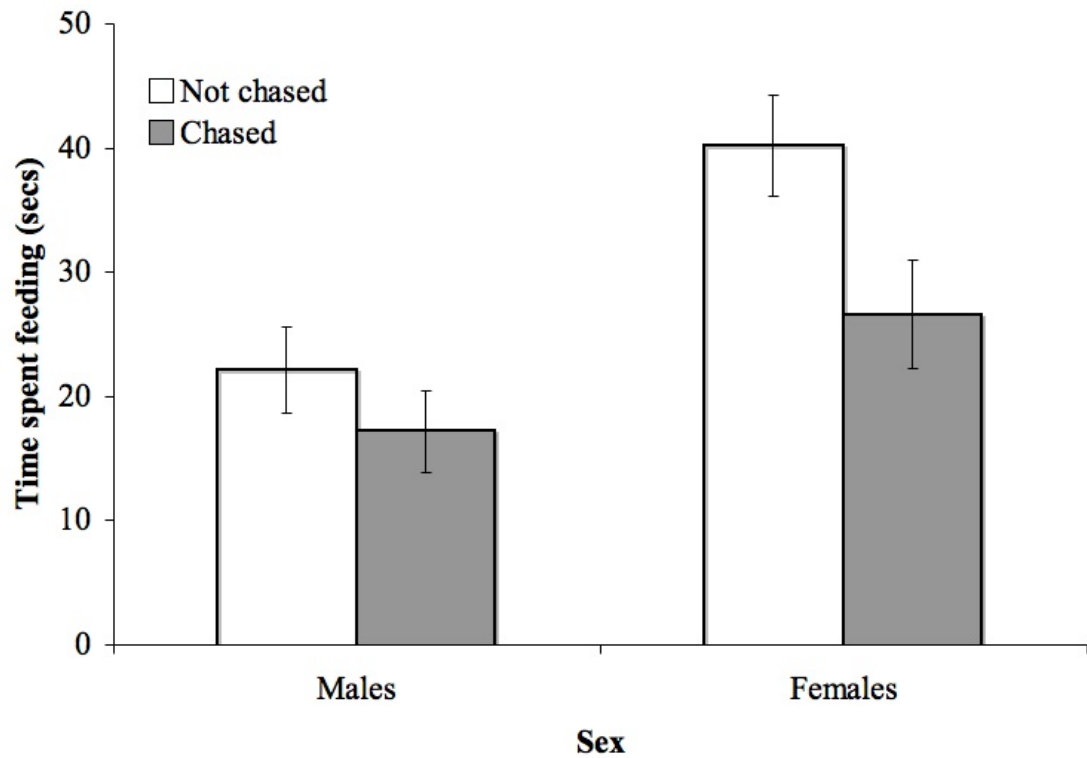


Figure 5: *The length of time birds fed for when they were not chased off the feeder by other birds (white bars) and if they were chased off the feeder by other birds (grey bars). Data are for males and females separately. N sites = 5.*

Discussion

The more birds visiting a site, the more sucrose was consumed, however, increasing the number of feeders at a site did not lead to more birds visiting that site (i.e. a garden). The number of visits to a site of both males and females was affected by air temperature, visits increased with decreasing temperature.

Increasing the number of feeders in a feeding array tended to reduce the number of birds visiting each feeder in an array. Reducing the number of birds at a feeder reduced aggression among individuals so that fewer birds of both sexes were chased off while feeding. However, this effect differed between the sexes, while the percentage of females chased remained constant as the number of birds around a feeder increased, the percentage of males that were chased increased. Birds that were chased fed for less time in one go than birds that were not chased, which means they either acquired less food on that feeding trip and would have had to return sooner, or expend time and energy moving between feeders. The reduction in time spent feeding by birds that were chased relative to those birds that were not chased was greater for females, which tended to drink for longer than males.

Variation in the number of birds visiting an array appears to be due mainly to air temperature. Birds may have visited feeders more when it was colder as they need more energy to keep warm when it is colder (Welch & Suarez 2008). In addition, they would have had fewer alternative food sources as fewer flowers open when it is colder and those that do produce less nectar than when in warmer conditions (Lyon 1992; Marina et al. 2004).

There is contrasting evidence as to how birds' use of artificial food changes with air temperature. For example, in cooler air temperatures dark-eyed juncos increase the amount of fat they lay down, presumably as a result of their higher food intake under these conditions (Rogers & Reed 2003). However, black-capped chickadees (*Poecile atricapillus*) do not appear to feed more as air temperature declines (Wilson 2001). As black-capped chickadees are food hoarders they may have been able to retrieve caches in poorer weather. Hummingbirds' high metabolic rate, combined with the comparatively high energetic costs of heating such a small body may make them more vulnerable to changes in temperature than other birds. It seems possible that differences in the availability of alternative resources and the body condition of foragers explain variation among studies in birds' use of supplementary food in responses to changes in air temperature.

Although adding feeders to an array did not appear to attract more individuals to sites as expected, it did reduce the number of birds around individual feeders. While all sites provided unlimited sucrose, access to the sucrose is limited both by the number of feeders and the number of competitors. Birds would chase each other even when there were free feeding holes at a feeder (there were 4-6 holes per feeder). Adding more feeders increased the dispersion of the resource such that food patches were further apart and harder for one individual to monopolise. Combined with a corresponding decrease in the number of birds at individual feeders, the increase in the number of feeders reduced aggression among individuals. These findings are consistent with those from studies of other species where resource distribution has been manipulated. For example, chickens (Leone & Estevez 2008), house sparrows (Johnson et al. 2006; Johnson et al. 2004), ruddy turnstones (Vahl et al. 2005; Vahl

et al. 2007) and blackbirds (Cresswell 1997) are all less aggressive when resources are more dispersed. Like the hummingbirds, blackbirds experiencing increased levels of competition decreased their food intake although unlike our hummingbirds, this was not due to direct interaction with others but simply in response to their proximity (Cresswell 1997).

I conclude that increasing the number of feeders provided at feeding sites can reduce aggression among small birds foraging at feeding sites in private gardens allowing individuals to forage for longer. This is important due to the high levels of food provision to small birds by the general public. The use of supplementary food was higher in colder conditions, which may mean that the maintenance of artificial feeding sites is likely to be particularly important to birds in cold weather. The number of feeders supplied did not affect the total number of birds visiting sites at least at the time scale of this experiment. It is entirely possible that increasing resource dispersion would eventually result in more birds visiting sites. More work is required to discover what site features may affect the number of birds using it.

Chapter 9 **Banding and recapture data: the breeding site
fidelity of rufous hummingbirds**

Abstract

In populations with high site fidelity there may be little gene flow among regions leading genetic structuring among these populations. In this case population dynamics in different areas may operate fairly independently and they may be treated as separate conservation units. The population of rufous hummingbirds is declining over much of its range but it is unclear how declines in some areas will affect the population as a whole. Here we use banding and re-capture data to investigate the site fidelity and possible dispersal distances of rufous hummingbirds breeding in North America. In general site fidelity appears to be fairly low (around 14%). However this may be due to birds moving only short distance that are not recaptured. Site fidelity, particularly for males, was higher at banding site that covered an area of several km rather than a point location. There were a number of long-distance movements (10s-100s km) both within and between breeding seasons that could have led to large scale population mixing if birds had bred in both locations. However, as banding data are limited genetic data would be useful to establish the degree of population structuring among regions.

Introduction

Rufous hummingbirds (*Selasphorus rufus*) are one of the most abundant species of hummingbird in North Western America. They are an important migratory pollinator that many native plant species depend on for pollination. They migrate from their breeding grounds as far north as Alaska to over winter predominantly in Mexico (Williamson 2001). From the breeding bird survey data, however, it appears that their population size is declining by several percent a year (Sauer et al. 2008), although the population changes are not the same over their entire breeding range. While the population is declining by around three percent in the core of the breeding range around British Columbia, the population appears to be increasing in other areas particularly the North (Alaska) and East (e.g. Montana; Figure 1; Sauer et al. 2008). Very little is known about the movements of these birds within and between breeding seasons or population structure. Migrant species that disperse shorter distance between breeding seasons appear to be more likely to be declining (Paradis et al. 1998), apparently due to increasing habitat fragmentation leaving the birds without suitable breeding sites nearby. Habitat fragmentation is considered to be one of several possible reasons for the decline in the *S. rufus* numbers (Healy & Calder 2006; Lehmkuhl et al. 1991). Whether or not population changes in one region will affect the population dynamics in other areas of the breeding range will depend partly on population structure.

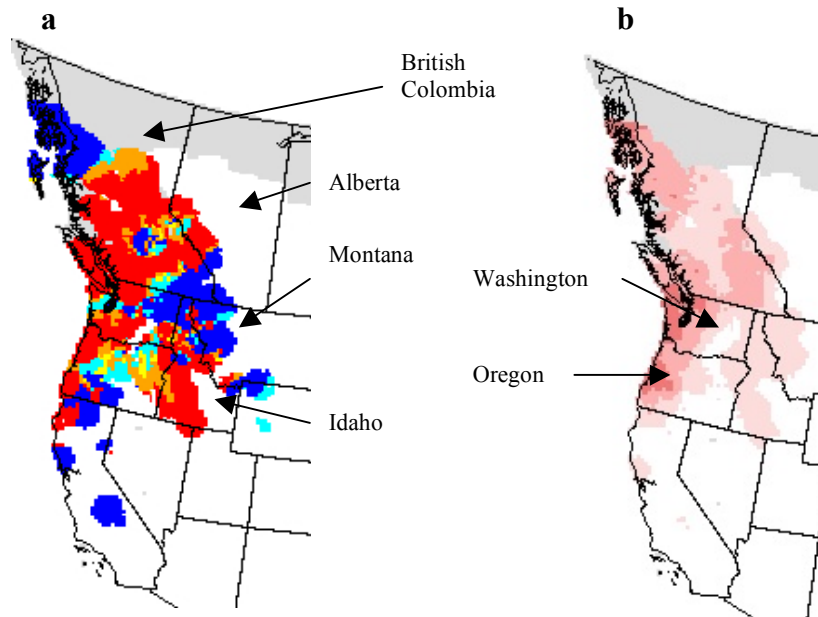


Figure 1 (a): The Breeding Bird Survey population trend map for rufous hummingbirds on their breeding grounds in North America 1966-2003. Data are percent changes per year in counts of birds along survey routes. Yellow to red colours represent areas of decreasing population density with red being most severe. Blues represent areas of increasing population density. (b) The Breeding Bird Survey population abundance map for rufous hummingbirds on their breeding grounds in North America 1966-2003. Data are mean counts of birds along survey routes. Darker red areas represent higher population densities. Sauer, J. R., J. E. Hines, and J. Fallon. 2008. *The North American Breeding Bird Survey, Results and Analysis 1966 - 2007. Version 5.15.2008.* USGS Patuxent Wildlife Research Center, Laurel, MD

The degree of structure in a population depends on gene flow among different parts of the population (Clobert et al. 2001; Wright 1943; Wright 1950). If very few individuals move from where they were born to breed elsewhere then populations may be highly structured such that individuals in each area are genetically distinguishable from those in other areas and the population dynamics of different areas will operate fairly independently (Clobert et al. 2001; Esler et al. 2006; Wright 1943; Wright 1950). In this case populations from different areas could be managed as different conservation units (Esler et al. 2006). Populations can become diverged via isolation by distance such that birds breeding within dispersal distance of each other will be more genetically similar than those breeding further away (Wright 1943). Population divergence may also occur because of geographic barriers such as mountain ranges that prevent dispersal and therefore gene flow among areas on each side (Fisher & Ford 1947; Lowe et al. 2004; Wright 1950). Where dispersal among breeding areas is common then animals from different areas will be genetically more similar and some degree of source-sink population dynamics may operate between such areas as individuals move between them (Esler et al. 2006). In this case changes in the population size in one area are likely to affect population sizes in other areas. Dispersal data may provide important clues about the possible genetic population structure of *S. rufus* that could be used in developing appropriate conservation strategies.

To infer typical dispersal patterns and whether populations might be structured at spatial scales of 10s or 100s of km, we need data on the movements of birds. Bird movements are typically monitored using banding and recapture data and, more recently, via telemetry. Although telemetry techniques provide a much more

certain record of the long distance movements of a few individuals in a population than banding data, rufous hummingbirds are too small to be fitted with radio-tracking devices. Thus far, then, the only data on their movements comes from banding returns. Large-scale long-term banding projects usually include records of long distance movements of both sexes and give good estimates of breeding site fidelity and dispersal for both sexes. In birds, females typically show lower site fidelity and disperse further than do males (Paradis et al. 1998).

Shorter-term studies (two or three-years), however, have been adding detail of birds' movements at much smaller spatial scales (e.g. a few square kilometres). These smaller scale data often include information on birds' ages and social status in addition to sex, which are often absent from larger-scale studies. Such small scale studies are also more likely to recapture birds that have only move a few kilometres but would not be picked up at large scale monitoring sites possibly leading to misleadingly low estimates of site fidelity. Data on age are relevant in the context of dispersal as juveniles typically disperse further than adults and thus contribute more to gene flow (e.g. Newton 2002; Paradis et al. 1998). The social status of birds also appears to affect dispersal such that non-territorial or non-breeding individuals are more likely to disperse than are territorial individuals within and between breeding seasons and thus probably also contribute more to gene flow than do territorial individuals (e.g. Danchin & Cam 2002; Forero et al. 1999; Hunt 1998; Kokko & Sutherland 1998; Rohner 1997; Shutler & Weatherhead 1994).

It appears from recently published data on the movements and site fidelity of rufous hummingbirds banded and re-captured in British Columbia that dispersal at least at the scale of tens of kilometers square, is probable (Finlay 2007). This

conclusion is based on the records of one hummingbird banding network operating in British Columbia. Their study includes records for 7,475 rufous hummingbirds captured between 1997 and 2006. Although up to 43% site fidelity has been reported for some sites in the past, it appears that the site fidelity for B.C. rufous females is only around 12% and for rufous males only 2.2% (Healy & Calder 2006). There are no good data for juveniles, as very few are caught and they cannot readily be distinguished from females (Finlay pers comm.) High site fidelity necessarily means that populations mix very little but such low site fidelity might mean that there is considerable movement of rufous around and beyond B.C. In addition, the study records twelve fairly long-distance movements (6-208km) by both males and females within and between breeding seasons. However, without evidence of birds breeding in two locations it is not possible to say if any of these movements resulted in gene flow among the areas they moved between. It is not clear, however, whether the way the birds move around British Columbia is typical for other regions.

Here I was interested in investigating dispersal patterns in regions additional to British Columbia so to infer possible within or between state/ province dispersal patterns and what implications such patterns may have for population trends in the different regions. In addition, data on movements at a much smaller scale may allow determination of whether particular individuals, sexes, territorial status or ages, in a population are more likely to move.

In the United States of America (USA), and in Canada, historic banding data, collected from licensed bird banders across the USA and Canada are available from The North American Bird Banding Program (BBL), which has data as far back as 1923 (Gustafson & Hildenbrand 1999). The BBL provides two sorts of data on the

distribution, movement and longevity of species: (1) Summary data and (2) Recapture data. The summary data are a record of a given site on a given date (e.g. the total number and sex of birds caught, not separate data for each bird). These data allows assessment of the number of birds caught across years, the time of year at which they are caught as well as locations of capture, which could point to possible migration routes. Re-capture data, perhaps most frustratingly for efforts to determine movements of rufous hummingbirds, include only recaptures from outside the 10-minute block of latitude and longitude where the individual was initially banded (equivalent to up to four miles from the banding site), hampering the significant quantification of adult site fidelity. However, the re-capture data do provide a record of movements made by individual birds rather than a more general pattern of movement that can be inferred from the timing of arrivals if different areas from the summary data. Such individual movements may allow identification of the maximum distances across which dispersal and, therefore gene flow, may occur (e.g. 10s to 100s of km) and at what scale we might expect to find population structure.

