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THE ROLE OF OPTIC VERSUS METABOLIC ODOMETRY IN HONEYBEE FORAGING ENERGETICS

By

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ABSTRACT

THE ROLE OF OPTIC VERSUS METABOLIC ODOMETRY IN HONEYBEE FORAGING ENERGETICS

By

Dina Leslie Grayson

Many animals adjust their foraging decisions in response to changes in the energetic profitability of food sources. However it remains unclear what mechanisms animals are using to estimate energetic profitability. This study investigates the mechanism that honeybees use to estimate the foraging costs associated with flight distance. The two possibilities tested in this study are the optic odometer hypothesis and the metabolic odometer hypothesis. I trained bees to fly down tunnels of identical length but differing optical patterns. Thus, I was able to keep the metabolic odometer's measure of flight distance equal between the two tunnels, while producing differing perceptions of flight distance as measured by the optic odometer. Analyzing the dance responses of bees to these two tunnels indicated a preference for the optically shorter tunnel, which implies that they that they were using an optic odometer to asses foraging costs associated with flight. The implications of this study for honeybee neuroethology and foraging ecology are discussed.

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KEY TO SYMBOLS

G = Gain (J) = SV (μl) • SC (μmol/μl) • 5.8 (J/μmol)

SV = Stomach Volume (μl) = amount of sucrose solution imbibed

SC = Sugar Concentration (μmol/μl)= concentration of the sucrose solution imbibed

$C = \text{Cost (J)} = \sum_{i=1}^{4} T_i \text{ (s)} \bullet MR_i \text{ (J/s)}$

T_i = Time (s) of each of the four segments of a foraging trip

T_1 = Time (s) of the flight from the hive to the food source

T_2 = Time (s) spent foraging at the food source

T_3 = Time (s) spent flying from the food source to the hive

T_4 = Time (s) spent at the hive upon returning from the food source

MR_i = Metabolic Rate (J/s) for each of the four segments of a foraging trip

MR_1 (J/s) = 0.003015 • UM (mg) $^{0.629}$ = metabolic rate of bee flying to the food

MR_2 (J/s) = 0.0026044 • UM (mg) $^{0.492}$ = metabolic rate of a bee sitting at the food

MR_3 (J/s) = 0.003015 • LM (mg) $^{0.629}$ = metabolic rate of a bee flying from the food

MR_4 (J/s) = 0.0026044 • LM (mg) $^{0.492}$ = metabolic rate of a bee sitting or walking in the hive

UM = Unloaded Mass (mg) = weight of a bee before it has imbibed any sucrose solution

LM = Loaded Mass (mg) = weight of a bee and the sucrose solution it has imbibed

INTRODUCTION

Since the inception of optimal foraging models in the 1960's, research into the foraging decisions of animals has flourished (for review see Stephens and Krebs, 1986). Many species have been found to be sensitive to differences in the profitability of alternative food sources. Profitability can be defined as the quality of a food source in terms its energetic gains and cost (Stephens and Krebs, 1986; Cuthill and Houston, 1997). Since animals respond to differences in profitability between food sources, it is clear they must be measuring profitability, but the mechanisms they use remain largely unknown and inaccessible to researchers. Honeybees provide a rare opportunity to elucidate a mechanism by which energetic costs associated with flight are perceived.

Honeybees are useful for addressing this question because foragers indicate their measure of food source profitability in the waggle dance. The waggle dance is used by foragers to communicate the direction and distance traveled to a food source (for review see von Frisch, 1967; Dyer, 2002). When a honeybee returns to the hive from a profitable food source she dances in a figure-eight shaped circuit. The midline between the two circles of the eight is emphasized by the honeybee 'waggling' her abdomen. The orientation of each waggling run indicates the direction to the food, and the duration of the waggling run signals the distance to the food.

