

HUMMINGBIRDS AT ARTIFICIAL FLOWERS MADE TO RESEMBLE ORNITHOPHILES VERSUS MELITTOPHILES

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Abstract—Certain floral characteristics are associated with specific pollinators. Hummingbird-pollinated flowers are usually red, lack a landing platform, lack nectar guides, and contain a high amount of dilute sucrose-rich nectar. Here we test hypotheses concerning the reasons for these characters to the extent that they involve hummingbird responses. An array was set up of 16 artificial plants, each with five artificial flowers. (1) Flowers made to differ only in colour elicited a slight preference for red. (2) When colour was associated with nectar offerings, and birds generally learned to visit flowers that provided much more nectar but did not associatively learn differences as little as 2 μL . (3) Birds were offered 8 μL of 12% sucrose versus 2 μL of 48% hexose, and they did *not* prefer the dilute nectar; they showed no evidence of discerning sucrose from hexose; however, they preferred 48% over 12% sucrose when both were offered in the same quantity. (4) Birds preferred flowers that lacked landing platforms over those with landing platforms. (5) Birds were offered flowers with nectar guides, associated with differing nectar volumes, and they did not associate the higher nectar reward with either flower type. In summary, the feedback from hummingbirds reflects some of the differences between bird- and bee-adapted flowers, but nectar seemed less predictive than expected. Factors other than the behavioural proclivities of hummingbirds, such as adaptation to discourage bees, are discussed as additional causes for the differences between the syndromes. We also discuss significance testing for field experiments involving one unreplicated array.

Keywords: *hummingbird, landing platform, nectar, nectar guide, pollination syndrome*

INTRODUCTION

Flowers adapted to birds are ornithophiles. Flowers adapted to bees are melittophiles. A suite of characters is associated with ornithophily as compared to melittophily. Most are red, often without much colour patterning, have high quantities of diluted sucrose-rich nectar, lack landing platforms, have long narrow floral tubes, and have outwardly exerted anthers and stigmas (Faegri & van der Pijl 1979; Mitchell & Paton 1990; Thomson et al. 2000). A number of factors might be responsible for the syndrome differences, namely, pollinator efficiency, floral costs, the deterrence of less beneficial visitors, and the behavioural proclivities of pollinators. Here we address hypotheses that involve the behavioural proclivities of hummingbirds visiting ornithophilous versus melittophilous artificial flowers.

In western North America there is a guild of ornithophiles that have reddish flowers (Grant & Grant 1968; Brown & Kodric-Brown 1979; Bleiweiss 2001). Using floral colour and colour patterns, pollinators are cued into nectar rewards, and colouration may also help pollinators extract nectar more quickly (Baker 1961). Though ornithophiles are usually red, hummingbirds visit not only red flowers. They can be conditioned to visit flowers of other colours, and when other colours are associated with nectar more to their liking, they show little

long-term preference for red (Bene 1941; Collias & Collias 1968; Stiles 1976). An alternative explanation to the view that hummingbirds simply prefer red has been well articulated by Rodríguez-Gironés and Santamaría (2004) who suggest that bees are less likely to visit red flowers because they lack red colour receptors that would allow them to easily distinguish between red flowers and green foliage. If birds are more efficient pollinators than bees, then flowers adapted to birds should have characters that deter pollen-wasteful bees from emptying the nectaries and scattering the pollen. Under this theory, birds are constantly finding that there is more nectar in red flowers, which have been under-visited by bees, so they come to expect the rule to hold (Raven 1972).

In ornithophiles nectar is the only floral reward, and it is often hidden deep in the flower. Hummingbirds rely on cues, such as colour, to locate which flowers are rewarding and which are not (Hurly & Healy 1996). If ornithophiles evolved partly or solely as a result of hummingbird preferences, then we would expect that hummingbirds should prefer to visit artificial flowers that mimic the nectar properties of ornithophiles as opposed to melittophiles. For example, the ornithophile *Ipomopsis aggregata* produces between 1 and 5 μL of 20-25% sucrose-rich nectar (Pleasants 1983; Irwin & Brody 2000). Ornithophilous penstemons, on average, offer 7.76 μL of 26% sucrose-rich nectar (Wilson et al. 2006). Aside from secreting copious nectar, the other properties of ornithophilous nectar have been explained as reducing viscosity (Baker 1975, cf. Roberts 1995, 1996). The thinking is that birds prefer less

Received 15 January 2012, accepted 23 April 2012

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viscous nectar because they can extract more energy per unit time. Although we expect birds to prefer ornithophilous nectar over melittophilous nectar, ornithophiles may not secrete what birds prefer. Nectar production can be costly for flowers. *Blandfordia nobilis* plants have been found to expend as much as 37% of their energy on nectar production (Pyke 1991), and nectar can be a substantial water expense (Baker 1975; Pyke & Waser 1981).

Ornithophiles tend to lack landing platforms compared with melittophiles (Faegri & van der Pijl 1979). It has been suggested that landing platforms would obstruct hummingbirds from extracting nectar quickly, and would make pollen placement less precise (Castellanos et al. 2004). In spite of the rule, some ornithophiles retain a lower lip, and Smith et al. (1996) present experimental evidence that it reduces the number of errors hummingbirds make in attempting to probe flowers. In other words, loss of what was a landing platform in a melittophilous ancestor should not be considered uniformly adaptive for ornithophiles.

The last feature we consider is spotting on a flower that is presumed to function as a "nectar guide" in melittophiles. Such patterns are often lost when a lineage shifts to ornithophily. For example, there is no patterning in the ornithophilous *Delphinium cardinalis*, in contrast to the many species of melittophilous *Delphinium*. Studying melittophilous *D. nuttallianum*, Waser and Price (1985) showed that patterning represents a nectar guide that speeds up handling time by bees. Medel et al. (2003) found disruptive selection on nectar guides in *Mimulus luteus* in which bees selected for larger nectar guides that point toward the mouth of the floral tube while hummingbirds selected for smaller heart-shaped nectar guides. Perhaps birds are less sensitive than bees to details that are seen only after the animals are very close to the flower (Gould & Gould 1988; Giurfa & Lehrer 2001).