The aim of this study was to determine the movements of rufous hummingbirds, to identify the scale at which dispersal and gene flow among regions may occur and which individuals are most likely to be moving. I did this using data from both a small geographic scale (my own banding records from birds caught within a single river valley) and from a larger geographic scale (data obtained from the BBL covering the whole of North America). I expected that movements recorded in the BBL data would follow a similar pattern to those in Finlay's data with more records of shorter movements (10s of km within states/provinces) than longer movements (100s km between states/ provinces). In addition, I expected to find that

longer distance movements tend to be in the expected direction of migration for that time of year. At a within-river-valley scale, I expected territorial males to show higher site fidelity than females and non-territorial/ intruding males as this is typical in most bird species (Newton 2008).

Methods

I banded hummingbirds on their territories in the Westcastle river valley in Alberta, Canada (Lat: 49.348975, Lon: -114.409486), between 2007 and 2009. A few birds were also banded at the same territories in 2005 and 2006 and using the same methods by T.A.H. Each territory was supplied with a sugar-water hummingbird feeder that territorial males defended against intruders, including females. Each territorial male was trapped at the beginning of the breeding season in late May or early June and colour marked on the upper-breast with non-toxic dye so they could be identified without capture over the rest of the breeding season. I used mesh cage traps to catch these birds. Although it was necessary to move the feeder slowly into the trap to catch territorial male, so long as the feeder was inside, intruders would fly straight in. Intruders and females were banded opportunistically but were not colour marked.

I obtained 'summary' and 'encounter' banding data from the BBL (1955-2004) for rufous hummingbirds banded on their breeding grounds (Alaska, Alberta, British Columbia, Idaho, Montana, Oregon and Washington). Summary data included the number and sex of birds banded on each date for each ten-minute banding block (approx. four miles square). The encounter data (recaptures of a

previously banded bird outside the ten-minute block where the individual was initially banded (approx. four miles square), were examined to look for within and between state/province movements within the breeding season in order to identify across what geographic scale (e.g. 10s to 100s of km) dispersal might occur. I expected to find a similar pattern of movements across the whole breeding range as described by Finlay for British Columbia. Longer distance (100s km) movements were expected to be in the same direction as migration patterns for that time of year.

Results

Westcastle banding data

Across the three years of the study, excluding within-year re-captures, one hundred and ten male and twenty-seven female rufous hummingbirds were caught in the Westcastle valley, a sex ratio of 0.25: 1.00 females: males. We also caught four male and eight female Calliope hummingbirds (*Stellula calliope*; Table 1).

Table 1: *A summary of the hummingbirds caught and banded in the Westcastle River Valley from 2007 to 2009, excluding within-year re-captures*

Species	Year	Males	Females	Total
Rufous	2007	20	16	36
	2008	28	6	34
	2009	49	5	54
Rufous total		97	27	124
Calliope	2007	2	4	6
	2008	0	2	2
	2009	2	2	4
Calliope total		4	8	12

Birds caught as territorial males were colour marked and were subsequently typically seen on their territories until at least the end of June. Across 2008 and 2009 $9 \pm 0.09\%$; $16 \pm 0.15\%$ in 2008 and 0% in 2009, of females caught (total= one recapture) and $14 \pm 0.04\%$; $21 \pm 0.08\%$ in 2008 and $10 \pm 0.05\%$ in 2009, of males caught (total =12 recaptures), were re-captures from previous years. Five territorial males were found to be defending the same territory on two consecutive years. One male held the same territory for three consecutive years and a second held the same territory for two years before moving to an adjacent territory the third year. One male captured as a territorial male in the second year was captured as an intruder at a different site in the third year. Two males captured as intruders were caught as intruders at different sites in subsequent years. One male captured as an intruder was captured as an intruder at the same site the following year. Ten males, both territorial and intruders, were recaptured at sites other than that of first capture within a season. One female was caught at the same site on the 2nd June 2007 and the 29th May 2008. She was carrying an egg on both occasions.

There were two foreign recaptures: one was a female caught on the 1st of June 2007 that was first banded on May 27th 2004 in Montana, USA (Appendix 1). The other was a male caught in Westcastle on the 1st of June 2008, apparently (from its band number) first banded at Mt Washington on Vancouver Island on the 22 of May 2006 as an adult female. This must have been a misreading of the band (read as C00639), which the other way up reads (C00936) a band belonging to a bird also banded on Vancouver Island: a male banded on the 8th of April 2006 as an adult. If this reading of the band number is correct. This latter recapture is the first evidence

that birds from British Columbia move to Alberta within or between breeding seasons.

To investigate how far birds were moving within the Westcastle valley and whether that was affected by sex or social status, I calculated the distances moved by birds between captures. I separated data for known territorial males and males caught as intruders on the basis that the breeding behaviour and success of these two groups might differ. As territorial males were known to be on their territories most of the time and were occasionally intentionally re-captured to allow us to re-mark them, I excluded re-captures of territory holders at the same site within years but not among years. Within years, intruders were typically re-captured about the same distance from their first site of capture, as were territorial males from their own territories (1435 ± 956 vs. 1717 ± 984 m respectively; unpaired t-test: $t_{5.57} = 0.205$, $p = 0.844$).

Between years, only one of nine territorial males changed territories, moving 150 m. However, intruders were not so site-faithful, moving on average 1287 ± 526 (N = 4) meters between years (Figure 1). Intruding males moved roughly the same distance within and between years (means = 1716.66 vs. 1287.50 m; unpaired t-test, $t_{3.13} = 0.385$, $p = 0.725$).

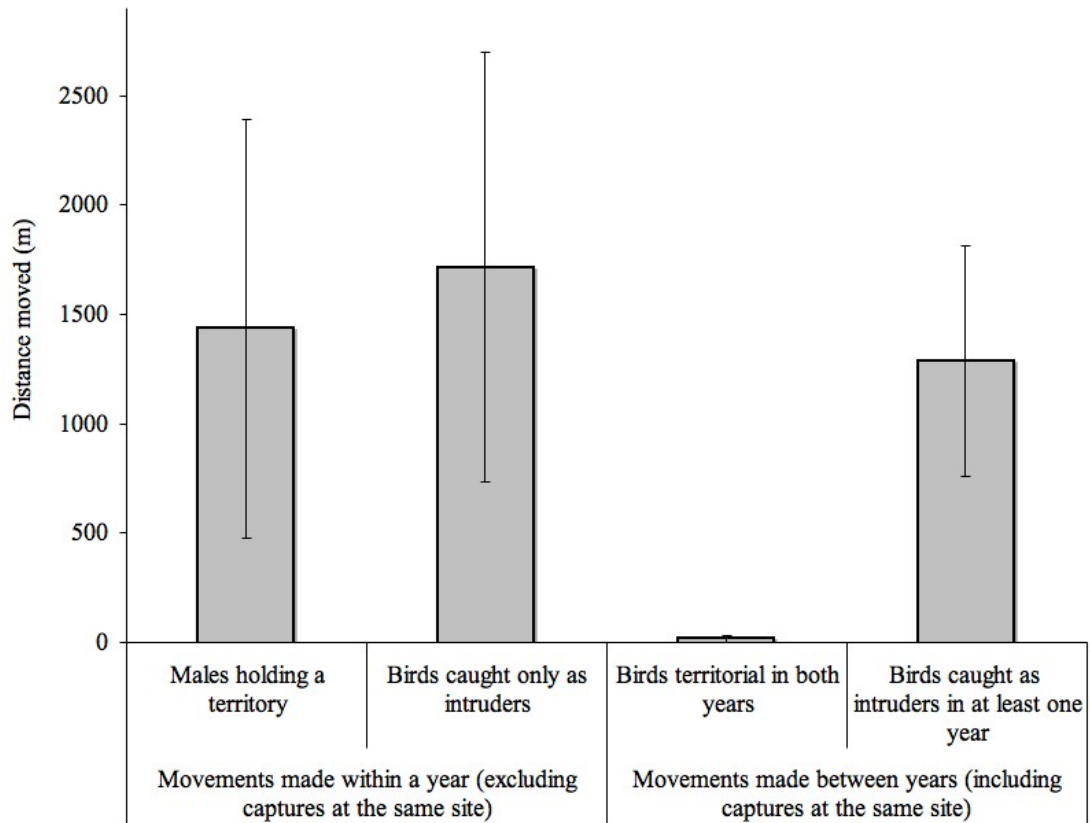


Figure 2: The distances moved by territorial males and intruding rufous hummingbirds within and between years. All birds were captured during the breeding season $N = 6, 3, 9$ and 4 , left to right respectively. Data are means and standard errors.

BBL data Banding effort

Across the whole area covered by the BBL, the number of birds caught and the number of master banding permits issued increased over this period (Figures 3a and b) despite a population decrease over this period. Master permit holders hold all the records for themselves and their sub-permit holders. Only master banders are allowed to train others to band birds.

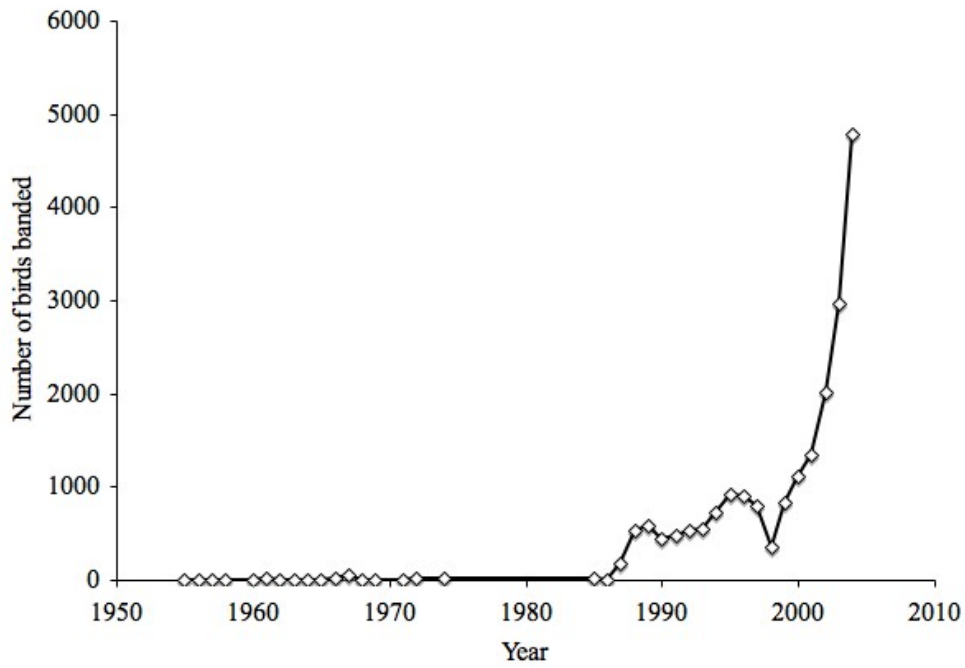


Figure 3a: The number of rufous hummingbirds banded each year on their breeding grounds (1955-2004). Data are totals for each year across the breeding grounds.

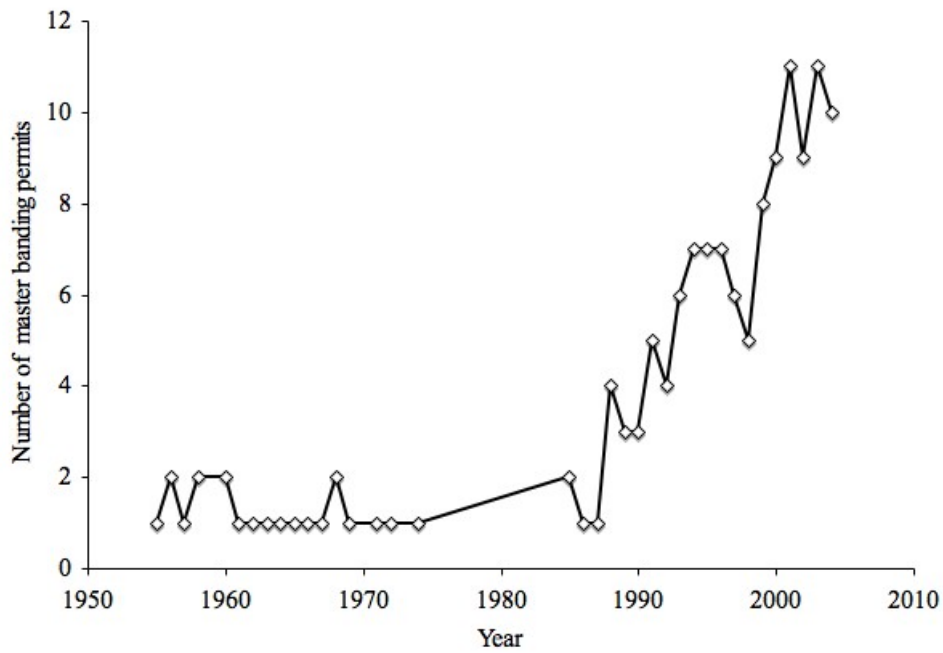


Figure 3b: The number of master banding permits for rufous hummingbirds, being used each year from (1955 to 2004). Data are totals for each year across the breeding grounds.

Sex ratio of banded birds

Across the seven states/provinces in the breeding range, significantly more birds identified as female were banded than were birds identified as male (females = 59.98 % \pm 0.003, males = 34.61 % \pm 0.003; Paired t-test: $t_6 = 5.73$, $p < 0.01$; Table 2), leaving 18% of birds banded with sex left unidentified. It is possible that this highly female-biased sex ratio may be, in part, due to juvenile males (which often cannot be distinguished from females) being misidentified as females. Even if all the birds of unknown sex were, in fact, male, more birds recorded as females were banded than were males (means= 59.98% females vs. 40.02% males + birds of unknown sex; $t_6 = 4.31$, $p < 0.01$).

Table 2: *The number and sex of rufous hummingbirds banded in their breeding grounds in each state/ province between 1955 and 2004.*

State/ Province	Females	Males	Unknown	Total	Sex ratio F: M
Alaska	90 \pm 6.14	37 \pm 5.31	28 \pm 4.79	155	2.43
Alberta	62 \pm 5.07	34 \pm 4.81	10 \pm 3.01	106	1.82
British Columbia	5459 \pm 42.32	2597 \pm 42.03	69 \pm 8.27	8125	2.10
Idaho	330 \pm 11.87	246 \pm 11.87	0 \pm 0	576	1.34
Montana	4464 \pm 45.01	3704 \pm 45.00	5 \pm 2.24	8173	1.21
Oregon	724 \pm 18.19	486 \pm 17.58	124 \pm 10.67	1334	1.49
Washington	1212 \pm 19.09	519 \pm 19.07	2 \pm 1.41	1733	2.34

Arrivals at and departures from the breeding grounds

The first arrival of rufous hummingbirds on their more south-western breeding grounds was in March (Oregon, Washington and British Columbia). On their more northerly and easterly breeding grounds, however, arrival is not usual until April (Montana) or May (Idaho, Alberta and Alaska: Figure 4). There is a single peak in abundance in Alaska, Alberta, British Columbia and Washington that is earlier in the south (around May) but later further north (in June and July). However, there is a double peak in Idaho, Montana, and Oregon, the first in late spring around May and the second in late summer around July, although the first peak is relatively small in Idaho and Oregon.