Honeybees indicate their measure of food source profitability by modulating the number of waggling tuns performed in the hive after each foraging trip (von Frisch, 1967; Seeley and Towne, 1992; Seeley, 1995; Seeley et al., 2000). A greater number of waggling runs are performed for more profitable food sources. Although several aspects of the dance are positively correlated with profitability (for review see Seeley, 1995;

Stabentheiner, 1996; Wainselboim and Farina, 2000a; Wainselboim and Farina, 2000b; Seeley et al., 2000) there are only two which honeybees have been shown to utilize. These are the probability of performing any dances and the number of waggle runs performed per foraging trip (Seeley and Towne, 1992). These variables determine the rate at which new bees arrive to exploit a food source through a complex set of individual and social decisions (Seeley et al. 1991; Seeley and Towne, 1992; de Vries and Biesmeijer, 1998). The process commences when bees that are ready to forage but have not yet found a food source (recruits) randomly choose a dancer to follow. They will follow a dancer for a few circuits and then leave to search for the site being indicated (Seeley et al., 1991; Seeley and Towne, 1992). Since recruits only follow a dancer for a few circuits, they do not have an opportunity to assess the profitability signal directly (Seeley and Towne, 1992). Yet, since the choice of which dancer to follow is random and new recruits are readily available to replace those that leave to forage, the number of recruits that are sent to a particular food source can be predicted by the proportion of dances in the hive that indicate that food resource (Seeley and Towne, 1992). Thus a colony allocates the majority of its foraging effort to the more profitable food sources even though no one bee has knowledge about the profitability of any food source except her own.

The question of how each forager decides the profitability of her food source can be broken down into three parts: (1) what variables affect gain? (2) what variables affect cost? (3) what combination of gain and cost, or currency, do bees use? Gains can be defined as the gross energy gained from consuming food. In nectar-foraging honeybees this is a function of the amount and concentration of sucrose solution imbibed, as is shown by studies demonstrating a correlation between these variables and dance variables associated with profitability (von Frisch, 1967; Seeley, 1986; Seeley et al., 1991; Seeley and Towne, 1992; Seeley, 1994; Seeley, 1997; Seeley et al., 2000; Waddington, 1982, Waddington, 1985, Waddington and Gottlieb, 1990).

Costs can be defined as the amount of energy expended to obtain and consume the food. In honeybees cost is influenced by two main factors: the distance of the food from the hive, and the handling time, or the amount of time it takes a bee to remove the nectar from the flower and to fly between flowers. Several studies have shown that bees judge a food source that is closer to the hive as being more profitable (von Frisch, 1967; Seeley, 1986; Seeley and Levine, 1987; Seeley et al., 1991; Seeley, 1994; Seeley, 1995). A decrease in handling time, induced by an increase in flow rate of the sucrose solution, has been shown to lead to an increase in dance duration (a variable strongly correlated with number of waggle runs) and probability of dancing (Farina, 1996). Other components of handling time, such as distance between flowers, have also been shown to affect dance response (Waddington, 1982).

Once honeybees have assessed the components of gain and cost, they must combine them into a measure of profitability or currency which maximizes honeybee fitness (Stephens and Krebs, 1986; Cheverton et al., 1985; Bateson and Kacelnik, 1998). Three currencies have been examined as the basis of honeybee foraging decisions (Kacelnik and Houston, 1984; Seeley, 1994; Schmid-Hempel et al., 1985). The most basic is net gains (Net), where Net is defined as total gains (G) minus total costs (C) of a foraging trip. The second possible currency is rate of net gain (Rate), where Rate is defined as [(G-C)/T], and T stands for the total trip time. The third possible currency is net efficiency (Efficiency), where Efficiency is defined as [(G-C)/C]. The majority of studies on this topic agree that efficiency is the currency honeybees maximize (Schmid-Hempel et al., 1985; Kacelnik et. al., 1986; Houston et al., 1988; Wolf and Schmid-Hempel, 1990; Seeley, 1994; but see Waddington and Holden, 1979; Waddington and Heinrich, 1979). However, the possibility that honeybees may use different currencies under different conditions can not be discounted (Seeley, 1994; Houston et al., 1988; Fewell et al., 1991).

The goal of my research is to discover the nature of the odometer by which bees measure the costs associated with foraging flight. Until recently it was reasonable to assume that honeybees measured the energy expenditure during flight between the hive and the food by measuring the calories consumed (for review see Esch & Burns, 1996). This assumption followed from von Frisch's (1967) suggestion that bees record flight distance for the dance by monitoring energy consumption during flight. However more recent evidence has shown that bees use optic flow to measure the distance reported in the waggle dance (Esch & Burns 1995, Esch & Burns 1996, Srinivasan et al., 1996; Srinivasan et al., 1997; Srinivasan et al., 1998; Srinivasan et al., 2000; Esch et al., 2001). So, are honeybees evaluating the energetic cost of flight by using an optic odometer or a metabolic odometer?