MATERIALS AND METHODS

General experimental set-up

An array of artificial flowers was set up during the years 2008 and 2009 at a site in the southern Sierra Nevada of California, near the unincorporated town of Kennedy Meadows (36° N, 118° W). Visiting the array were hummingbirds of several species, sexes, and age classes: male and female *Archilochus alexandri*, male and female *Calypte anna*, and female *Selasphorus rufus*. Species and sex were recorded, but analyses were done pooling together all birds. As detailed in Haley (2010), the array consisted of 16 artificial plants spaced 1 m from one to the next. Each plant was a stalk with five artificial flowers spaced at intervals of 7.62 cm. Petals were made of brightly coloured ribbon fabric, and artificial nectar was pipetted into plastic nectar spurs made of 200 µL PCR tubes. The 16 plants typically differed by treatment. For example, on certain experimental days half of them were red with 2 µL of nectar and the other half were purple with 4 µL of nectar, while on other experimental days half were purple with 2 µL and the other half red with 4 µL. At the start of each hour of an experimental day, we replaced artificial plants with fresh

models that had just been filled with nectar. Within experimental hours, each observation period was 40 minutes long. During that period hummingbird visits were followed and video taped. Generally, there were six 40-minute observation periods during a day (though on one stormy day, we only observed for five periods).

We scored four dependent variables (Wilson & Jordan 2009), which are reported on fully in Haley (2010): the kind of plant upon which a bird *initiated* a bout of foraging at the array; the number of *plants visited* of the two kinds of plants offered; the *number of probes* at each plant of the two types; and the *time per probe* at each plant. (Time per probe, or "handling time," is not expected to be minimized since birds with longer handling times were often extracting more calories, i.e., it is not "handling efficiency." In general, we are not trying to measure the physiological costs and benefits to the bird on a per visit basis, merely the feedback that the pollination environment gives in response to floral offerings.) Here we condense our treatment to just *plants visited* and *time per probe*. In general, *bout initiations* gave similar results to *plants visited*, and in general *number of probes* gave similar results to *time per probe* (Haley 2010). That *bout initiations* were similar to *plants visited* indicates that birds are able to adjust their behaviour not only within a bout but also between bouts. That *number of probes* was similar to *time per probe* was less expected since *number of probes* is an inverse part of *time per probe*, so the similarity in the responses of these two variables must mean that the duration of visits counteracted the inverse part-whole relationship.

Experiment 1: colour preference

Red is associated with ornithophily, whereas melittophilous flowers are often purple (or yellow: Haley 2010). To get started, we set up the 4 × 4 array with half of the plants having red flowers, and the other half having purple flowers. Equal amounts of nectar (2.5 µL of 23% sucrose solution) were pipetted into each of the artificial flowers at the beginning of each hour of study. Flower type was alternated within the array, with the first row being red-purple-red-purple, the second row being purple-red-purple-red, etc. On the next experimental day, the colour of alternating plants was switched. This experiment was run for six days.

Experiment 2: nectar volume

A second set of experiments was done in order to test preference between different amounts of 23% sucrose solution associated with different colours. Nectar of different amounts (0 versus 2 µL; 0 versus 4; 0 versus 6; 2 versus 4; 2 versus 6; 4 versus 6; 2 versus 8) was placed into differently coloured flowers. Birds were expected to use floral colour as a cue to find nectar when the difference in amount was large enough. On half of the days, red flowers contained one nectar volume and purple flowers contained the other nectar volume, and on the other half of the days the colour of the cue was reversed. Each nectar volume combination was set up for a span of four experimental days. Positions of the different treatments were rotated on different days. The various sub-experiments were done

consecutively with different combinations of nectar amounts contrasted.

Experiment 3: effects of nectar composition

Three sub-experiments were done in order to test bird preferences for or against nectar concentrations and the size of sugar molecules, both of which affect nectar viscosity. Three solutions were made: 12% sucrose weight to volume, 48% sucrose, and 48% hexose comprised of half glucose and half fructose. Each sub-experiment consisted of a difference in nectar. In Experiment 3A, we used nectars differing in volume *and* concentration *and* sugar molecule size to see if hummingbirds show a preference for a higher volume of lower concentrated sucrose nectar. We used 8 μ L of 12% sucrose versus 2 μ L of 48% hexose. The goal was to have two solutions offering equal amounts of energy but different amounts of water. This sub-experiment confounds the volume difference with the concentration difference and with the chemical differences in the same way that natural syndromes confound these three variables, and the multifaceted difference was deemed a priori the most likely to have an effect. In Experiment 3B, birds were offered equal amounts of the same concentration of differing sugar types: 5 μ L of 48% sucrose versus 48% hexose. In Experiment 3C, birds were given the choice between two solutions that only differed by concentration, and had equal volumes of the same sugar: 8 μ L of 12% sucrose versus 48% sucrose. Each of the three sub-experiments was replicated on four days, switching flower colours and positions in all combinations.

Experiment 4: preference against landing platforms

Landing platforms are generally present in melittophilous flowers and often absent in ornithophilous flowers. An array was set up so that half of the plants consisted of artificial flowers that had landing platforms (i.e., an elongated tube that was cut to resemble a flower with five petals), whereas the other half of the stakes consisted of artificial flowers without landing platforms (i.e., elongated tubes with the bottom two petals cut out: Haley 2010). Each artificial flower in the array contained 5 μ L of 25% sucrose solution. The artificial petals were always red. This experiment was run for four days in 2008 and two days in 2009. As usual, inflorescences were set up in an alternating spatial pattern, and positions were rotated each day.

Experiment 5: patterning on the petals

The purpose of the final experiment was to test if birds could use a flower cue other than the colour of the perianth as a whole to associate with great nectar volume. An array was set up where half of the plants had a pattern on their petals – the pattern was two black spots on the perianth – whereas the other half of the stakes had unmarked flowers (Haley 2010). This was an associative-learning experiment in which varying nectar reward was associated with presence or absence of pattern. All perianths were red. This experiment was replicated during four days in 2008 and four more days in 2009. Stakes were set up in an alternating pattern, where flowers contained 8 or 2 μ L. The flower

types and nectar amounts were rotated to ensure that flower types were in every position, solutions were in every position, and solutions were combined with each flower type in every position. Nectar in 2008 was 21% sucrose solution, and in 2009 was 23% sucrose solution (the difference being unintentional).

Statistics, with worries about replication

Our null hypothesis H_0 was generally that manipulations in flower characters would not elicit preferences by hummingbirds. There would be a 1:1 ratio for plant visits, and birds would spend the same amount of time at a plant per probe regardless of experimental treatment. Our expectations were as follows:

1. H_A based on the systematic differences between ornithophiles and melittophiles, along with past experimental results, we expected hummingbirds to visit red more than purple, and we did not have expectations for time per probe.