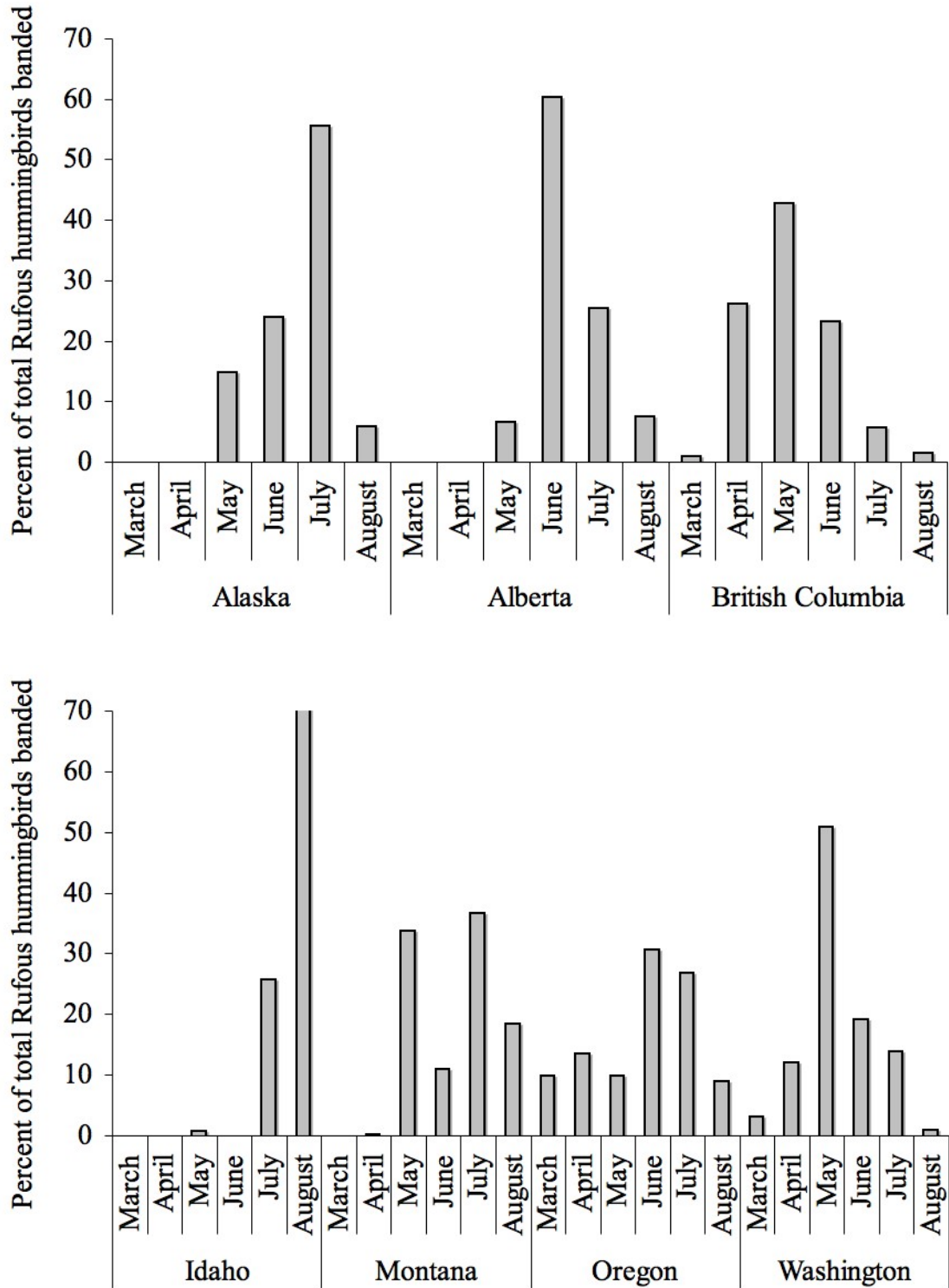


Figure 4: The percent of the total number of hummingbirds banded each month in each state/ province of their breeding grounds between 1955 and 2004.

Movements within and between breeding seasons

There were 199 individuals in the BBL encounter records, from which I included only encounters out-with the initial 10-minute block of banding. Recaptures occurred from zero to eight years after birds were first banded. The mean number of years between recaptures was 2.14 ± 0.63 for males and 2.71 ± 0.15 for females (data are means and standard errors).

(a) Movements within state/ province of initial banding

I first looked at movements within states/provinces. Of the 55 available records of birds recaptured in the same state in which they were banded, 21 were from birds recaptured in the same 10-minute block as initial banding and for six records it was not possible to tell if the encounter was in the same or a different 10-minute block from initial banding. These 27 records were, then, excluded from analysis. The remaining 28 individuals (seven males and 21 females) were caught and recaptured within the breeding season between March and August. Of these, five females moved outside the block in which they had been banded within the same year. Four of these females moved within the same month they were banded, one in May and three in June. The fifth bird was banded in April and encountered in May of the same year. The remaining 23 birds were re-captured in years subsequent to the year in which they were banded. Sixteen (two males and 14 females) of these birds moved within British Columbia, six (four males and two females) moved within Montana and one male moved within Washington.

(b) Movements between states/ provinces

As I was particularly interested in movements that may result in population mixing (birds encountered outside the state or province in which they were first banded), I examined records where both first captures and later encounters were within the breeding season and at breeding grounds. There were only three records of interest. All three suggest that rufous hummingbirds move inland from the coast into the Rocky Mountains later in the season. The first bird, a female, was caught in British Columbia on the 16th of June 2006 and recaptured in Idaho on the 19th of July 2006. In the other two records banding and encounters were a year apart. Both birds (males) were banded in Montana in July and recaptured the next season in May, in British Columbia. Assuming they followed the same pattern of movement in both years, as they were in British Columbia earlier in the season this would mean that they moved from British Columbia to Montana over the summer before moving south then back north to British Columbia the next spring. However, they may also have followed different routes each year and only visited one of these states in each breeding season. A summary of links between all different states/provinces both within and out with the breeding are shown in Figure 5. These encounters demonstrate that at least some birds breeding both side of the Rockies overlap in their migration routes and at least spatially.



Figure 5: A map of long distance movements made by rufous hummingbirds in North America. Each thick black line links states/provinces between which at least one individual rufous hummingbird has moved. Data are only for encounters outside the initial state of province of initial banding and are from 1988 to 2005. Darker shading represents progressively higher altitudes around mountain ranges. States and provinces are outlined in white. States in the southeast USA are encircled as one general area for simplicity. Map modified from:

<http://johomaps.com/na/nablank.html>

Discussion

It appears from recaptures and re-sightings of marked individuals within a single river valley in Alberta during the breeding season, that males holding territories tend to move off their territories very infrequently whereas intruding males are much more mobile. Territorial males appear to return to exactly the same or an adjacent territory across years. The sole recaptured female also returned to the same territory she was banded at the previous year. However, intruding males were much more likely to be caught on different territories from where they were initially banded than were territorial males. No intruding males became territory holders in subsequent years although one territory holder became an intruder the following year. Intruding males could have been defending unobserved territories with poor food resources or they could have been non-territorial. Males without territories are thought to have a lower mating success than do territorial males, as territories provide uninterrupted access to intruding females and a display arena, although there are no data to confirm this supposition (Armstrong 1987). Despite having more limited mating opportunities than territorial males, non-territorial males may contribute more to gene flow within the river-valley as they are more mobile. The recapture of the same female (gravid on both occasions) on the same territory in two successive years suggests high nest-site fidelity for this individual. However, as I recaptured only one female, and banded relatively few females at all, I cannot say if this is typical behaviour. Site fidelity to successful nesting sites is fairly typical in many species of birds and may enhance breeding success (e.g. Newton 2008;

Newton & Marquiss 1982; Payne & Payne 1993; Winkler et al. 2004). However, in most species, females, especially younger females, are more likely to change breeding sites and to move further than are males (e.g. Greenwood 1980; Newton 1993; Winkler et al. 2004). Therefore, more banding data for females are necessary at this spatial scale before we can be sure of how common nest site fidelity is and what is the typical dispersal distance among breeding seasons.

At a larger scale, rufous hummingbird breeding-site fidelity appears to be quite variable. Estimates for female breeding site fidelity tend to be higher (up to 43%) than those for males (up to 19%) in studies where banding is carried out at large provisioning sites (Finlay 2007). However, this may reflect the banding procedures used rather than differences between the sexes in site fidelity. In the Westcastle valley, for example, where banding was done on male territories, I found a higher return rate (or recapture rate) for males than for females. Indeed, the site fidelity of males in many bird species is typically higher than that of females (e.g. Greenwood 1980; Murphy 1996). Given that expected values for the percentage of recapture each year (site fidelity) for other bird species are 30-60% for passerines and 60-90% for non-passerines (Newton 2008; pp 490 & 534), average recapture rates of 12-18% for rufous hummingbirds are relatively low, unless mortality is around 80% a year. The low recapture rates may be due to birds moving short distances between seasons such that they are still within a few km of the previous year but do not visit the same feeding sites and are, therefore, not re-encountered. This seems possible as small study areas, such as the hummingbird feeding stations, which provide much of the data in this chapter, are typically associated with lower site fidelity than are larger sites, as they fail to detect individuals that move only

short distances (Newton 2008). Alternatively, low adult site fidelity may represent a tendency for longer distance movements between seasons. Longer distance movements may result in gene flow over greater distances and therefore have important implications for population structure. More data from areas closer to major banding sites may help detect more females if they are only moving short distances.

In terms of investigating how birds' dispersal patterns may affect gene flow and population structure, all the data discussed here have a major weakness, which is the absence of any data on natal site fidelity or juvenile dispersal in rufous. Such data are important as juvenile birds typically disperse further than adults and thus contribute more to gene flow (e.g. Newton 2002; Newton 2003; Paradis et al. 1998). This deficit is due in partly due to poor identification of juveniles in the hand, however, there is some suggestion that they are also harder to trap (Finlay pers. comm.). Whatever the reason, more effort to accurately identify and band juveniles would greatly enhance our understanding of the contribution of juvenile dispersal relative to adult dispersal to gene flow.

Banding at large provisioning sites, which are used predominantly by females (personal observations 2007 & 2008: see chapters 7&8), may explain the strong female-biased sex ratio in the banding data from the BBL. However, it is more typical for a bias in avian adult sex ratios to be towards males rather than females, apparently due to higher female mortality (Donald 2007). It may be, then, that male rufous have a shorter life span than do female rufous. If, atypically for birds, a male rufous' life expectancy is, indeed, shorter than it is for a female, this would explain the female-biased sex ratio.

In the spring, rufous hummingbirds arrive earlier in their more southern and western breeding grounds than in the north and east. It appears that most rufous hummingbirds move north along the coast of North America from Mexico before moving inland (Healy & Calder 2006). The recapture of several individuals within a breeding season that have moved from the coast east into the mountains supports this interpretation of arrival times although it is unclear how far east they will move. It is possible that they follow along the mountains and will move up mountain valleys but not cross over mountain ranges unless there are mountain passes such as the Colombia river valley between Washington and Montana. Such east to west movements may simply represent post-breeding migration and may not result in gene flow. Furthermore, it is conceivable that birds actually breeding in the east of the Rockies may arrive by a different route. These easterly breeding birds may be the small portion of the population that follow a more inland route north in the spring. This inland movement can be seen in the data as comparatively low first peak in abundance in Idaho and Montana in May. This route would not require birds to cross over the Rocky Mountains to reach places such as Alberta as there are several mountain passes between Idaho and Montana. As the distance and direction of birds migration is partially under genetic control and can be a distinctive trait of sub-populations, it would be interesting to see if the birds following the inland route north are unrelated to those following the main coastal flyway (Helbig 1991; Pulido 2007).

Second peak abundances in Idaho, Montana and Oregon after the breeding season show that in the autumn, birds from further north and, in the absence of a second peak abundance along the westcoast, also from the west move south along an

inland route following the mountain ranges. This, suggest that birds breeding along the west coast probably move east after breeding before flying south.

However, the timing of arrivals in more easterly breeding areas is after the peak abundance or at the end of the breeding season in more westerly areas. It is also about the same time that birds from westerly areas would be expected to be moving east into the mountains before heading south. It is possible that birds that have already bred on the coast intermix with breeding birds in the east of the range. For example, birds start leaving British Columbia between May and June, which is about the same time rufous arrive in Alberta and Alaska. We have no reason to believe that post-breeding birds from the west would not breed again with more easterly nesting birds on their way back south or even continue north to breed again. Indeed, several bird species that have multiple broods will breed at several points along their migration routes (e.g. the European Quail *Corturnix corturnix*, (Aebischer & Potts 1994; Newton 2008)). Many other species will breed several times within a season following the seasonal abundance of food between different locations such as: the Zebra finch, *Taeniopygia gutta* (Zann & Runciman 1994) and a nectivore the Regent Honeyeater, *Xanthomyza phrygia* (Gerring & French 1998). Having a second clutch is certainly plausible as rufous females that have been trapped carrying an egg at the beginning of the breeding season have been recaptured, again carrying an egg, sufficiently later in the same season for the first brood to have fledged (Finlay 2007). Alternatively, this may represent re-nesting after the failure of the first nesting attempt rather than the start of a second brood (Finlay 2007). Second nesting has been recorded in several other North American hummingbirds, such as the Anna's hummingbird, which will start incubating a second clutch whilst still feeding

fledglings from the first (Scarfe & Finlay 2001). However, as yet there is no evidence of hummingbirds moving locations between breeding attempts. Breeding in several locations seems more plausible for male rufous hummingbirds who play no role in rearing young and so are more free to move than it is for females (Healy & Calder 2006). If this was the case then there might be gene flow among breeding locations in quite distant parts of the breeding range.

Decreases in the population of rufous hummingbirds in the core of their breeding range (around British Columbia) are evident from the Breeding Birds Survey data. This is consistent with the decrease in the number of birds banded relative to banding effort reported by Finlay 2007. The decrease in numbers has been attributed, in part, to habitat fragmentation as rufous hummingbird population densities are positively correlated with old-growth forest area and negatively associated with more complex forest patch shapes (Lehmkuhl et al. 1991). If dispersal distances are typically short, this may exacerbate the affect of habitat loss on the breeding rufous population as birds may not disperse far enough from their natal location to find suitable breeding habitat. Migrant birds with shorter dispersal distances seem to be more likely to decline than those with longer dispersal distance for this reason (Paradis et al. 1998). However, it is unclear why populations may be increasing in many parts of the north and east of their range. It is possible that this represents a range shift possibly linked to environmental factors such as changes in habitat or climate. It may be possible to use genetic markers such as microsatellites both to establish if population growth is due to increased breeding success in those areas or immigration from other regions and to confirm whether some long distance movements may result in gene flow. If long distance dispersal and immigration are

common, then we would expect to find only low levels of genetic population structure among regions, meaning that the population dynamics of different areas would be unlikely to operate fully independently.

In summary, it appears that despite some site fidelity of both male and female rufous hummingbirds both in Alberta and British Columbia, individuals do move both within and between breeding seasons. Such movements may lead to gene flow and therefore a lack of strong population structure at scales of 10 to 100s of km. However, without evidence of individuals actually breeding in several locations we cannot say if such movement do actually result in gene flow. If the rufous hummingbird population is not highly structured then it is unlikely that the population dynamics in different geographic locations will not operate independently from other areas and it is likely that some sort of source sink population dynamics may operate among regions. In the absence of sufficient banding data, genetic data may help to answer this question.

Appendix 1: *The movements of rufous hummingbirds within the Westcastle River*

valley within and between years. Data are for males (territory holders and intruders separately) and females.