2. H_A we expected birds to learn to prefer any colour associated with a higher volume of nectar and to take more time to extract more nectar.

3. H_A when presented with high versus low concentrations of nectar, both containing the same amounts of sugar, we predicted birds would prefer flowers that contain higher volume (less concentrated) nectar. Flowers that have a higher volume of more dilute nectar were predicted to have a decreased time per probe because their nectar is less viscous (though perhaps this prediction is naïve since a higher volume would take more time to consume if concentration had been equal).

4. H_A birds were expected to prefer flowers that lack landing platforms and to have increased time per probe when visiting flowers with landing platforms.

5. H_0 for the final experiment on nectar guides, we predicted that the null hypothesis would be accepted. Because ornithophilous flowers often lack nectar guides compared to close relatives that are melittophilous, hummingbirds ought *not* to distinguish patterns on the perianth.

We summarize visits to plants as the percentage of visits that were to red inflorescences. This is a categorical variable that we analysed with G^2 goodness-of-fit tests to 1:1. Heterogeneity in goodness-of-fit was tested to see if days were similar enough to be pooled ($P > 0.25$), or if they had to be separated. When pooling was permitted, we report on the simplified analysis. Time per probe is a continuous variable, so we used ANOVA, and scrutiny of residuals compelled us to do analyses on $\log(\text{time per probe})$. Before doing the tests, we searched for outliers, and as noted in the Results, a few were winsorized to the next highest number. The ANOVAs often started out split-plot mixed-models because day was a random variable whereas treatments such as colourcue and nectartype were fixed variables. Days were nested within cues, and plants were replicates within nectartype. We calculated F -values as in Quinn and Keough (2002, p. 314). For these split-plot designs, the effect of

nectartype, which is the within-plot treatment, is the one of primary interest, and in the full model it would be tested over $\text{nectartype} \times \text{day} \subset \text{cue}$. When $P > 0.25$, terms were sequentially pooled. For example, if $P > 0.25$ for $\text{nectartype} \times \text{day} \subset \text{cue}$, then the effect of nectartype could be tested over the pooled residual with far more degrees of freedom. Cue, the among-plots treatment, was not of primary interest, and in the full model it would be tested over $\text{day} \subset \text{cue}$, which would be a weak test because there were few days. Pooling higher order terms when $P > 0.25$ often allowed for a much simpler and more powerful final model than the original split-plot design.

In using these significance tests, we are pretending as though each visit to an artificial plant composed of five flowers were a replicate representing a larger universe of such visits. However, by the very nature of these types of experiments (in which animals are acquiring information as they forage) a visit to a plant by a bird is not a unitary indicator-of-behaviour that is independent of the other visits to that array by that bird or by other birds that are visiting the array. Unfortunately, there was only one array. A dozen arrays each with different birds surrounded by naturally varying flowers would have allowed arrays to be used as replicates. Then a variate would have been the percent of visits to, say, sucrose-filled plants in an array or the average time per probe at sucrose-filled plants (Rodríguez et al. 2004). Such an effort was far beyond our means.

Many other pollination biologists have done array experiments using a level of replication below whole arrays. Schemske and Ågren (1995), Meléndez-Akerman et al. (1997), Wesselingh and Arnold (2000), Gómez et al. (2008) and Owen and Bradshaw (2011) all chose to do significance tests such as we did using the visit as a replicate. Carlson (2008), likewise, used visits as the unit of replication, although her experiments have higher level blocking factors (like our day effect), but she does not take the average response in a block as a variate. Another option would be to use an observation period as a replicate. Majetic et al. (2009) used 10-minute observation periods as replicates, and Hersch and Roy (2007) used 45-minute observation periods. The analog in our study would have been to use our 40-minute observation periods. This would not have eliminated pseudoreplication. Also presumably number of visits in 40 minutes would not have followed a normal distribution being a meristic variable with a low mean, which would have compelled using generalized (rather than merely general) linear modelling. Observation period is arbitrary in the sense that it is fixed by the scientist, so pollination biologists sometimes choose to use foraging bout as a replicate (Aldridge & Campbell 2007, Campbell et al. 2010), but foraging bouts are still pseudoreplicates of the same individual pollinator who is accumulating experience with the treatments. If bout were used, the outcome would then be reported as the mean of a sample of numbers like 1/1, 1/5, 2/3, 10/10 each based on counts with continuity problems and lack of normality. Also, a great deal of data would be gathered on a very few individuals while many individuals would be represented by only one or two bouts, so using bout would be a poor approximation of using

individual bird, which itself was nearly as far beyond our means as replicating experimental arrays.

The most sensible minimization of pseudoreplication that is feasible within the confines of using one array for only two years is to use each “plant” as a replicate (Schemske & Bradshaw 1999, Fenster et al. 2006). In addition to the analyses we report using visits as replicates, we also elaborate on selected results using the positions in the array during the course of a day as a replicate. This is akin to using plant except that we replaced the artificial plants each hour. Using positions as replicates, *visits to plants* becomes *number of visits to a position*, and *log(time per probe)* becomes *mean log(time per probe)* averaged across all visits observed in a day at that position in the array. There were $n = 8$ such replicates for each of two treatments during any one day. Position is less pseudoreplicated than visit, but it is still not equivalent to replicating the experiment. We cling to using visits as replicates in our primary analyses for several reasons. First, it seems the most natural unit of response by which birds interact with plants. A bird is more or less making decisions to visit one plant or another and to take time during its probes at a plant, whereas the number of visits during six 40-minute observations on a day or the average time per probe during those visits is a mere artificial aggregation; it's not fitness because the flowers are plastic, and it's not about how a representative bird treats a plant behaviourally. Second, we have a number of non-significant results for which it seems best to report the most powerful test, which is the test that uses visits as replicates, so only when there is a significant and interesting result at the lower level of replication do we feel compelled to check the significance at the level of positions within a day. Third, one would not expect the various “plants” that occupied a position during the course of a day to be particularly similar to one another. In fact, looking at the data there was generally no evidence of such an effect; for example, in the experiment with the most replication (Experiment 5), a nested ANOVA of $\log(\text{time}/\text{probe})$ yielded $P = 0.991$ for position nested within treatment-day combinations. Values of $P > 0.25$ are widely accepted as permission to pseudoreplicate (Quinn & Keough 2002).