Bird/ Band number	Sex	Year	Day- Month 1 st capture	Site 1 st capture if own territory	Site 1 st capture if not own territory	Day Month 2 nd capture	Site 2 nd capture	Distance move within year (m)	Distance moved from previous year (m)
Birds caught on two or more consecutive years									
C00833	M	2007	29-May	WC01		2-Jul	WC10	830	
		2008	28-May	WC01		13-Jun	WC0d	270	0
C00835	M	2007	29-May	WC02					
		2008	28-May	WC02					0
C00836	M	2007	29-May	WC04					
		2008	31-May	WC04					0
		2009	11-Jun	WC03					150
C00839	M	2007	31-May	WC24					
		2008	29-May	WC24					0
		2009	30-May	WC24					0
C00845	F	2007	2-Jun		WC28				
		2008	29-May		WC28				0
C00855	M	2007	22-Jun	WC03					
		2008	27-Jun	WC03					0
C99630	M	2008	28-May	WC23					
		2009	30-May		WC28				2470
C99632	M	2008	29-May	WC26					
		2009	30-May	WC26					0
C00830	M	2006		WC15					
		2007	2-Jun	WC15					0
C00837	M	2007	29-May		WC15	3-Jul	WC25	3600	
		2009	31-May		WC20				1700
C00846	M	2007	2-Jun		WC25				
		2008	29-May		WC24				980
Birds caught at more than one site in the same year only									
C00840	M	2007	29-May	WC16		16-Jun	WC14	830	
C00843	M	2007	1-Jun	WC26		18-Jun	WC10	6180	
C99649	M	2008	20-Jun	WC0e		13-Jun	WC13	280	
C99677	M	2009	31-May	WC20		31-May	WC19	220	
C99637	M	2008	29-May		WC13	31-May	WC04	280	
C00842	M	2007	1-Jun		WC25	3-Jun	WC23	1270	

**Chapter 10 Microsatellite variation in rufous hummingbirds: evidence
for a weakly structured population**

This chapter was written in collaboration with Sue Healy and Josephine Pemberton who provided many helpful comments on experimental protocols and the text.

Abstract

The rufous hummingbird population is declining for unknown reasons in some areas but not in other. Understanding the genetic population structure of this species could be useful in understanding its dispersal behaviour and whether particular geographical areas may be worth considering as separate conservation units. As there were no existing microsatellite markers for rufous hummingbirds we tested 60 universal bird markers and 16 markers designed for other hummingbird species for amplification in rufous hummingbirds. Twenty-seven of these markers were useful for genotyping rufous hummingbirds. Markers developed for other hummingbird species typically amplified better and were more polymorphic in rufous hummingbirds than the universal bird markers. Using six microsatellite markers I then investigated the population structure of rufous hummingbirds breeding in Canada. We found that the population was weakly structured such that birds breeding in central British Columbia could be distinguished from those breeding in Alberta and on Vancouver Island several 100km away. However, birds breeding on either side of Vancouver Island, a distance of around 40km, were indistinguishable from each other. More data are required to establish whether bird movements resulting in gene-flow are in any way age or sex specific.

Introduction

Conservation of species, particularly those with a wide distribution, can be greatly enhanced through knowledge of the genetic structure of their populations. Highly structured populations may need to be managed as a number of independent populations whereas more panmictic populations may be considered as a single conservation unit (Esler et al. 2006). Such structuring within populations is dependent on the number of individuals that disperse away from their natal sites and the distance that they travel (Clark et al. 2004; Walters 2000). While banding and recapture data provide us with information on the typical movement and dispersal behaviour of avian species, we often fail to detect low numbers of movements among parts of the populations that otherwise appear to be separate (Alcaide et al. 2009). When supposedly separate sub-populations are examined using genetic approaches, however, they appear to be panmictic. Observations of such mismatches between banding and genetic data are increasingly common (Pearce & Talbot 2006).

The rufous hummingbird *Selasphorus rufus* is one of the most common and widespread hummingbird species in western North America. Its population is estimated to have been declining by about three percent for the past thirty years, equivalent to a population decline of over fifty percent in that period (Healy & Calder 2006), the reasons for which are unknown. However, the decline is not equal across all parts of the species' breeding range (Sauer et al. 2008): it is less severe in Alaska and in eastern parts of the range including eastern Washington, northern Idaho, Montana and Alberta. As there are no data on rufous hummingbird population structure, it is unclear if declines in one area affect the size of populations breeding

elsewhere or whether some areas should be treated as separate conservation units. It is possible that mountain ranges such as the Coastal and Rocky mountains act as barriers to movement between breeding populations as they do in other species (e.g. Swanson's thrush (*Catharus ustulatus*) Ruegg et al. 2006), and that populations on either side should be considered separately. However, it is also possible that they can readily cross such barriers by following the few east west flowing rivers that cut thru them such as the Columbia and Fraser rivers.

From banding and recapture data of adult rufous hummingbirds it appears that both sexes are generally faithful to their breeding sites as adults (Finlay 2007). High site fidelity would reduce gene-flow (the movement of individuals from one population to breed in a different population), between regions and we would expect to find a structured population. Dispersal on the other hand increases gene-flow and reduces population structure. Avian dispersal is typically longer and more common in juveniles than adults (Newton 2008). However, there are few data from individuals banded as juveniles and, thus, no real indication of levels of natal dispersal. However, there are also records of adults travelling fairly long distances (e.g. 87 km) within the breeding season indicate that a few birds may move among breeding populations (Finlay 2007). As the mean number of years between captures in the banding records for rufous hummingbirds in North America is 2.14 ± 0.63 for males and 2.71 ± 0.15 for females (data from The North American Bird Banding Program; Gustafson & Hildenbrand 1999) birds are living long enough for both juvenile dispersal and the movement of adults between as well as within years to affect population structure. Rufous hummingbirds are believed to follow a circular migration route, north in the spring, east into the mountains in late summer, then south along the mountain ranges in autumn (Williamson 2001), and it is possible that birds, particularly males, while migrating to more northerly or easterly breeding

sites, may mate en route with birds on breeding grounds to the south and west. Birds arrive in British Columbia in March and start leaving between May and June, in Alberta they do not arrive until about this time, they then leave Alberta mostly in July. Given the timing of their arrivals and departures it seems possible that some birds from British Columbia may cross the Rockies into Alberta and breed again. Alternatively, the Rockies may act as a barrier to movement between British Columbia and Alberta, limiting gene flow between them. Given the limited data on the birds' movements, banding and recapture data alone are insufficient for inferring whether or not the population is likely to be highly structured.

Population structure due to isolation by distance or barriers to gene flow such as mountain ranges is best inferred using highly variable markers such as microsatellites (Guillot et al. 2005). Microsatellite markers are particularly useful due to their high variability (up to 50 alleles at a single locus) (Parker et al. 1998) and they are now one of the most common markers used in studies of population structure and landscape genetics (Selkoe & Toonen 2006). Although developing microsatellite markers for a new species can be time consuming and many loci are not transferable between species (Parker et al. 1998), increasing numbers of markers are being identified with highly conserved flanking regions that will amplify in multiple species (Dawson et al. 2009a; FitzSimmons et al. 1995; Parker et al. 1998).

I sought, firstly, to identify polymorphic microsatellite markers that would be useful for investigating the population structure of rufous hummingbirds. Secondly, I looked for genetic variation among breeding populations within British Columbia and tested whether the Rocky Mountains act as a barrier to gene flow between the rufous populations in British Columbia and Alberta. I expected to find population structure at least among breeding populations separated by mountain ranges.

Methods (1) Identifying polymorphic microsatellite loci

Markers

I tested 60 ‘universal’ bird microsatellite markers and sixteen microsatellite markers developed for other hummingbird species for amplification and polymorphism in rufous hummingbirds. D. Dawson (University of Sheffield) provided fluorescently-labelled primer pairs for the universal bird markers (Dawson. pers comm; Dawson 2007; Dawson et al. 2009b; Slate et al. 2007). The methods used to identify the target loci involved comparing zebrafish (*Taeniopygia guttata*) microsatellite sequences to the chicken (*Gallus gallus*) genome sequence to identify homologous sequences. Homologous chicken and zebra finch sequences were aligned and mismatching bases and gaps were replaced with the code n* for unknown base. These hybrid zebra finch-chicken sequences were then used to design primer sets that were identical in zebra finch and chicken and that did not include any degenerate bases (Dawson et al. 2009b).

Of the sixteen hummingbird markers tested, six had been developed for blue-throated hummingbird *Lampornis clemenciae* and primer sequences which were obtained from G. Segelbacher (University of Freiburg). These markers were isolated via an enrichment protocol (Kundernatsch et al. 2009). They had not been screened for polymorphism in any hummingbird species.

The remaining ten hummingbird markers were developed from a genomic library constructed for the broad-tailed hummingbird *Selasphorus platycercus*. Primer sequences were obtained from S Oyler-McCance & J St John (University of Denver) and I bought fluorescently-labelled primers pairs. These markers did not

become available until testing of the other markers was nearly complete but by then they were known to amplify microsatellites in rufous, Allen's *Selasphorus sasin* and Anna's hummingbirds *Calypte anna* (Oyler-McCance & St. John pers.comm).

Samples and extraction

Both feather and tissue samples were tested. Feather samples were collected from rufous hummingbirds between 2007 and 2008 at three traditional banding sites in British Columbia, two on Vancouver Island and one in central British Columbia. Samples were also collected from one site in Alberta, in the Westcastle River valley. Feathers were stored dry in paper envelopes for up to eighteen months before DNA extraction. Tissue samples were of breast muscle collected opportunistically from birds found dead throughout the project. Causes of death were not known for all individuals but included flying into windows of buildings and collisions with traffic. Dead birds were initially frozen at -20°C for around six months while export permits were acquired. Muscle tissue was then removed and stored in 100% ethanol before shipment to the UK. In total, muscle tissue came from seven individuals, five from British Columbia and two from Alberta.

Initial testing of the universal bird markers was done on DNA extractions from tail feathers collected from six rufous hummingbirds (two males and four females) and control samples from chicken, *Gallus gallus* and zebra finch, *Taeniopygia guttata*. Only the first 1cm of the feather shaft was used. The remainder of the feather was used in a complementary project using stable isotopes to determine the over-wintering areas of rufous hummingbirds from different breeding locations (Jonathan Moran, University of Victoria, British Columbia).

Feather extractions were done using the Quiagen DNeasy tissue extraction kit and protocol with the following variations: samples were lysed with proteinase k for

over 12 hours and only one final elution step was used, to maximise the concentration of the final DNA. Further testing was done on the muscle tissue samples when they became available. Extractions from tissue samples were done in the same way but were lysed for only three hours.

The 60 universal bird markers and the 10 markers developed for *S. platycercus* were amplified using a standard PCR protocol with the annealing temperatures suggested by their originators. Each 10 μ l polymerase chain reaction (PCR) contained 1 μ l of DNA (concentration as extracted), 1 μ l of each forward and backward primer (10pmole concentration), 3.94 μ l of water, 1.25 μ l of Bioline 10* NH_4 reaction buffer, 1.25 μ l of dNTP (concentration 2mM), 0.5 μ l of MgCl_2 (concentration 50mM) and 0.06 μ l Bioline TAQ DNA polymerase (concentration 5u/ μ l). The PCR program used was of the form: 94 °C for 3mins, then 35 cycles of: 94 °C for 30s, annealing temperature for 30s, 72 °C for 30s, and finally: 72 °C for 10mins followed by 8 °C until removed from the machine.

For the six *L.clemenciae* loci I used unlabelled primers and identified appropriate annealing temperatures by using the same reaction mixture as above and a touchdown PCR program: 95 °C for 5 mins, then 30 cycles of: 94 °C for 30s, gradient within thermal cycler from 48 °C in well one to 60 °C in well twelve for 1min30s, 72 °C for 1min30s, and finally: 60 °C for 30min followed by 4°C until removed from the machine. Visualization of PCR products of these six markers was carried out on 4% agarose gels stained with ethidium bromide. Only four of these six markers (L1, L2, L4 and L5) amplified and were subsequently fluoro-labelled at the 5' end of the forward primer before being tested for polymorphism. All PCRs were performed using DYAD peltier thermal cyclers (Genetic Research Instrumentation).

The universal bird and *S. platycercus* markers in *S. rufus* were also fluorescently-labelled at the 5' end of the forward primer. All markers (including the four amplifying *L. clemenciae* markers) were tested for polymorphism using six to twelve samples (for more promising loci) from *S. rufus*. The PCR reactions used for markers differed only in the annealing temperature used.

All fluorescently-labelled PCR products were run on an ABI3730 capillary sequencer (Applied Biosystems) together with an internal size standard Genescan LIZ 500 (Applied Biosystems). PCR products were diluted 1 part reaction to 100 parts water (tissue samples) and 1 part reaction to 10 parts water (feather samples). Each 10µl GeneMapper plate well contained 1µl of diluted PCR product and 9µl of HiDi LIZ 500 size standard mix (1µl size standard in 1ml HiDi formamide). Analysis of the fragments was carried out using the software Genemapper version 4.0 (Applied Biosystems).

Results (1)

Of the 60 universal bird markers, four failed to amplify and 26 were monomorphic. The remaining 30 markers were polymorphic 16 of which showed strong peaks and had very little stutter, a characteristic nuisance phenomenon due to slippage during amplification of microsatellites. The outcome of the trial for each locus is shown in Table 1, the 16 'best' loci were TG06009, TG01 148, TG03 098, TG13 017, TG05 046, TG04 012a, TG04 004, TG03 035, TG12 015, TG01 147, TguGga z002, TG22-001, DkiD126 ZEST, Pte24 CEST, ApCo46 ZEST and Tgu06. TguGga z002 is a sex marker and correctly sexed all of the individuals tested. Pte24 and Tgu06 amplify the same locus (D. Dawson pers. comm). I was not aware of this at the time of testing,

however but, as expected, they gave the same genotypes for all birds tested. The number of alleles for the universal bird markers ranged from one to five (mean = 1.78 ± 0.16 ; Table 1).

Of the sixteen markers developed for other hummingbird species, the ten developed for *S. platycercus* all amplified, while four of the six developed for *L. clemenciae* amplified. Eight of the *S. platycercus* markers (markers: H1, H2, H3, H6, H8, H9, H10, H15) and three of the *L. clemenciae* markers (markers: L1, L2 and L4) were polymorphic with strong peaks in *S. rufus*. Of the hummingbird markers that amplified, the number of alleles ranged from one to ten (mean = 3.88 ± 0.78 ; Table 2), higher than the mean number of alleles found in the universal markers. Two of the primers cloned from *L. clemenciae* did not amplify a product of the expected size (L3 and L6). Details of the outcome for all markers are shown in Table 2 with a summary in Table 3.

Table 1: A summary of test results for rufus hummingbirds using microsatellite markers developed as universal bird markers. Markers that were both polymorphic and showed strong clear peaks are highlighted in bold.