To reiterate, then: tests at neither level allow for inferential statistics beyond the circumstances of our one array visited by a small number of birds with relatively scant access to naturally occurring flowers at our arid field site. Significance tests such as ours for experiments of an array merely provide a conventional cut-off for credibility that a pattern is non-random in that one context.

RESULTS

Experiment 1: colour preference

Birds had a slight preference for red. Plant visits: On days 1 and 2, birds significantly preferred red inflorescences ($\text{day}_1 = 69.6\%$ red; $\text{day}_2 = 69.0\%$; $P_{\text{day}_1} < 0.001$; $P_{\text{day}_2} = 0.012$), but on days 3 through 6, birds showed no significant preference for either flower colour ($\text{day}_3 = 61.9\%$; $\text{day}_4 = 52.3\%$; $\text{day}_5 = 55.6\%$; $\text{day}_6 = 59.3\%$;

TABLE 1. Nectar volume experiments.

		% visits to inflorescences (<i>n</i>)	nectar quantity ^a	log(handling time, s/probe)	nectar quantity ^b
2 vs 0 μ L	red with 2 vs purple with 0	70.4 vs 29.6 (206)	***	-0.135 vs -0.140	NS
	purple with 2 vs red with 0	58.2 vs 41.9 (184)	*	-0.168 vs -0.150	
4 vs 0 μ L	red with 4 vs purple with 0	63.8 vs 36.3 (323)	***	-0.079 vs -0.147	***
	purple with 4 vs red with 0	56.6 vs 43.4 (258)	*	-0.104 vs -0.149	
6 vs 0 μ L	red with 6 vs purple with 0	69.0 vs 31.1 (168)	***	-0.038 vs -0.185	***
	purple with 6 vs red with 0	64.7 vs 35.3 (269)	***	-0.091 vs -0.144	
4 vs 2 μ L	red with 4 vs purple with 2	50.4 vs 49.6 (500)	NS	-0.055 vs -0.102	***
	purple with 4 vs red with 2	51.7 vs 48.3 (208)	NS	-0.063 vs -0.101	
6 vs 2 μ L	red with 6 vs purple with 2	58.0 vs 42.0 (226)	*	-0.081 vs -0.150	NS
	purple with 6 vs red with 2	55.9 vs 44.1 (195)	†	-0.076 vs -0.105	
6 vs 4 μ L	red with 6 vs purple with 4	50.9 vs 49.1 (340)	NS	-0.037 vs -0.081	*
	purple with 6 vs red with 4	50.2 vs 49.8 (251)	NS	-0.057 vs -0.061	
8 vs 2 μ L	red with 8 vs purple with 2	63.1 vs 36.9 (425)	***	-0.078 vs -0.114	NS
	purple with 8 vs red with 2	55.5 vs 44.5 (290)	†	-0.111 vs -0.169	

NS $P > 0.1$; † $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

^agoodness-of-fit test to 1:1; on different days for each experiment, nectar quantity preference may have been complicated by colourcue preference; days were heterogeneous for colourcue and often could not be pooled

^bsplit-plot ANOVA; for 6 vs 4 μ L nectar quantity \times colourcue yielded $P < 0.05$; otherwise the interaction and colourcue yielded $P > 0$.

$G^2_{\text{heterogeneity}} = 6.89$, $df = 5$, $P = 0.229$). Pooling all days, red was preferred ($G^2_{\text{pooled}} = 19.29$, $df = 1$, $P < 0.001$). Replicating with position, colour was still significant ($P_{\text{colour}} = 0.013$). **Time per probe:** The interaction of colour \times day yielded $P = 0.161$ so could not be pooled. Generally, birds spent significantly more time at red than at purple flowers ($P_{\text{colour}} = 0.009$), and days varied in the amount of time birds spent per flower ($P_{\text{day}} < 0.001$). Replicating with position, colour \times day yielded $P = 0.010$, which meant that the colour effect had to be tested over the interaction, and it signalled no significant effect beyond the interaction ($P_{\text{colour}} = 0.073$). Further results with colour combinations involving yellow are given in Haley (2010).

Experiment 2: nectar volume

Zero versus some nectar: Table 1 shows that birds significantly preferred flowers with nectar over empty flowers. **Plant visits:** They visited inflorescences that contained nectar significantly more for each sub-experiment. **Time per probe:** Birds spent significantly more time at flowers that had 4 or 6 μ L versus 0 μ L, but there was no significant difference when the filled flowers had only 2 μ L.

Some versus more nectar: When nectar differed by a larger amount (≥ 4 μ L), birds chose the higher nectar volume (Table 1). **Plant visits:** They showed a significant preference for higher nectar volumes when the volumes differed by at least 4 μ L. In other cases, the difference was not significant. **Time per probe:** Birds spent significantly more time at the higher flower volume when the two volumes differed by only 2 μ L. There was no significant difference when flowers differed by ≥ 4 μ L, which was an unexpected result.

Experiment 3: effects of nectar composition

2 μ L 48% hexose versus 8 μ L 12% sucrose: A difference in viscosity elicited very little difference in bird behaviour (Fig. 1). **Plant visits:** There was not a consistent preference for plants with one of the nectar types over the other. If anything, there is simply a suggestion that birds prefer the flowers that are red regardless of nectar type. For the analysis with positions as replicates, day within colourcue interacted with nectar type ($P_{\text{nectartype} \times \text{day} \subset \text{colourcue}} = 0.003$), but simpler terms involving nectar type were not significant ($P < 0.05$). **Time per probe:** Using plant visits as replicates, birds seemed to take significantly more time at 2 μ L 48% hexose than at 8 μ L 12% sucrose ($P_{\text{nectartype}} = 0.002$). However, replicating with position, no term involving nectartype was significant ($P < 0.05$).

Sucrose versus hexose: Birds seemed unaffected by sugar type (Fig. 2). **Plant visits:** They showed no significant preference for sucrose versus hexose ($P > 0.05$ for each day). **Time per probe:** One datum was winsorized from 0.4293 log(seconds/flower) to 0.2975 log(seconds/flower). The interaction of nectartype \times day \subset colourcue yielded $P = 0.197$, so this term could not be pooled. Nectartype \times colourcue yielded $P = 0.685$. There was added variance in how much time was spent at sugar types on different days ($P_{\text{day} \subset \text{colourcue}} < 0.001$), but birds did not significantly spend more or less time depending on sugar type ($P_{\text{nectartype}} = 0.544$) or the colour of the cue ($P_{\text{colourcue}} = 0.984$). The analysis of position as the unit of replication did not change the outcomes of these tests.

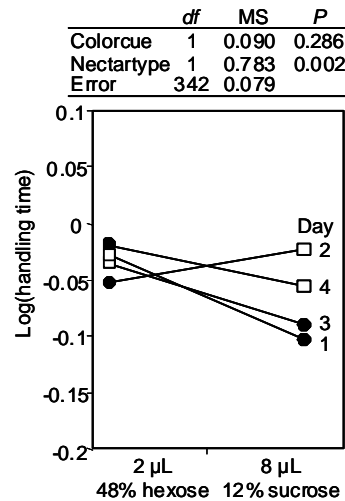
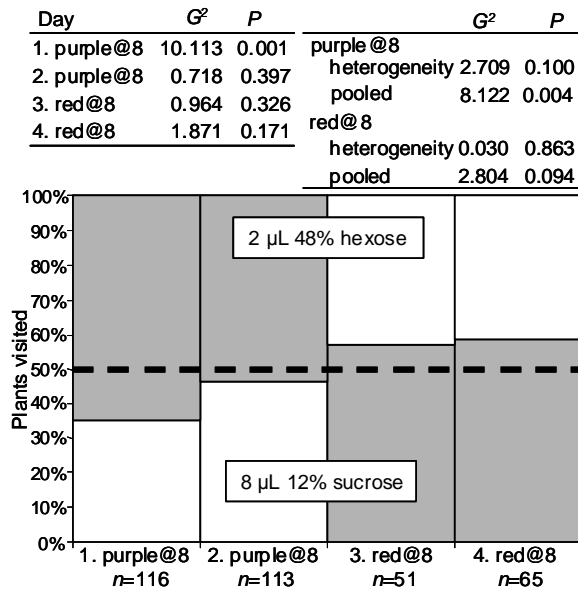


FIGURE 1. 2 μL 48% hexose versus 8 μL 12% sucrose. This experiment tested preferences for bee-versus bird-type nectar. (grey bars: red flowers; white bars: purple flowers; ● red flowers; □ purple flowers)

12% versus 48% sucrose: Birds generally preferred 48% over 12% sucrose (Fig. 3). **Plant visits:** Overall, birds significantly preferred 48% sucrose on two days, and showed marginal significance on one day. Replicating by position, there was a highly significant preference for the higher concentration ($P_{\text{nectartype}} < 0.001$). **Time per probe:** Birds spent significantly more time visiting more concentrated nectar ($P_{\text{nectartype}} < 0.001$). They also spent different amounts of time depending on the colour of the cue ($P_{\text{colourcue}} = 0.017$). Replicating with position, they still took significantly more time per probe ($P_{\text{nectartype}} < 0.001$).

Experiment 4: preference against landing platforms

As expected, birds preferred flowers lacking landing platforms (Fig. 4). **Plant visits:** Birds significantly visited inflorescences without landing platforms over those with landing platforms on five of the six days ($P < 0.05$), and on the non-significant day, the deviation was in the same direction. There was significant heterogeneity among days ($P = 0.020$), but the total G^2 for all days was highly significant ($P < 0.001$). This effect was significant when positions were used as replicates ($P_{\text{flower type}} = 0.002$, based on dividing $MS_{\text{flower type}}/MS_{\text{interaction}}$ in mixed model).

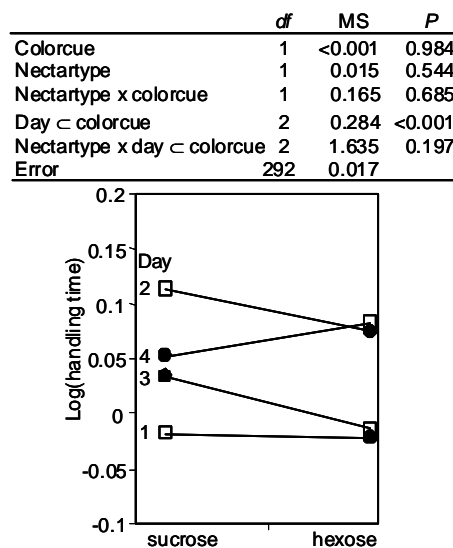
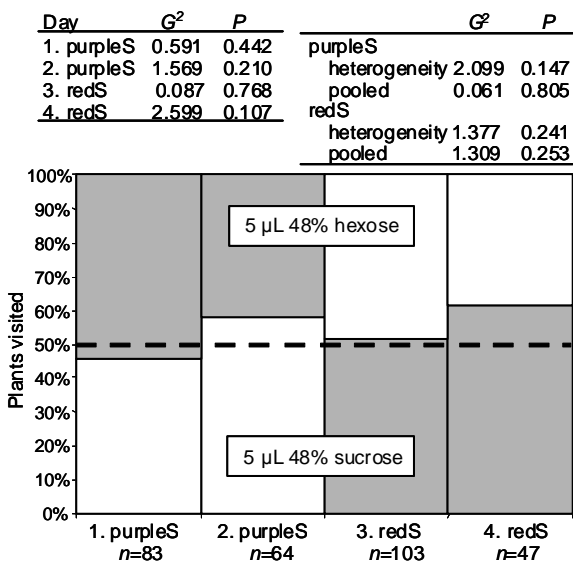


FIGURE 2. Sucrose versus hexose. This experiment tested preferences for sugar type. (grey bars: red flowers; white bars: purple flowers; ● red flowers; □ purple flowers)