Locus	N alleles	N birds	Allele sizes	Observed Heterozygosity	Comment	Reference
TG04-012	No amplification			0		Dawson et al. 2009
TG01-058	2	7	297, 298	0.143	Peaks<400 counts	Dawson et al. 2009
TG01-000	5	8	234, 235, 236, 237, 238	0.875	Peaks<400 counts	Dawson et al. 2009
Tgu-Gga z040	1	5	130	0		Dawson 2007
TG01-124	1	4	391	0		Dawson et al. 2009
TG05-053	2	12	226, 229	0	All birds homozygous	Dawson et al. 2009
TG06009	2	8	116, 119	0.5		Dawson et al. 2009
TG04-012a	2	7	237, 239	0.571		Dawson et al. 2009
TG01-148	3	12	185, 189, 200	0.5		Dawson et al. 2009
TG01-040	1	2	277	0		Dawson et al. 2009
TG02-120	1	5	234	0		Dawson et al. 2009
TG03-002	1	5	130	0		Dawson et al. 2009
TG03-031	1	10	196	0		Dawson et al. 2009
TG03-034	1	5	175	0		Dawson et al. 2009
TG01-114	1	9	177	0		Dawson et al. 2009
TG02-078	1	6	289	0		Dawson et al. 2009
TG03-098	4	9	224, 225, 227	0.111		Dawson et al. 2009
TG04-004	4	12	158, 160, 162, 166	0.333		Dawson et al. 2009
TG01-092	1	9	178	0		Dawson et al. 2009
TG02-088	1	6	248	0		Dawson et al. 2009
TG03-035	5	10	222, 226, 227, 228, 229	0.4		Dawson et al. 2009
TG09-014	1	6	151	0		Dawson et al. 2009
TG11-011	1	5	212	0		Dawson et al. 2009
TG13-016	1	6	154	0		Dawson et al. 2009
TG13-017	2	6	205, 209	0	Some stutter	Dawson et al. 2009
Tgu-Gga z037	1	6	162	0		Dawson 2007
TG04-061	3	5	198, 199, 201	0.2	Peaks<400 counts	Dawson et al. 2009

Locus	N alleles	N birds	Allele sizes	Observed Heterozygosity	Comment	Reference
TG05-030	1	6	181	0		Dawson et al. 2009
TG07-022	1	12	442	0		Dawson et al. 2009
TG08-024	1	6	163	0		Dawson et al. 2009
TG12-015	4	6	283, 285, 287	0.667		Dawson et al. 2009
TG13-009	1	6	193	0		Dawson et al. 2009
TG22-001	2	10	251, 255	0.1		Dawson et al. 2009
Tgu-Gga z002	2	10	220, 223	0.6		Dawson 2007
TG01-077	1	7	155	0		Dawson et al. 2009
TG01-147	5	5	280, 283, 285, 287, 289	1		Dawson et al. 2009
TG04-041	1	4	172	0		Dawson et al University of Sheffield
TG05-046	2	6	329, 334	0.167		Dawson et al University of Sheffield
Tgu-Est 09-005	3	6	160, 164, 168	0.667	Peaks<400 counts	Dawson et al University of Sheffield
Tgu-Est 09-018	1	6	281	0		Dawson et al University of Sheffield
ApCo46-ZEST	2	5	222, 227	0.2		Dawson et al University of Sheffield
Tc11B4E(2)-CEST	1	6	408	0		Dawson et al University of Sheffield
Tc11B4E-CEST	1	5	461, 464	0		Dawson et al University of Sheffield
Ase55-CEST	4	5	286, 290, 292, 294	0.2	Peaks<400 counts	Dawson et al University of Sheffield
AviAAGG30-ZEST	1	4	163	0		Dawson et al University of Sheffield
BFPO8-ZEST	2	6	93, 97	1	High stutter	Dawson et al University of Sheffield
Calex-08-ZEST	2	2	216, 218	0	Peaks<400 counts	Dawson et al University of Sheffield
DcyAAGG142-ZEST	1	6	208	0		Dawson et al University of Sheffield
DkiB102-ZEST	2	6	204, 209	1	High stutter	Dawson et al University of Sheffield
DkiD126-ZEST	2	6	165, 169	0.167		Dawson et al University of Sheffield
Pdo23-ZEST	2	2	145, 149	0	Amplified in < half birds	Dawson et al University of Sheffield
Pij14-23-CEST	2	4	151, 161	0.5	High stutter	Dawson et al University of Sheffield
PmaGAn30-ZEST	2	3	126, 135	0	Peaks<400 counts	Dawson et al University of Sheffield
Pte24-CEST *	2	6	220, 224	0.167		Dawson et al University of Sheffield
Tgu-06*	2	6	161, 164	0.167		Slate et al 2007
SAP47-ZEST	?	6	?	-	High stutter	Dawson et al University of Sheffield
Tgu-Est 09-021	?	6	?	-	High stutter	Dawson et al University of Sheffield
MSLP4-ZEST	No amplification			0		Dawson et al University of Sheffield

Locus	N alleles	N birds	Allele sizes	Observed Heterozygosity	Comment	Reference
Tgu-Est 09-025	No amplification			0		Dawson et al University of Sheffield
DkiD12ZF Chrom 9	No amplification			0		Dawson et al University of Sheffield

* These two loci are the same (see text)

Table 2: A summary of test results for rufous hummingbirds using microsatellite markers developed for other hummingbird species.

Markers that were both polymorphic and showed strong clear peaks are highlighted in bold.

Locus	N alleles	N birds	Allele sizes	Observed Heterozygosity	Comment	Reference
L1	2	11	145, 149	0.09		Gernot Segelbacher, University Freiburg
L2	7	9	138, 142, 143, 147, 151, 155, 159	0.67		Gernot Segelbacher, University Freiburg
L3	No amplification		-	-		Gernot Segelbacher, University Freiburg
L4	3	11	113, 115, 117	0.55		Gernot Segelbacher, University Freiburg
L5	1	11	181	0		Gernot Segelbacher, University Freiburg
L6	No amplification		-	-		Gernot Segelbacher, University Freiburg
H1	3	8	137, 141	0		S. Oyler-McCance & J. St John, University of Denver
H2	3	8	142, 146, 150	0.75		S. Oyler-McCance & J. St John, University of Denver
H3	3	8	150, 152, 156	0.63		S. Oyler-McCance & J. St John, University of Denver
H6	10	8	144, 147, 150, 162, 168, 171, 174, 180, 183, 210	0.88		S. Oyler-McCance & J. St John, University of Denver
H7	2	8	152, 155	0.38	High stutter	S. Oyler-McCance & J. St John, University of Denver
H8	5	8	123, 126, 129, 132, 135	0.75		S. Oyler-McCance & J. St John, University of Denver
H9	7	8	96, 102, 106, 108, 116, 122, 124	0.88		S. Oyler-McCance & J. St John, University of Denver
H10	8	8	116, 121, 130, 133, 139, 142, 146, 167	0.50		S. Oyler-McCance & J. St John, University of Denver
H14	1	8	188	0		S. Oyler-McCance & J. St John, University of Denver
H15	7	8	132, 140, 146, 150, 154, 158, 162	0.50		S. Oyler-McCance & J. St John, University of Denver

Table 3: A summary of results for microsatellite loci from different sources tested with a view to genotyping rufous hummingbirds, *S. rufus*.

Source	Species loci designed for	Number of loci tested in <i>S. rufus</i>	Number % loci amplified	Number % loci polymorphic	Mean alleles per locus	Number % of loci considered useful for genotyping <i>S. rufus</i>
D. Dawson, University of Sheffield	Universal bird markers	60	56 93%	30 50%	1.78±0.16	16 27%
S. Oyler-McCance and J. St John, University of Denver	Broad-tailed hummingbird, <i>Selasphorus platycercus</i>	10	10 100%	9 90%	4.90±0.94	8 80%
G. Segelbacher, University Freiburg	Blue-throated hummingbird, <i>Lampornis clemenciae</i>	6	4 66%	3 50%	3.25±1.31	3 50%

Methods (2): Investigating population structure

Samples

To investigate the species' population structure, I genotyped feather samples from 190 individuals captured in the species' breeding range in Canada. Feather samples were gathered at traditional banding sites by the hummingbird-banding network in B.C. (West of the Rocky Mountains) and by me, from birds caught in the Westcastle River Valley in Alberta (East of the Rocky Mountains). Birds captured were categorized as coming from four broad geographical areas: Alberta (40 males and 19 females), Central B.C. (21 females), Eastern Vancouver island (17 males and 42 females) and Western Vancouver Island (24 males and 30 females; Figure 1).

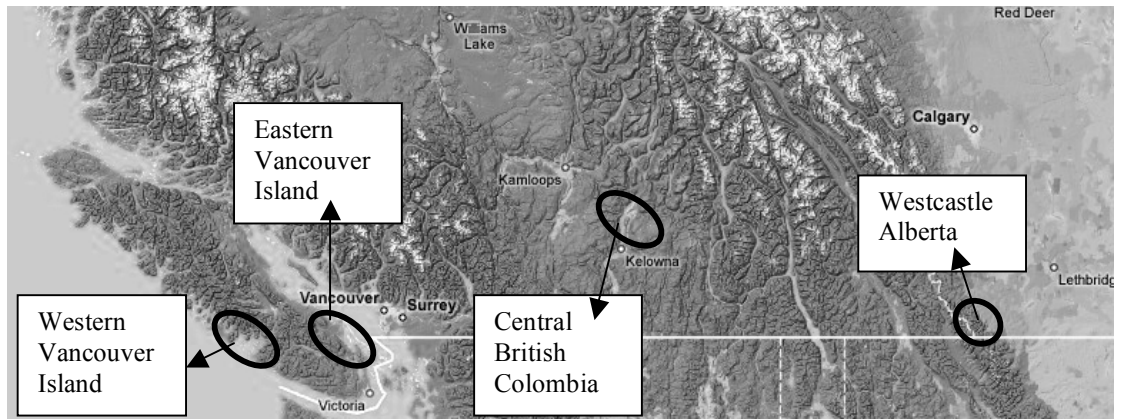


Figure 1: The four Canadian geographical areas from which feather samples were collected to investigate the population structure of rufous hummingbirds.

Markers

I used ten of the markers developed for other hummingbird species to investigate population structure. These were chosen because they showed the highest polymorphism and peak sizes in testing (H1, H2, H3, H6, H8, H9, H10, H15, L2 and L4). The same extraction, PCR and analysis protocols as described above were used. The forward primers were labelled with one of four fluorescent dyes depending on the size of their PCR products (ideally loci with overlapping ranges had different colours) to test whether they could be co-amplified and co-loaded to reduce time and money requirements: The colours assigned to the primers were: PET, red (L4, H10, H8); 6-FAM, blue (L2, H2); VIC, green (H1, H3, H6, H9) and NED, yellow (H15). I tested which markers would co-amplify and which could be co-loaded by multiplexing for PCRs for individuals with known genotypes and then co-loading multiplexed PCRs on sequencer runs. Results were examined for consistency of genotypes between the markers when multiplexed and co-loaded in groups to when they were amplified and loaded individually.

Analysis

Deviations from Hardy-Weinberg equilibrium and null alleles for each locus were tested for using goodness of fit tests to Hardy-Weinberg proportion in the program CERVUS 3.0 (Marshall et al. 1998), with Bonferroni corrections made for multiple comparisons. Molecular variance within and among populations and an estimate of genetic divergence among populations were calculated via F_{ST} AMOVA in the program ARLEQUIN (Excoffier et al. 1992; Schneider et al. 2000; Weir & Cockerham 1984). Analysis of molecular variance was done using 100000 permutations. Population pairwise F_{ST} values were calculated using 100000 permutations for significance and 1000 permutations for Mantel tests. Exact tests of population differentiation used 100000 Markov chain steps and 10000 Dememorisation Steps. Deviations from Hardy-Weinberg equilibrium for each locus and sampling population were calculated alongside this analysis using exact tests (Guo & Thompson 1992).

Further analysis of population structure was carried out using two Bayesian methods. Firstly, I analyzed the data with the more conservative program STRUCTURE 2.2 using a Bayesian clustering algorithm (Falush et al. 2007). This program assumes there are K populations each with a characteristic set of allele frequencies at each locus. Hardy-Weinberg and linkage equilibrium of loci is assumed within populations. Individuals are assigned probabilistically to a population, or populations (in the case of an admixed individual), resulting in a number of clusters of individuals as close to Hardy-Weinberg and linkage equilibrium as possible (Pritchard et al. 2000). The most likely number of

populations (K) in the data set was estimated independently in five replicates of $K=1-6$. The model was run using a burn-in period of 2×10^4 and a run of 5×10^5 Markov chain Monte Carlo steps, under the standard model of admixed ancestry and the model of correlated allele frequency ($\lambda=1$). The occurrence of null alleles was estimated simultaneously, using the recessive alleles model. After the release of STRUCTURE 2.3 I used the new function LOCPRIOR designed to improve the detection of weak population structure to reanalyze my data (Hubisz et al. 2009). LOCPRIOR uses sampling location information as prior information to aid clustering and is designed for use on data sets where population structure is too weak to be found using standard STRUCTURE models (Hubisz et al. 2009).

At values of $F_{ST} < 0.03$, such as over half the F_{ST} values calculated among populations in this study, STRUCTURE tends to underestimate the probable number of clusters of individuals (Latch et al. 2006). Therefore, I used an additional clustering program BAPs (BAPs 3.1, Corander et al. 2004), as, conversely to STRUCTURE, BAPs tends to overestimate the number of populations at similarly low values of F_{ST} (Latch et al. 2006). BAPs works in a similar way to STRUCTURE but uses a stochastic optimization algorithm in place of a Markov chain Monte Carlo randomizations to infer the best model and runs much faster than STRUCTURE (Corander et al. 2006). I used the ‘groups of individuals’ model. This model uses both individual genotypes and the *a priori* information about the allele frequencies of sample groups (in this case the sampling region in which feathers were taken) to infer population clusters (Corander et al. 2006). I modelled the data in this way using both mixture models where individuals are assigned completely to one of the identified clusters and admixture models where an individual can be assigned

entirely to one group or partially to several groups (Corander & Marttinen 2006; Corander et al. 2006).

Results (2)

Through testing of co-amplification and co-loading of markers, I initially reduced the number of PCRs from ten (one for each marker) to six (H9 and H3, annealing temperature 60°C; H15, H2 and H6, annealing temperature 60°C; L2 and L4, annealing temperature 56°C; H10, annealing temperature 52°C; H8, annealing temperature 58°C and H1, annealing temperature 60°C). It later appeared, during testing of large numbers of samples, that at least two of these markers were not working well (H15 and L2, see later) these were excluded from further testing. I then regrouped the markers into five PCR groups (H9 and H3, 60°C), (H2 and H6, 60°C), (H8 and L4, 58°C), (H10, 52°C) and (H1, 60°C). These PCR groups were combined to give three groups that could be co-loaded; Group 1: H1- loaded alone as it tended to swamp and interfere with the signal of other markers; Group 2: H9, H10 and H3 and Group 3: H2, H6, H8 and L4.

Of the ten loci screened in the larger sample of birds, initially two, and subsequently four, of the markers were eventually excluded. In three cases this was because they failed to amplify in most individuals (H2, H6, H15) and in one case because reliable scoring was made very difficult due to high levels of stutter (L2). The six remaining loci amplified in 139 to 185 of the 190 samples tested and the number of alleles ranged from 6 to 25 (Table 4).

Across the 190 samples, only 165 gave a clear genotype at three or more of the six markers. These 165 samples were split among the four geographical regions as follows: Alberta (45 individuals), Central B.C. (17 individuals), Eastern Vancouver island (53 individuals) and Western Vancouver Island (50 individuals). Only data from these 165 individuals were used in the analysis of population structure. However, this still left 1.2 to 18.0% missing data for each locus.

Table 4: Summary of the six loci used in the analysis of population structure for the 190 individuals sampled. * = a significant deviation from Hardy-Weinberg equilibrium. The tests are across the whole data set using the programme Cervus.

Locus	No alleles	No individuals	Heterozygotes Observed	Heterozygotes Expected	Hardy Weinberg	Null allele frequency estimate	% missing data
H1	6	185	0.054	0.074	$\chi^2= 5.53$, $p= 0.477$	0.145	1.2
H3	10	148	0.547	0.696	$\chi^2=14.506$, $p= 0.002^*$	0.123	12.0
H8	16	161	0.696	0.799	$\chi^2= 4.389$, $p= 0.624$	0.065	9.0
H9	11	139	0.705	0.840	$\chi^2= 3.266$, $p= 0.352$	0.083	18.0
H10	25	171	0.550	0.921	$\chi^2= \text{Infinity}$, $p<0.001^*$	0.249	6.0
L4	7	155	0.426	0.546	$\chi^2= 12.383$, $p<0.001^*$	0.134	14.4

Analysis in ARLEQUIN revealed that most genetic variation was found within populations but there was a small, significant amount of variation among populations (Table 5).