Day	G ²	P	G ²	P
1. purple@48	3.221	0.073	purple@48	
2. purple@48	5.437	0.020	heterogeneity	0.213 0.644
3. red@48	1.950	0.163	pooled	8.445 0.004
4. red@48	9.942	0.002	red@48	
			heterogeneity	1.912 0.167
			pooled	9.979 0.002

	df	MS	P
Colorcue	1	0.111	0.017
Nectaratype	1	1.647	<0.001
Error	264	0.019	

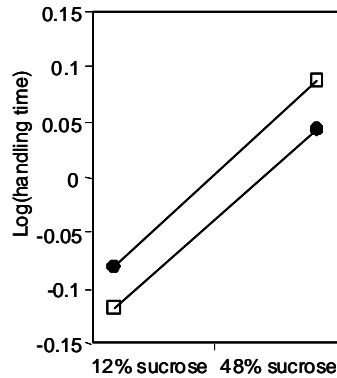
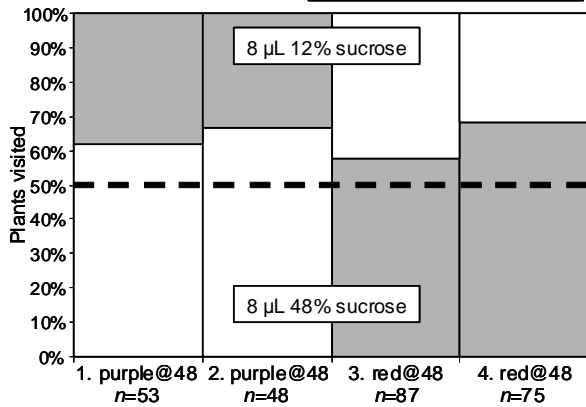


FIGURE 3 12% versus 48%. This experiment tested preferences for nectar concentrations. (grey bars: red flowers; white bars: purple flowers; ● red flowers; □ purple flowers)

Time per probe: Birds took significantly less time per probe visiting flowers lacking landing platforms than flowers with landing platforms ($P_{\text{flowertype}} < 0.001$). This was also highly significant when using position as a replicate ($P_{\text{flowertype}} < 0.001$).

Experiment 5: patterning on the petals

Birds did not use patterns as a cue to find the higher nectar source, although they definitely paid attention to higher nectar volumes once they were at an inflorescence (Fig. 5). Plant visits: They visited the two flower types

comparably, regardless of which volume was associated with patterning ($P_{\text{all days pooled}} = 0.933$). Using positions as replicates, nectaratype did not interact with the pattern of the cue ($P_{\text{nectaratype} \times \text{patterncue}} = 0.0765$), nor did nectaratype have an effect ($P_{\text{nectaratype}} = 0.977$ after pooling), and the effect of the pattern of the cue was not significant when testing over the highly significant day effect ($P_{\text{patterncue}} = 0.363$ after all other terms had been pooled). Time per probe: One datum was winsorized from 0.9841 to 0.3971 log(seconds/flower). Birds spent significantly more time at 8 µL than 2 µL, but the amount of time spent varied by day ($P_{\text{nectaratype} \times \text{day} \subset \text{patterncue}}$

	G2	P
2008 day 1	21.482	<0.001
2008 day 2	10.743	0.001
2008 day 3	6.180	0.013
2008 day 4	0.978	0.323
2009 day 5	18.536	<0.001
2009 day 6	12.172	<0.001
total	70.091	<0.001
pooled	56.760	<0.001
heterogeneity	13.331	0.020

	df	MS	P
Flowertype	1	2.501	<0.001
Error	604	0.021	

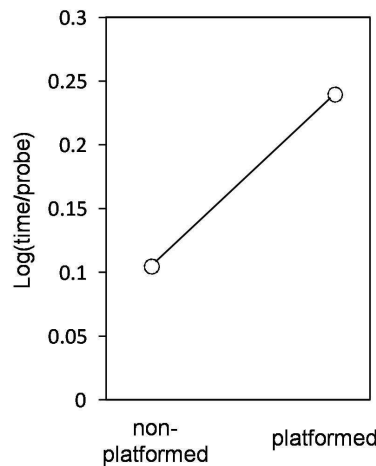
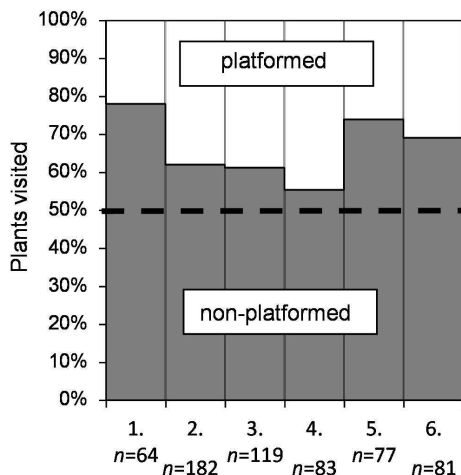


FIGURE 4. Platformed versus non-platformed. This experiment tested preferences against landing platforms. (○ pooled days)

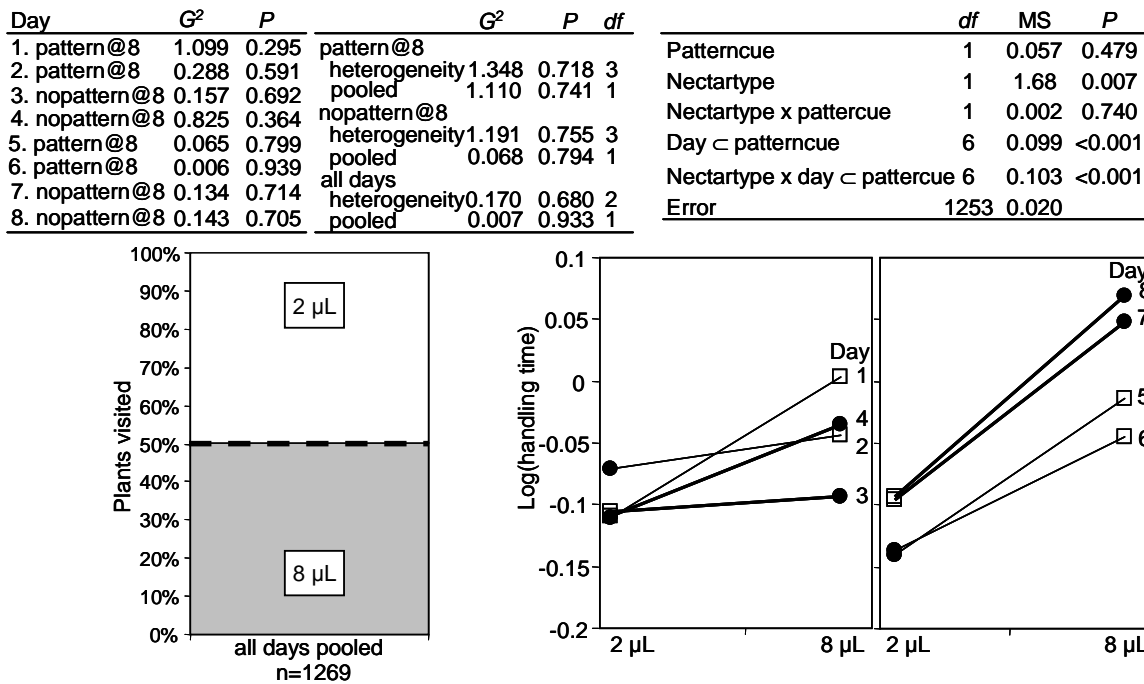


FIGURE 5. Patterned versus non-patterned flowers. This experiment tested whether hummingbirds would use pattern cues to find more rewarding nectar. (grey bar: non-patterned flowers; white bar: patterned flowers; ● non-patterned flowers; □ patterned flowers; — days that were patterned@ higher volume; - days that were non-patterned@ higher volume)

< 0.001). Furthermore, there was no interaction between nectartype and the pattern of the cue ($P_{\text{nectartype} \times \text{patterncue}} = 0.740$), though replicate days varied greatly by the amount of time spent at a pattern type ($P_{\text{day} \subset \text{patterncue}} < 0.001$). Birds spent more time at flowers with 8 μL over those with 2 μL ($P_{\text{nectartype}} = 0.007$), and they spent similar amounts of time regardless of the pattern of the cue ($P_{\text{patterncue}} = 0.479$). Using position days as replicates, significance tests were the same. Most notably, there was no significant interaction between nectar amount and patterncue ($P_{\text{nectartype} \times \text{patterncue}} = 0.848$) and there was a significant effect of nectar amount ($P_{\text{nectartype}} = 0.004$).

DISCUSSION

In our experiments hummingbirds sometimes showed a slight preference for red over purple. They definitely preferred flowers with nectar over nectarless flowers. When nectar volumes were 2 μL or more, birds were not consistently discerning between nectar volumes in terms of visits to inflorescences. The effects seemed more consistent for time spent per probe. When nectar was energetically equivalent, birds generally visited the two types of flowers similarly with a slight bias for red cues. Birds visited flowers with sucrose and hexose in equal frequencies when the two sugar types were offered in equal amounts and concentrations. They generally preferred 48% sugar over 12% sugar. Birds preferred flowers without landing platforms, and took less time probing flowers without landing platforms. Regardless of the presence or absence of spots on the flowers, birds visited both types of flowers equally, evidently failing to associate the spots with the difference in nectar volume. The constitution of these

specific hummingbird-flower characteristics (flower colour, a higher nectar volume, and the lack of landing platforms) seems to be, in part but only in part, due to the behavioural proclivities of hummingbirds for the flowers with ornithophilous features. The main inconsistency with simple predictions stemming from the recognition of pollination syndromes is that birds visiting our array did not always prefer flowers with nectar at the extreme of hummingbird-adapted flowers.

How did the traits associated with ornithophily evolve? Floral characters might be adaptations to principle pollinators, either because their pollinators have specific behavioural preferences such as we studied or because of the mechanics of pollen transfer (Wilson 1995). Alternatively, ornithophiles might be adapted to discourage visits by bees that take rewards while transferring less pollen (Castellanos et al. 2004). And there are many environmental factors other than visitor preferences that affect floral evolution, such as herbivory (Kang et al. 2008; Gomez et al. 2009), temperature stress (Tyrrell & Tyrrell 1985), and length of reproductive season (Elliott & Irwin 2009).

Comments on ornithophilous features one at a time

Colour. Floral colour is a cue, or advertisement, that pollinators use to find rewards (Waser 1983). The behavioural propensity for red was evident, but after a short period of time birds were able to recognize purple flowers as rewarding and visit them in nearly equal frequencies. Using Stiles' (1976) terminology, there appears to be a weak holdover period for colour preference. The remaining curiosity is that birds spent more time per probe at red flowers than equally rewarding purple flowers of similar

construction. This was also true of red compared to yellow flowers (Haley 2010).

Nectar quantity. Hummingbirds will under-visit flowers that in the past they have found to be empty (Hurly & Healy 1996). Our birds preferred flowers with nectar over those without nectar, but any threshold below which birds disdain flowers of a certain appearance would seem to be less than 2 μL . Gass and Roberts (1992) pointed out that some ornithophiles produce large volumes of nectar, whereas others produced very small volumes. In our experiments, birds showed no preferences for higher nectar volumes when flowers differed by 2 μL . Carlson (2008) reports similar results. The choice of volumes in our experiments may have clouded our efforts to test other aspects of bird preferences: 1 versus 4 μL might have yielded different results than 2 versus 8 μL . One might expect amount of reward to be the most important property affecting hummingbird preferences during pollination (Blem et al. 2000) and for there to be some volume at which the flower and the pollinator come to an evolutionary compromise (Zimmerman 1988). Ornithophiles should produce a high enough volume to entice birds into becoming frequent visitors, some amount above the birds' minimum threshold, but it should not be vastly above that number because doing so would waste energy and water (Southwick 1984; Pyke 1991) and would fail to encourage the birds to move on to the next plant. Our study suggests that a nectar volume of 2 μL may be more than enough to keep birds coming back to flowers. We could speculate, however, that in less arid environments the minimum reward might well be higher than at our field site.

Nectar viscosity and its components. Melittophilous tend to have a much higher sugar concentration than ornithophiles: on average 35% but in some species as high as 80% sugar (Baker 1975; Pyke & Waser 1981), and melittophilous nectar tends to be more hexose-rich (Baker & Baker 1983a; Wilson et al. 2006). Possibly bees under-visit flowers with dilute nectar (Harder 1986; Cnaani et al. 2006). The extra water in more dilute nectar takes longer for bees to get rid of. In contrast, hummingbirds have extremely efficient kidneys that excrete water quickly (Tyrrell & Tyrrell 1985). Since hovering is expensive, hummingbirds are expected to minimize the amount of time expended while foraging, hence to prefer flowers with less viscous nectar (Baker & Baker 1983b; Powers and Nagy 1988; Wilson et al. 2006). As nectar viscosity increases, the amount of nectar extracted per lick decreases and licking takes longer. Kingsolver and Daniel (1983) suggested that if a bird were able to extract all floral nectar in a single lick (1.9 μL for a Rufus Hummingbird), then it would prefer lower concentrations. However, Roberts (1995, 1996) casts doubt on whether energy intake rate calculated over the duration of a visit to a flower would actually be higher at lower concentrations for flowers with 4–8 μL . Our findings were not particularly consistent with the Kingsolver–Daniel theory. In Experiment 3, birds showed little response to different nectar types except when the amount of sugar contained within them was different. Stiles (1976) showed that hummingbirds had a preference for sucrose-containing nectar, rather than solutions that contained glucose or fructose. Contrary to Stiles, our birds had no preference for