Table 5: Sources of variation in microsatellite molecular data.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	3	13.225	0.036	2.41
Within populations	330	488.913	1.482	97.59
Total	333	502.138	1.518	
Fixation Index F_{ST} among populations= 0.0241, $p < 0.001$				

There was slight but significant differentiation between birds breeding on Vancouver Island and those breeding on the mainland ($F_{ST} = 0.081$ to 0.015 , $p < 0.001$ to 0.006 ; Table 6). There was no difference between birds breeding on the East and West of Vancouver Island ($F_{ST} = 0.003$, $p = 0.369$). The birds breeding in Central British Columbia were not quite significantly different from those breeding in Alberta after Bonferroni correction for multiple comparisons between populations ($F_{ST} = 0.025$, $p = 0.013$, $\alpha = 0.008$).

Table 6: Pairwise differentiation among populations (F_{ST} below diagonal) and probability tests for allele frequency differences (P value above diagonal).

Bonferroni correction for six tests ($\alpha = 0.008$). F_{ST} values significantly different from zero are in bold.

Population	Western VCI	Eastern VCI	Central B.C.	Alberta
Western VCI	-----	0.369 ± 0.001	$< 0.001 \pm 0.000$	0.004 ± 0.000
Eastern VCI	0.003	-----	$< 0.001 \pm 0.000$	0.006 ± 0.000
Central B.C.	0.081	0.068	-----	0.013 ± 0.000
Alberta	0.018	0.015	0.025	-----

In all populations locus H10 deviated significantly from Hardy-Weinberg equilibrium (Table 7). Except for H1 all other Loci deviated from Hardy-Weinberg equilibrium in at least one population. In all cases deviations from Hardy-Weinberg were due to fewer than expected heterozygotes. Locus H1 was in Hardy-Weinberg equilibrium in all populations except in Central B.C. where this locus was monomorphic. However, as on average only seven percent of individuals were heterozygous at this locus we would only have expected to find one heterozygous individual among the 17 individuals sampled from this population.

Table 7: Deviations from Hardy-Weinberg equilibrium for each sampling location and locus. VCI= Vancouver Island. Significant P values are highlighted in bold.

Area		Locus					
		H1	H3	H8	H9	H10	L4
West VCI	Heterozygotes Observed	0.04	0.62	0.69	0.70	0.56	0.26
	Heterozygotes Expected	0.10	0.67	0.78	0.84	0.92	0.41
	Hardy-Weinberg P value	0.06	0.86	0.28	0.01	< 0.01	< 0.01
East VCI	Heterozygotes Observed	0.04	0.56	0.67	0.73	0.40	0.38
	Heterozygotes Expected	0.06	0.70	0.79	0.86	0.92	0.43
	Hardy-Weinberg P value	1.00	0.01	0.03	0.06	< 0.01	0.12
Central B.C.	Heterozygotes Observed	Monomorphic	0.38	0.70	0.60	0.40	0.75
	Heterozygotes Expected		0.72	0.87	0.68	0.94	0.64
	Hardy-Weinberg P value		< 0.01	0.44	0.24	< 0.01	1.00
Alberta	Heterozygotes Observed	0.09	0.53	0.71	0.71	0.68	0.53
	Heterozygotes Expected	0.11	0.71	0.82	0.84	0.91	0.58
	Hardy-Weinberg P value	1.00	0.01	0.11	0.04	< 0.01	0.05

The most likely number of population clusters of individuals (populations) identified by STRUCTURE 2.2 was $K = 1$ (average $\ln P(X|K) = -2762.28$). For values of $K > 1$, the $\ln P(X|K)$ values decreased and the variation among the independent runs increased (Figure 2). It, therefore, seems unlikely that the birds sampled came from more than one population. There was no clear genetic distinction among any of the sampling sites with all individuals being partially assigned to all populations. This was also the case when I simulated just the two populations as would be expected if differentiation occurred due to the Rocky Mountains (Figure 3).

Removing the locus that deviated most severely from Hardy-Weinberg equilibrium (H10) from the analysis did not affect the most likely number of population clusters of individuals identified. $K = 1$ was still had the highest $\ln P(X|K)$ value indicating the most likely number of clusters was one (average $\ln P(X|K) = -1973.26$). For values of $K > 1$, the $\ln P(X|K)$ values similarly decreased and the variation among the independent runs increased. The LOCPRIOR model from STRUCTURE 2.3 also identified $K=1$ as the most likely number of clusters (average $\ln P(X|K) = -2760.12$; Figure 4). However, the $\ln P(X|K)$ values decreased much less steeply with increasing K than in the STRUCTURE 2.2 model. In addition, the average $\ln P(X|K)$ value increased between $K=5$ and $K=6$; because of this I ran the model again for $K=7, 8$ and 9 to check that this increase did not continue. It did not.

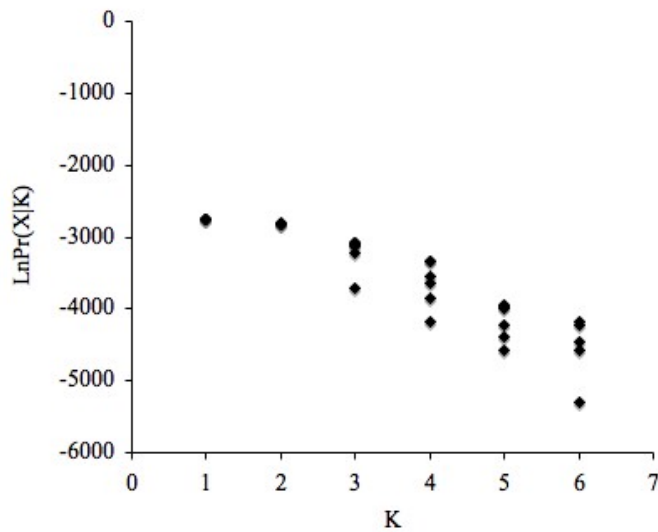


Figure 2: The likelihood of each value of $\text{LnPr}(X|K)$ for five independent runs of $K = 1$ to 6. Analysis conducted using a burn-in period of $2 \cdot 10^4$ and a run of $5 \cdot 10^5$ Markov chain Monte Carlo steps, under the standard model of admixed ancestry and the model of correlated allele frequency ($\lambda=1$), in the program STRUCTURE 2.2.

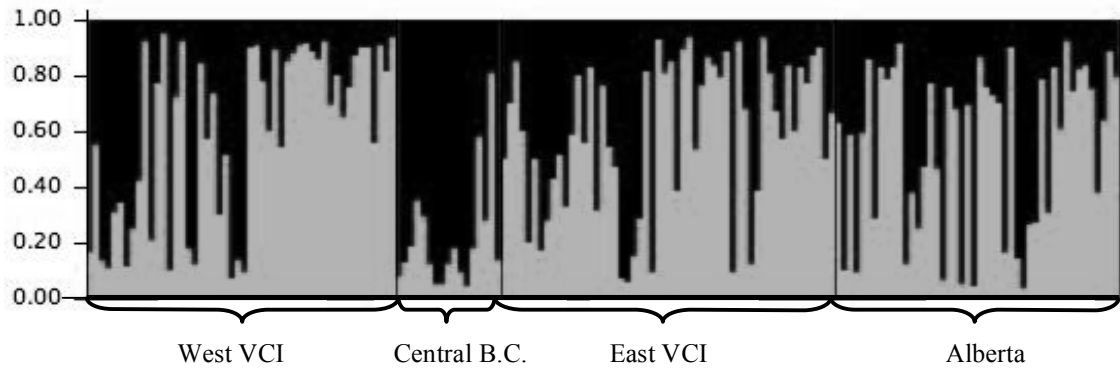


Figure 3: *The estimated membership coefficient, for each individual ($n = 165$), to each cluster of genotypes (when $K=2$). The vertical lines, each representing an individual, are split into black or grey each representing the proportion of that individual assigned to each simulated population/ characteristic set of allele frequencies for each locus (all individuals have genotypes which partially match the characteristic allele frequencies of both populations). Individuals are grouped by location along the x-axis. VCI = Vancouver Island. Birds from each geographical location are indistinguishable from the other populations.*

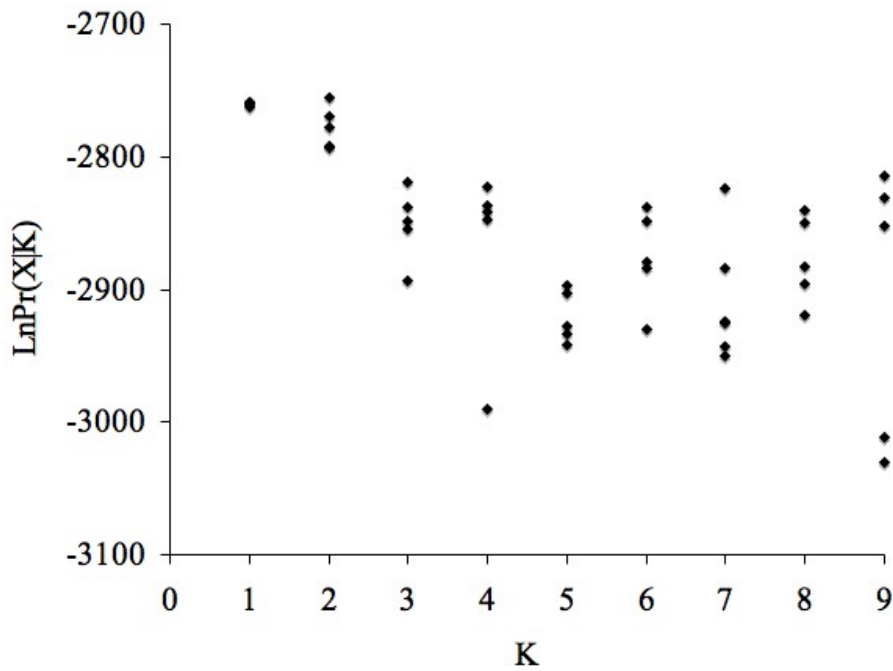


Figure 4: The likelihood of each value of $\text{LnPr}(X|K)$ for five independent runs of $K = 1$ to 9. Analysis conducted using a burn-in period of $2 \cdot 10^4$ and a run of $5 \cdot 10^5$ Markov chain Monte Carlo steps, under the standard model of admixed ancestry and the model of correlated allele frequency ($\lambda=1$) using the model *LOCPRIOR* in the program *STRUCTURE 2.3*.

The same data were analyzed using BAPs 3.1. The model was run both with and without admixture for 10 iterations and the same number of K ($K= 1$ to 6). Both of these models predicted two populations, with all the birds from Vancouver Island and the birds from Alberta in one population and the birds from Central British Columbia forming the second population. The mixture model predicted two populations (Log(marginal likelihood) of optimal partition= -2882.08, probability of two clusters = 1: figure 5a). The admixture model assigned all but two of the 165 individuals to one of two populations with a probability of 1, and the remaining two

individuals to both populations with probabilities of 0.10 and 0.12 (Figure 5b). Both of these individuals were sampled in Alberta. As ARLEQUIN suggested there may be a third population (a nearly significant difference between Central B.C. and Alberta), a mixture model in BAPs was used to estimate the probability of the existence of three populations. This model was significantly worse than the optimal model of $K = 2$, (Log(marginal likelihood) of partition for $K=3$: -2926.47). However, this model did identify the same possible populations as the ARLEQUIN analysis: Vancouver Island, Central British Columbia and Alberta. Allowing admixture with K set at three provided the same result as the mixture model for $K = 3$ but identified one individual from Western Vancouver Island that was split between the Central B.C. and Alberta populations ($p = 0.02$).

As locus H10 was not in Hardy-Weinberg equilibrium in any of the sampled populations, I re-ran the analysis in BAPs excluding this locus. I again found an optimal two clusters of individuals, with all the birds from Vancouver Island and the birds from Alberta in one population and the birds from Central British Columbia forming the second population: mixture model (Log(marginal likelihood) of optimal partition= -1962.881, probability of two clusters = 1. Similarly when this second analysis was run for $K=3$ it was still significantly worse than the $K=2$ model and identified the same populations: Log(marginal likelihood) of partition for $K=3$: -1988.824. Furthermore, both admixture models, the optimal model $K=2$ and the model for $K = 3$, found similar results to when locus H10 was included. However, different individuals were assigned partially to several populations. Only one individual, rather than two individuals, were assigned partially to both populations in the $K=2$ model. This individual was from a different sampling group than the

individuals assigned to both clusters in the model where H10 was included, originating from the East Vancouver Island sampling group rather than the Alberta group. The admixture model for $K=3$ again assigned one, but a different individual, from Western Vancouver Island to both the Central B.C. and Alberta populations, but a different individual and in addition assigned one individual from East Vancouver Island to both the Central B.C. and Alberta populations.

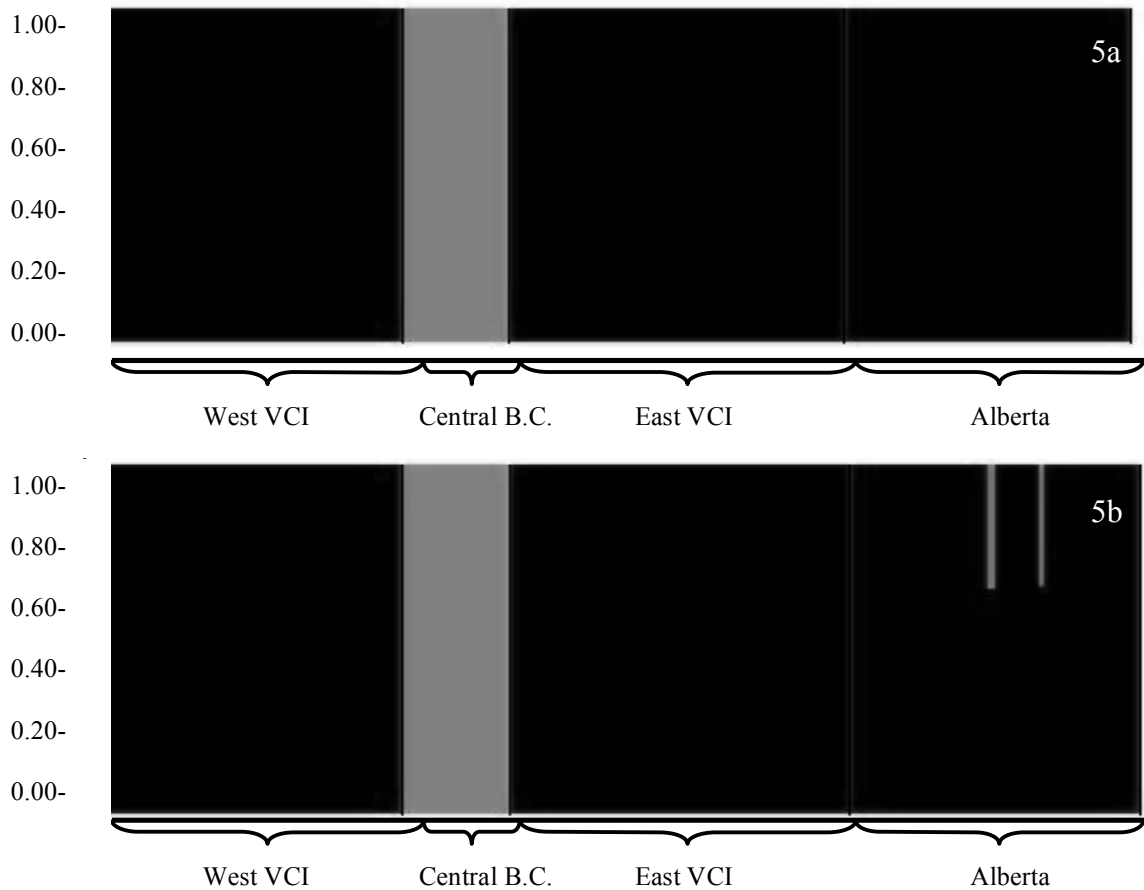


Figure 5: (a) The assignment of each individual from each geographic location ($n = 4$) to one of each simulated cluster of genotypes ($K=2$), using a mixture model in BAPs. (b) The estimated probability, of each individual ($n = 165$), belonging to completely or partially each cluster of genotypes (K) using an admixture model (with $K = 2$) in BAPs. The vertical lines each represent an individual. In each case, the vertical lines are either black, grey or both representing the proportion of that individual assigned to each cluster (genotype cluster one= black, and genotype cluster two= grey). Partial assignment to both clusters is only possible in the admixture model. Individuals are grouped by location. VCI= Vancouver Island.