sugar type, but similar to Stiles, they preferred more concentrated nectar. It is possible that the dilute nectar of ornithophiles is to discourage bees, because bees tend to have a lower net energy gain when extracting dilute nectar (Harder 1986), so the discrepancies we see between bird behaviour and the syndrome difference might be due to bee preference, not bird preference. An explanation for the syndrome difference not based on viscosity has been put forward by Schondube and Martínez del Río (2003). They suggest that hummingbirds prefer sucrose only at high concentrations and that they do so because sucrose has a lower osmotic potential than does hexose at the same concentration. Their birds preferred sucrose when artificial nectars were presented at 40%. Our birds failed to confirm this preference when presented nectars at 48% sugar.

Landing platforms. The lack of landing platforms in ornithophiles could be explained in a number of ways. First, landing platforms might obstruct hummingbirds from extracting nectar (Sutherland & Vickery 1993; Castellanos et al. 2004), thereby making them prefer flowers without landing platforms, as they did in Experiment 4. Consistent with this, birds took more time extracting nectar when there were landing platforms than when landing platforms were absent. Second, hummingbirds may be sensitive when flowers obscure their view of potential predators and competing hummingbirds (Lima 1991). Third, the absence of landing platforms might not be a response to bird preference but instead to deter inferior visitors. In other words, the absence of landing platforms may be to decrease competition between hummingbirds and bees for nectar (Valido et al. 2002; Rodríguez-Gironés & Santamaría 2004) thereby reducing visits by bees that tend to waste more pollen (Castellanos et al. 2003). Fourth, landing platforms might hinder the loading of pollen onto and the pickup of pollen from the bird's head. Landing platforms make the contact against anthers and stigmas imprecise (Castellanos et al. 2004; Fenster et al. 2004; Armbruster et al. 2009), so selection for increased pollen transfer efficiency would eliminate the landing platform. Studying the melittophilous *Mimulus lewisii*, Owen and Bradshaw (2011) found that mutants without landing platforms were under-visited by bumblebees, and even when the bees did visit, they did not crawl into the mutant flowers in the usual way, so were not transferring pollen as effectively. The theory that landing platforms are not valuable in ornithophiles was not, however, supported by Temeles and Rankin (2000) who compared ornithophilous *Monarda didyma* flowers with the lower lip removed to normal-lipped flowers. They found that removing the lip nearly halved the amount of pollen picked up by hummingbirds. Thus, lips are maintained in *M. didyma*, unlike in many ornithophiles. Questions remain, but we can say that, when given the choice, birds avoided flowers with landing platforms, plausibly because it takes longer to extract nectar.

Nectar guides. In shifts from melittophily to ornithophily, flowers sometimes lose colour patterning. Waser (1982) suggested that the presence of patterns on perianths in melittophiles might be used as nectar guides to lead bees to rewards, thereby improving the bees' handling time and making the flowers more attractive. Waser and

Price (1985) supported this proposition with the finding that foragers have increased handling times when visiting albino flowers that lack nectar guides. In *Mimulus lewisii*, mutants without nectar guides are under-visited compared to wildtype flowers (Owen & Bradshaw 2011). Bees seem to easily discern rewards based on associated patterning (Gould 1985). Perhaps birds are not guided the way bees are. In Experiment 5, hummingbirds did not use black dots to find rewarding nectar, although when they visited flowers with 8 μ L, they not only probed them more often (Haley 2010), they spent more time per probe. It would be good to confirm hummingbird inattention to nectar guides using other markings (such as white stripes), and using reward differentials to which hummingbirds are more consistently sensitive (not 2 versus 8 μ L). However, our results taken at face value are consistent with the syndrome difference. The lack of patterning in ornithophiles could be a result of hummingbird negligence – not recognizing patterns that might help them find rewarding nectar. Nectar guides may be at a visual scale that birds are insensitive to. Our results do not suggest that hummingbirds would select *against* nectar guides. In contrast, the results of Medel et al. (2003) suggest that hummingbirds do notice the red against yellow nectar guides of *Mimulus luteus*, and select them to be smaller and of a different shape than would be optimal for attracting bees.

Ending

Can the results from the various experiments be integrated? Bird preferences for some characters seemed stronger than others. For example, bird preference for higher volumes in Experiment 2 seemed not as definitive as preference for higher concentrations in Experiment 3C. This result follows a concentration-before-volume criterion hierarchy in bees (Cnaani et al. 2006). Stiles (1976) likewise suggested that hummingbirds have a preference hierarchy whereby birds take into account factors most important to them first and less important factors later. Smith et al. (2008) supported this hypothesis in showing that hummingbirds consider nectar reward and number of flowers in an inflorescence as more important than floral colour. Our study further supports this idea, suggesting that amount of sugar might make birds change behaviour, whereas nectar with almost any amount of water is treated similarly in terms of teaching birds to visit flowers. Hexose versus sucrose would be the least important aspect of nectar. In contrast, the landing platform difference was responded to quickly, strongly and stably, perhaps because it didn't require an assessment of hidden rewards. Comparing our several results is provocative when speculating on bird behaviours, but it does little to address the topic of multi-trait integration in pollination syndromes (Fenster et al. 2004). For example, reddish colouration might be considered the *sin qua non* of ornithophily in the western U.S., but our results do not indicate that red colouration is necessary for hummingbird pollination. Hummingbird behavioural proclivities *add* some characters to the syndrome, but to the extent that there is a functional *interaction* between traits at their optimum it would seem to be based on a harmony of several morphological characters working mechanically well together in transferring pollen

onto and off of particular pollinators (Reynolds et al. 2010).

ACKNOWLEDGEMENTS

Thanks to Sigma Xi for funding, to M. Fernandez for help with sugars, to C. Ryan, M. Steele, F. Hertel and various anonymous reviewers for comments on experimental design and various other topics, and to the following family members for support and assistance: T., A., L. and E. Haley, A., S., J. and E. Baldwin, and N. Guzman.

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