Discussion

We identified 27 markers that would be useful for genotyping *S. rufus*. Markers developed for other hummingbird species proved to be more useful for genotyping *S. rufus* than did the universal bird markers. Indeed, the low levels of polymorphism observed using these markers greatly reduces their utility compared to the hummingbird markers tested (see table 3). This is not surprising as the transferability of genetic markers (both amplification and polymorphism) declines with increasing genetic distance between species (Dallimer 1999; Dawson et al. 2000; Hughes et al. 1998; Primmer et al. 1996; Primmer et al. 2005). For example, of 103 loci developed for passerines only 13% were useful for genotyping zebra finch *Taeniopygia guttata* (Dawson et al. 2006). However as, the position of the apodiformes (swifts and hummingbirds) relative to the galliformes (e.g. chickens) and passeriformes (e.g. zebra finches) in the most recent bird phylogeny based on comparative anatomy, is between the Galliformes and the Passeriformes (Livezey & Zusi 2007). It is unsurprising that the majority of the highly conserved microsatellites that amplify in both galliformes and passeriformes (universal bird markers) also amplify in hummingbirds. However, as the universal marker sequences have not changed over a long evolutionary period, it is possible this may be because these genomic regions are under selection and mutation, or at least polymorphism, has been suppressed (Primmer et al. 2005). If this is the case there may be concerns about the use of these markers in studies which assume neutral evolution even if they were usefully polymorphic (Primmer et al. 2005).

Rufous hummingbirds breeding on Vancouver Island had significantly different genotypes from those breeding in central British Columbia. However, it was more difficult to distinguish Alberta birds from the birds from these two regions. This lack of clarity is probably due to the very high level of within population variation compared to among population variation. In addition, it is important to note that with such low F_{ST} values both BAPs, that found two populations and STRUCTURE that found only one, may give misleading results (Latch et al. 2006). BAPs is more likely to overestimate the number of clusters and STRUCTURE to underestimate them (Latch et al. 2006). However, taking results of both Bayesian clustering methods and the F_{ST} analysis together, it seems probable that there are at least two genetically distinguishable sub-populations. Clarifying the situation with such weak population structure would require analysis of the samples with more markers than were available to this study.

The apparent weak but significant population structure among regions suggests that the adult breeding site fidelity, recorded in the banding data at around 12% (Finlay 2007), and levels of natal dispersal (unknown) are sufficient to create population structure among the geographically more distant populations. However, enough individuals must be moving between populations at smaller geographic scales e.g. on Vancouver Island, to maintain gene flow and a panmictic population. This movement may be due to the dispersal of juveniles, the movement of non-territorial males throughout the season or to females re-locating within and between seasons. This is consistent with the few long distance movements on the scale of up to 87km recorded in the banding data within a breeding season (Finlay 2007).

As migration direction and distance has a heritable component at least in one species but environmental factors and body condition may also influence on migration behaviour, it is possible that *S. rufus* movements and therefore gene-flow among populations may result from a combination of these factors (Berthold & Helbig 1991; Helbig 1991). It is possible that some birds from the west, particularly those that fail to breed early in the season and males who typically leave the breeding grounds earlier than females, move on and make a second attempt further along the migration route (see Aebischer & Potts 1994 for an example of similar behaviour in quail). Migration for western individuals up a more easterly route in some years or *vice versa* cannot be ruled out, but given both banding evidence showing the majority of birds move along the west coast and probable genetic tendency to follow a certain migration pattern, this seems less likely (Helbig 1991).

It is not clear whether movements are in any way age- or sex-specific as the banding and re-capture data so far analysed contains too few examples of individual bird movements for useful comment. Furthermore, the sample sizes in this study were too small to make testing for sex differences worthwhile (Kalinowski 2005). When F_{ST} is 0.05 or more a sample size of twenty individuals per population is sufficient but when F_{ST} is around 0.01, as in this study, closer to one hundred individuals per population is advised; in order to test for sex differences in dispersal, this number would need to be doubled (Kalinowski 2005).

Among the loci screened in up to 165 hummingbirds, several loci deviated significantly from Hardy-Weinberg equilibrium due to a deficit of heterozygotes. However, the exclusion of the locus most severely affected by this (H10) from analyses of population structure did not significantly affect the results. The deficit of

heterozygotes may be due to the presence of null alleles or poorly amplifying alleles or, which can be a problem when amplifying DNA from low samples containing low quantities of DNA such as feather extractions, further genotyping errors can arise due to the generation of false alleles during PCR (Gagneux et al. 1997; Gerloff et al. 1995; Navidi et al. 1992; Segelbacher 2002; Taberlet et al. 1996). The heterozygous deficit could also be attributed to the Wahlund effect where structure within populations can lead to an overall deficit of heterozygotes (Wahlund 1928), or inbreeding, although this seems unlikely given the apparent lack of strong structure in the wider population. A final possibility is that, too few individuals were sampled to allow an accurate estimate of expected allele frequencies, particularly of rare alleles, leading to an overestimate of expected heterozygotes (Guo & Thompson 1992). This a particular problem when using goodness-of-fit tests to Hardy-Weinberg proportion, as used in CERVUS, and with loci with more than ten alleles (Guo & Thompson 1992). However, the exact tests used in ARLEQUIN are more robust to this problem, (although not completely, Lauretto et al. 2009), but still showed some significant deviations from Hardy-Weinberg equilibrium (Guo & Thompson 1992). It thus seems probable that problems with scoring and amplification of some alleles are the most probable explanation for deviations from Hardy-Weinberg equilibrium in this case.

It may be possible to reduce deviations from Hardy-Weinberg equilibrium due to the presence of poorly amplifying alleles by employing a multi-tube approach (Goossens et al. 1998; Segelbacher 2002; Taberlet et al. 1996). A multi-tube approach requires repeating each experiment several times for each extraction and each locus (Navidi et al. 1992; Taberlet et al. 1996). However, this approach is very

time consuming and expensive (Segelbacher 2002). The reliability of genotypes can also, in the case of plucked hairs, be increased by using more than one hair from each individual in each extraction (Goossens et al. 1998). In the case of rufous hummingbirds it may be worth assessing the quantity of DNA that can be obtained from body feathers rather than tail feathers as these could be plucked in greater number with less risk to the birds. Indeed, initial results suggest that enough DNA can be extracted from around six broad-tailed hummingbird (*S. platycercus*) breast feathers to obtain reliable genotypes (Lisa Goldberg, University of California, Davis, Dept Animal Science, pers. comm).

To clarify the degree of differentiation among populations and possible effects of geographical barriers in more detail, a study including samples from more geographical locations would be necessary. Sites should be placed at a minimum of three locations on both sides of the Rockies so that multiple comparison of genetic distance between populations along and across the Rockies can be made. As I encountered problems with amplification, such a study should collect additional samples so that samples that fail to amplify well can be discarded. As the quantities of DNA extracted from feather samples are low, which can lead to genotyping errors I would recommend using at least six breast feathers in each extraction rather than a single tail feather and a multi-tubes approach to increase the reliability of genotypes. Due to the high within-population variation, using more than the six markers used here would increase the chance of detecting weak population structure. I also recommend using a larger sample size, closer to 100 birds per location, due to the small F_{ST} values found in this study (Kalinowski 2005).

In conclusion, we found 27 microsatellite markers that could be useful for genotyping *S. rufous*. Using six of these we found that the breeding population of rufous hummingbirds in Canada has very weak genetic structure that is not clearly related to geographical barriers such as the Rocky Mountains. While banding data has allowed us to see where birds move between, our genetic data suggest that at least some of these movements result in gene flow among populations. More work is necessary using improved protocols, more samples and more than six markers before we can say if these movements are in any way age or sex specific.

Variability in food rewards

Despite having an optimal meal size that differs with the sucrose concentration (DeBenedictis et al. 1978), hummingbirds unexpectedly chose not to change immediately how much they drank when a new concentration was encountered. Instead, they continued to feed as they had before the change, at least until post-ingestive feedback became available. As hummingbirds are on fairly tight energy-budgets, I predicted that they should always try in one way or another to maximise the efficiency of their foraging, by always drinking close to the optimal volume of any sucrose concentrations encounter (DeBenedictis et al. 1978; Hainsworth & Wolf 1972; Lopez-Calleja et al. 1997). Therefore, I expected that they would immediately change how much they drank in the direction of the optimal bout volume for that concentration, drinking more of poorer concentrations and less of higher concentrations, a change they would make based on the taste of the new solution (DeBenedictis et al. 1978). Alternatively, they could have treated a change as a probable one-off event. In that case they were expected to avoid drinking lower concentrations and to drink more of higher concentrations. This prediction was based on two concepts, firstly, the hedonic value associated with concentrations higher or lower than that of the past concentration would be greater or smaller respectively (Costa et al. 2007). Thus, animals would be expected initially to drink more of the higher or less of the lower concentration. Secondly, there is a general trend for animals to avoid poorer rewards if they believe they can wait and obtain a better

reward in the near future (Stephens & Krebs 1986). This tendency would also lead to hummingbirds initially drinking less or none of the poorer concentration.

That they did neither i.e. did not change how much they drank initially on encountering a new concentration, suggests that, despite being able to taste the change, the birds prefer to rely on post-ingestive feedback to adjust how much they drink when encountering new concentrations. Post-ingestive feedback can give animals more accurate information than taste can on their energetic state and the quality of ingested food (Sclafani 2004; Yearsley et al. 2006). Thus post-ingestive feedback may be a better sort of information than taste on which to base decisions about how much to drink when resource quality changes. When there has been no change in resource quality, the amount drunk at the previous bout may be a sufficiently accurate guide of how much to drink at the next. However, if resource quality changes rapidly i.e. between every meal then post-ingestive feedback on the food currently being drunk will not be available. In this case, animals must base their decisions on probably less accurate information such as taste, which may result in them making less appropriate foraging decisions.

The context in which I investigated what information hummingbirds were using to decide how much to drink was one in which the concentration remained reasonably stable. Most of the time, under such circumstances, basing how much to drink on roughly how much was drunk at the previous bout would be sufficient to ensure about the optimal volume of that concentration was consumed at every visit. However, where the concentration drunk was constantly changing between bouts as in risk-sensitivity experiments, how much was drunk at previous bouts and the post-ingestive feedback provided from those bouts would be a much poorer guide of how

much to drink at the next visit, increasing birds' reliance on current information such as taste.

It seems that current information was indeed used to decide how much to drink in the risk sensitivity experiment discussed in chapter 5. In that experiment hummingbirds drank a mixture of concentrations at each bout and altered how much they drank relative to differences in the mean sucrose concentration. In general, greater reliance on current information when the environment is less predictable is not a novel observation and has been shown in other species including bees and pigeons (Biernaskie et al. 2009; Shettleworth & Plowright 1992). But, considering this result in the context of risk-sensitivity may point to an important but previously un-explored explanation for animals' general preference for resources that provide more constant or less variable rewards. Consider an animal that prefers to base its decisions on its previous behaviour or, at the very least, post-ingestive feedback. Then consider how it might choose between a resource that allows it to forage in just such a manner (the constant option) or one that does not, i.e. a variable concentration or volume. It then seems possible that animals' preference for the constant option may stem from a general preference to forage from locations that require less reliance on current information to exploit that/those resource(s) efficiently. It is not clear why current information might be less preferred, but it may be because current information (e.g. taste for these hummingbirds) is more difficult to assess accurately (in energetic terms) than is post-ingestive information. More variable resources will also be less efficiently exploited on the basis of past behaviour and may increase costs, possibly in time or memory load, associated with assessing resources and in the decision-making process itself (Chittka et al. 2009). Thus, while the constant and

variable options appear to provide the same mean reward, there may be hidden costs that make the constant option more economical.

However, where the possible cost of errors in assessing the value of a variable resource relative to a constant resource is smaller and thus choosing between the two is difficult, then there is likely to be a trade off between information gathering and learning and exploitation (Chittka et al. 2009; Niv et al. 2002). Knowledge of resources and learning speed can directly affect foraging performance (e.g. Inglis 2000; Raine & Chittka 2008) and, as the adaptive significance of learning is increasingly being considered in many models of animal foraging, the apparent ignoring of learning as important to risk-sensitive foraging behaviour seems surprising (Eliassen et al. 2009). The potential value of learning from exploration of the variable option in risk-sensitive foraging situations may lead to animals preferring or at least investing some effort in foraging from the variable resource in order to learn about it perhaps in the hope of learning to predict the occurrence of above mean rewards.

Some support for this notion comes from the evidence that hummingbirds were attempting to learn about the low variance option in the risk sensitivity experiment discussed in chapter 5. When choosing between a constant reward and a low variability reward but not between a constant and high variability reward, birds increased their foraging on the variable reward as would be expected if they were trying to learn about it. Assessing the variance in the low variability reward was probably not associated with much cost. Indeed, unlike the higher variability treatment in the same experiment, the birds did not change the amount they drank depending on the mean concentration they had drunk. This means that they could

have been drinking a fairly standard amount based on past behaviour rather than using on current information. Additionally, bout lengths decreased with increasing experience in the highly variable option but not in the low variability option where they were slightly lower. This suggests at least some time cost to foraging on the more variable options. Visiting more constant wells under such conditions would have reduced both the time requisite to compare the constant and variable options and birds' reliance on current information.

In addition to being more risk averse when the variable option is more variable, animals are also more risk averse when they are less hungry. This has typically been explained using the energy-budget rule (Stephens 1981). The energy-budget rule and modifications of it are based on minimising the random chance of falling below various energetic thresholds such as survival or reproduction (McNamara et al. 1991; Stephens 1981). This means that as an animal's energy level changes it should switch between risk proneness and risk aversion as it nears and crosses these boundaries (Kacelnik & Bateson 1996; McNamara et al. 1991). However, there is a general trend for animals on lower energy budgets simply to be less risk averse than those on higher energy budgets (Kacelnik & Bateson 1996). It seems possible that tradeoffs between at least time and information gathering may help explain this. Risk-sensitivity is not the only context in which energy-budgets affect choice between different food rewards. In the context of contrafreeloading animals choose to work for food from a hidden resource in order to learn about it rather than from a freely available alternative (Inglis & Ferguson 1986; Talling et al. 2002). Under such conditions animals prefer constant or free rewards when hungry but variable rewards when satiated (Inglis & Ferguson 1986; Talling et al. 2002).

This is the opposite trend to that observed in risk-sensitivity experiments where birds forage more from variable resources when hungrier. However, in contrafreeloading the costs of foraging from the hidden resource rather than the free one may be substantial compared to the benefits of learning about it. Whereas, in risk-sensitive foraging situations the costs of foraging from the variable resource may be comparatively small compared to the benefits and animals could gain were it able to learn to predict the pattern of rewards. Being able to predict patterns of rewards in the variable options and achieve above mean intake would be more beneficial when an animal is hungrier, therefore we would expect animals to forage more from the variable rewards when their energy budgets are lower even if this incurs some slight cost in time or energy.

It seems possible that animals will always invest some effort in learning about resources regardless of their predictability so long as there is no great cost to doing so. The predictability of patterns of variation in natural foraging situations may vary. In some situations, similarly to risk-sensitivity experiments, patterns may not be predictable. Indeed, it is very unclear at what level of resource unpredictability an animal would be expected to decide it is not worth trying to predict. There is some evidence that animals respond to predictable and unpredictable variation similarly although not necessarily identically (Bateson & Kacelnik 1997; Talling et al. 2002). At least in the case of hummingbirds it seems probable that there are some predictable patterns of variation under natural foraging conditions that they could benefit from learning about such that information gathering would be expected to be an integral part of their foraging behaviour. For example, within a plant species there is variation among plants in the nectar rewards they produce and variation among

flowers on the same plant, some of this variation is predictable (McDade & Weeks 2004). A plant that supplies above mean rewards in its flowers or more flowers one day will probably do so the next day and flowers that are emptied predictably produce more nectar than other flowers (McDade & Weeks 2004; Ordano & Ornelas 2004; Ornelas & Lara 2009; Pleasants 1983). Furthermore, flowers known to have had longer to accumulate nectar are likely to hold more nectar than those which have been emptied more recently (Wolf & Hainsworth 1986). Thus, where resource patterns are highly predictable animals would be expected to exploit this predictability and to forage from the most profitable sources only, however, where predicting the occurrence of good patches is harder animals would be expected to continue sampling variable patches as well as more constant ones.

The ease of determining variation in a resource will depend on animals' perceptual and learning abilities. For example, if variation is spatial and an animal has good spatial memory then it should be able to learn the locations of above mean rewards and then forage predominantly from those. However, if discriminating among the good and bad rewards is harder then the animal will need to sample more to decide which are the best rewards. Thus, predictable variation in the sorts of information that animals find easy to discriminate between and learn about will result in more exploitation of good rewards and less sampling of poor rewards than if discrimination and learning are hard. It seems probable that concentration is easier to learn about than volume and that timing is even harder to learn about. If this is the case, then the similar response of starlings to predictable and unpredictable variation in delay to rewards is unsurprising as learning to predict such timing patterns may be very hard (Bateson & Kacelnik 1997). Indeed, while hummingbirds readily learn the

spatial location of higher concentrations in a flower array, their ability to remember the refill rates of flowers seems much less accurate (Healy & Hurly 1998; Henderson et al. 2006).

In none of the experiments in this thesis did I address risk-sensitivity where variation is in the delay to reward. Thus, predicting how information gathering might affect risk-sensitive choice in this context is purely speculative. If animals do indeed become increasingly risk prone as discrimination among rewards becomes harder, and assuming discrimination among time periods is harder than discrimination among amounts, I would predict risk proneness as observed in the majority of studies where variance is in delay to reward. There is possibly some conflict here with the idea that animals prefer to rely less on current information than on past behaviour when making decisions. An animal foraging from a resource of constant quality that always eats the same amount is likely to digest that amount and to feed again at fairly regular intervals. While a resource with constant delays would allow this if the delays between resource renewal matched the animals desired return rate, it would not if the renewal rate did not match the desired return rate. Under conditions of such a mismatch, it is hard to predict if animals should alter their meal sizes to match the renewal rate of resource or look elsewhere. If the animal could learn the timing of reward, this would allow the animal to establish a regular feeding pattern such that they fed at the most profitable places at appropriate times. Not only do at least some animals (e.g. the hummingbirds I tested) appear capable of doing this, it is probably one of the advantages of behaviours such as defence of feeding territories and of traplining (repeated sequential visits to a series of feeding locations), where knowledge of resources allows individuals to profit more from them than

opportunists who are less familiar with the area (Henderson et al. 2006; Ohashi & Thomson 2009). Traplining has been observed in many species including bumblebees (Comba 1999), hummingbirds (Garrison & Gass 1999), pied wagtails (Davies & Houston 1981), primates (Janson 1998), and bats (Lemke 1984), so the ability to use both resource quality and timing information may be widespread.

The most obvious reason why an unpredictable renewal rates may be encountered under natural conditions is because competitors exploit resources between a forager's visits. This would lead to further tradeoffs between competing for rapidly renewing resources and exploitation of more slowly renewing ones visited less frequently by competitors. Indeed, having to wait before being able to choose an alternative resource where resources that renew at constant or variable rates are present, as animals must do in risk-sensitivity experiments, seems quite unlikely in nature. Associative learning may also play a role as shorter delays in the variable reward are likely to be disproportionately strongly associated with reward, so that the strength with which a delay is associated with reward asymptotes as the delay increases. Thus, the average associative strengths of the delays in the variable option will be higher than that of the constant option and it will be preferred (Kacelnik & Bateson 1996).

Past experience and risk-sensitivity

In the risk experiment described in chapter 4 I discussed the choices hummingbirds made between a constant resource and a resource that varied in the amount of reward where the concentration of the food had either varied or been more constant over time. I found that both the concentration of sucrose birds had fed from

for two days before participating in the risk-sensitivity tests and the concentration used in the tests both affected birds' choices between the constant and variable options. Birds were most risk averse when the past and test concentration were high and least risk averse when both the past and test concentrations were low. When past and test concentrations differed the birds' preference fell between the two extremes. There were two possible explanations for this, which I could not distinguish with that experiment. Firstly the energy-budget rule: as the concentration birds had experienced before the test concentration could have contributed to differences in their energy-budgets, differences in energy-budgets may explain those results. The other possibility was what I termed the contrast effect. Here I expected that a bird's expectation about the concentration of the test sucrose would be affected by the concentration on which they had fed in the past, in a similar manner as decisions about how much to drink appeared to be in the experiment described in chapter 3. Given that the stability of the past can significantly affect animals' choices (Shettleworth & Plowright 1992), it would be interesting to see how these birds' choices would be affected by rapid changes in test concentrations (e.g. every three bouts). By controlling the length of resource stability between changes in concentration such that on average energy-budgets across the day were equal it may be possible to determine whether past experience alone affects risk-sensitive decisions. In this case longer periods of past stability would be associated with choices that lie somewhere between those associated with the past and current concentrations, whereas, where resource stability is shorter I would expect choices to more closely reflect those associated with the current concentrations.

If risk-sensitive choices do, indeed, depend on an animal's past experience, independently of their energy budgets, then we might expect difference in behaviour between territorial individuals that have predictable access to food to show different levels of risk-sensitivity to intruders whose access to food is unpredictable. While territory owners have the luxury of being able to establish fairly stable foraging patterns intruders must be much more opportunistic and must rely more on current information than the territory holder (Sandlin 2000; Tiebout 1996). Indeed, subordinate individuals tend to explore more than dominant individuals and typically have lower energy reserves (Sandlin 2000; Stahl et al. 2001). In the case of hummingbirds, intruders, unlike territorial males, will fill their crops suggesting that under situations of highly unpredictable food availability there is little advantage of trying to develop stable foraging patterns based on past experience and post-ingestive feedback. Animals on lower energy budgets like intruders, which could profit greatly from learning to predict or to find the best rewards and that are unable to benefit from resources allowing the establishment of a stable foraging pattern, should be more risk prone than are territory holders. This would be particularly likely to be the case if territory holders defend their preferred constant resources more heavily than less preferred variable resources. However it is possible that the provision of supplementary food by increasing the predictability of intruders' access to food could make them less risk prone.

Resource defence

It appears that expectation may also affect hummingbirds' resource defence behaviour. When I altered the concentration of sucrose in birds' feeders they reduced

the distance they perched from the feeder before there was any change in the number of intruders. They remained perching closer to their feeder until the feeder concentration was reduced and the intrusion rate decreased. This suggests they may have long standing expectations about how competition will vary depending on the relative quality of the resource they are defending. Many animals have been shown to increase their defence of higher quality food resources due to associated increased in intrusion pressure for example by reducing territory sizes (e.g. Camfield 2006; Chapman & Kramer 1996; Eberhard & Ewald 1994). However, as far as I am aware this is the first evidence that birds may change their defence of a food resource in response only to its quality rather than any change in intruder pressure. However, it is very unclear how common this may be. A similar experiment using Anna's hummingbirds found no affect of food quality alone on territory size, such that territory size was only adjusted in response to changes in intrusion pressure (Eberhard & Ewald 1994).

Foraging around artificial feeders

Where intrusion pressure is very high, defence of food resources can become uneconomical and territoriality can break down (Carpenter 1987). The provision of artificial feeders to rufous hummingbirds under some circumstances appears to prevent affective defence of those feeders (chapters 7 & 8). However, it does not appear to cause a breakdown in territoriality as males continue to defend areas territories immediately adjacent to such feeding sites. This is probably because territories of male rufous hummingbirds in the breeding season are predominantly display territories rather than feeding territories. Such territorial systems have been

referred to as exploded lekking systems and have also been described for Calliope and Anna's hummingbirds (Armstrong 1987; Powers 1987). Indeed, Anna's hummingbirds will continue to defend territories even after all food resources have been removed from them (Powers 1987). The benefit of these territories is believed to be two-fold: Firstly, they provide an area where males can perform display flights relatively undisturbed and, secondly, they allow males to have almost exclusive access to any females that enter the territory (Armstrong 1987; Powers 1987). Holding a territory, close to a feeding site that cannot be defended but is visited by many females, may then be more beneficial than holding a territory with easily defended resources elsewhere as fewer females pass through it. Competition for such territories may explain the generally smaller size of territories close to provisioning sites visited by many rather than a few females.

It remains unclear what determines how many birds will use a feeder. I found no evidence that birds in British Columbia, where many birds often use feeders, were any less aggressive than were the birds in Alberta, where feeders are usually defended by a single male. The difference in feeder use between these two regions is probably due to a higher population density in British Columbia. However, within British Columbia, the difference in the number of hummingbirds visiting provisioning sites initially appeared to be greater at sites providing more feeders. This was unrelated to general location as a single feeder on side of a house could be almost exclusively defended by a single male while an array of feeders the far side of the house might be visited by many birds each minute. It, therefore, seemed probable that arrays of multiple feeders might be harder to defend, making them easier targets for intruding birds. Indeed, more dispersed resources have been found to be harder to

defend than more clumped ones in a number of species (Johnson et al. 2004; Kim & Grant 2007; Vahl et al. 2005). The more intruders that then chose to visit the array, the more difficult defence of the feeder would become giving intruders the advantage of numbers as well as a more distributed resource such that they could move between feeders if disturbed (e.g. Chapman & Kramer 1996).

However, when I attempted to test this hypothesis a year later (chapter 8), I found that the addition of feeders to small arrays of one or two feeders did not change the number of birds visiting sites. It is possible that provisioning location features such as cover may explain differences in the number of birds visiting arrays of feeders. However, this seems unlikely as the position of busy feeding sites ranged from very sheltered positions such as between a building and vegetation covered bank to a balcony on a building in the middle of a field. One feature shared by all of these feeding sites was that they had been used by birds for many years, some for more than thirty years. One possibility is that birds return to provisioning sites they used the previous year in the spring and if nothing has changed that they then continue to use those sites for the rest of the season such that alterations of the distribution of feeders at the site is unlikely to affect how many birds choose to forage there. In this case, setting up new feeding stations with different resource distributions before birds arrive may give a better indication of whether resource distribution play any role in determining the number of birds visiting sites. Adding more feeders to arrays did, however, significantly reduce the number of birds visiting each feeder and allowed more birds to finish feeding undisturbed. Improving our understanding of what affects animals use of provisioning sites and how aggression and over-crowding at such sites can be limited, may greatly improve our ability to

plan effective supplementary feeding programmes, especially those used to improve the survival of endangered species (e.g. Meretsky & Mannan 1999).

Dispersal, population trends and population structure

How dependant hummingbirds are on artificial feeders is not clear. It has been suggested that such provisioning sites increase local populations above natural levels and that this may contribute to population declines in some areas (Healy & Calder 2006). Supplementary food can certainly improve reproductive success (Reynolds et al. 2006; Robb et al. 2008). In addition, there is some evidence that where supplemental food provision is common, such as in urban environments, population densities can increase to the level where defence of food is impossible and all individuals get less than they require to maintain a healthy condition (Shochat 2004). However, Australian magpies (*Gymnorhina tibicen*) and rusty-margined guan (*Penelope superciliaris*) living in urban environments choose natural food at least 76% of the time and are not dependent on the ready supply of supplementary food (O' Leary & Jones 2006; Ottoni et al. 2009). It is possible that supplementary food is only critical to birds' survival and reproduction during particularly lean times such as during summer snowfalls (Robb et al. 2008). However, if such rare events would normally result in high mortality the provision of supplementary food might prevent this and be enough to raise the population beyond its natural level.

It seems unlikely that, in the case of rufous hummingbirds, feeders are responsible for population changes, as data from the breeding birds survey data show population changes over the past thirty years, although negative in some areas have been positive in others despite the increasing popularity of hummingbird feeding

across North America (Sauer et al. 2008). It, therefore, seems more likely that population changes are due to large-scale environmental changes such as habitat fragmentation. Indeed, the abundance of rufous hummingbirds is greater in larger areas of old growth forest but is much lower in more fragmented forest areas (Lehmkuhl et al. 1991). Whether or not populations in some areas could become extinct or act as sink populations depends on the species dispersal patterns.

A species with fairly long dispersal distances may be more robust to habitat fragmentation than a species with shorter dispersal distances as dispersing individuals are more likely to find somewhere suitable to breed (Paradis et al. 1998). My analysis of banding data revealed fairly low site fidelity, possibly due to data being collected at small single sites rather than from a larger area such that the majority of individuals that moved only short distances were never recaptured (Newton 2008). I found that some birds move 10s to 100s of km within and between breeding seasons, suggesting that birds may disperse considerable distances, which would lead to a panmictic population structure and reduce the independence of population dynamics in different parts of the range. However, there was no banding evidence of birds actually breeding in more than one location or of juveniles dispersing to other areas to breed, at least one of which would be necessary for gene flow to occur.

Microsatellite data however, suggest that some gene flow is occurring across quite large distances (chapter 10). This gene flow is sufficient that population differences were only just detectable between areas as distantly separated Vancouver Island and Alberta (over 1000 km). There was no detectable difference between populations breeding on either side of Vancouver Island (approx 40km separation).

Although there were some problems with the genotyping data due to poor amplification of DNA and the presence of null alleles I do not believe these were sufficient to change the general pattern of population structure detected. From the markers tested during this work there are now around 27 that are potentially useful for genotyping rufous. This gives a wide choice of markers that could be used to expand my study to get a more accurate and detailed understanding of the species' population structure. For example, looking at male and female genotypes separately to identify whether male and female dispersal patterns are different. Future genetic studies should sample several breast feathers rather than a single tail feather to increase the amount of DNA available for amplification. Using a multi-tubes approach, although more expensive and time consuming, would help to reduce genotyping errors (Navidi et al. 1992; Taberlet et al. 1996).

Conclusions

In conclusion, the combined results of my experiments investigating how hummingbirds adjust their behaviour to changes in resource quality over time and how such responses might affect risk-sensitive foraging, show that animals seem to prefer to make foraging decisions based on past behaviour/information or on post-ingestive feedback rather than on current information such as taste. I suggest that this is because this requires less decision-making time and is less error prone. When an animal forages from a variable resource it must base its decisions more heavily on current information and may not be able to regulate its intake and foraging efficiency as accurately as when post-ingestive feedback is used. This could make foraging from a constant alternative with the same mean preferable. However, when

variability is sufficiently low that the costs of foraging from a variable resource are negligible then animals may feed as readily from the variable reward as from a constant reward and may even feed more from the variable reward.

Unexpectedly, I did not find any effect of adding more feeders to supplementary feeding sites on the number of individuals visiting. However, I still feel it is probable that resource distribution plays a role but that this may be hard to detect after birds have established their favoured feeding sites in the spring. It seems unlikely that such feeding sites are largely responsible for population changes. However, as there seems to be enough dispersal within the population to maintain a fairly panmictic population structure the effects of population changes in one area are likely to affect the population as a whole.

I recommend furthering the investigations of a possible role for information gathering in explaining risk-sensitive behaviour. I also believe that given the very limited knowledge of this species' dispersal patterns that extending the work on population structure could greatly enhance our understanding of their ecology.

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