The optic odometer functions by using optic flow. Optic flow can be described as movement of visual contours over the visual field. The movement may result from movement of objects relative to a stationary animal, or by movement of the animal through the environment. Much of the work done to prove the optic flow hypothesis was been carried out in tunnels, into which bees are trained to find food (Srinivasan et al., 1996; Srinivasan et al., 1997; Srinivasan et al., 1998; Srinivasan et al., 2000; Esch et al., 2001). By changing the width, height, and wall and floor patterns in the tunnels it is easy to manipulate a honeybee's perception of optic flow in order to make her estimate that she has traveled much farther than the actual flight distance in the tunnel. It has also been verified that recruits watching the dances can't tell that the dancer had been in a tunnel and would search for food in the field at the distance that was optically simulated in the tunnel (Esch et al., 2001).

To address the question of whether bees use an optic or metabolic odometer in their profitability assessment I constructed two tunnels equal in length but different in the optical pattern presented on the walls and floor. The optical pattern of one tunnel simulated 300 m of distance, and the other tunnel's optical pattern simulated 100 m of distance even though both tunnels were only 10.2 m long. I trained separate groups of bees to find food at the ends of these two tunnels and recorded their dances while keeping the sugar concentration equal in both tunnels. With the sugar concentration and lengths of the tunnels equal the metabolic odometer's measure of profitability should have also been equal. On the other hand, if bees were using an optic odometer they should have judged the optically shorter tunnel to be more profitable.

METHODS

All of the experiments included in this study were conducted at the Inland Lakes Research and Study Center on the campus of Michigan State University. The experiments were conducted within a section of a hoop-house measuring approximately 24.4m long, 5.7m wide at the ground, and 2.4m high in the middle. The hoop-house consists of semicircular metal hoops covered by a grass-green 30% shade cloth. The hoop-house ends were covered by wooden walls allowing researchers access by doors.

For honeybees to adapt to life in an enclosed space, it is necessary that they have no outside flight experience. Therefore, at the start of the study, brood were taken from hives containing Italian-derived bees (*Apis mellifera ligustica*) and raised in an incubator until they emerged. Upon emergence the honeybees were placed in a four-frame observation hive with a queen and extra brood and pollen from other colonies. The observation hive was placed in the middle of the hoop-house against the East side, so that the entrance faced West. Both sides of the observation hive consisted of wooden doors opening onto glass panes to allow easy viewing and recording of honeybee activity. The entrance to the observation hive was fitted with a shunt that forced all of the honeybees to enter onto one side of the comb, so that all dances could be observed through one window.

Throughout the experiments we attempted to keep the observation hive condition as constant as possible. Specifically, colony population, stored honey, and stored pollen were regularly visually checked to see if adjustment was required. Colony population was adjusted through the addition of honeybees raised in the incubator, or the addition of a frame of brood. Stored honey was removed if excess, and the colony was fed with a stock feeder in the hoop-house if their supply was inadequate. Pollen was added through the addition of pollen frames and via a pollen feeder in the hoop-house. The pollen feeder consisted of a petri dish filled with a pollen and sugar water mixture, which foragers ingested and carried back to the hive. All of these adjustments to colony state were carried out on days prior to experiments.

During the experiments honeybees were trained to reach food by flying down one of two tunnels. The walls and floor of the tunnels were made of wood and lined with an optical pattern. The tunnel ceiling was covered with black nylon mesh which allowed a view of the sky, but prevented bees from escaping. Each tunnel was 10.2 m long and consisted of 3 tunnel sections 3.4 m long each. The tunnels had an inside height of 20 cm and a width of 11 cm.

The two tunnels differed in the optical pattern presented on the walls and floor. The optical patterns were chosen to provide the honeybees with differing impressions of how far they had flown to reach the food. In the 'julesz' tunnel a random Julesz pattern of 1 cm^2 black and white squares covered the entire length. According to previous studies in tunnels of the same width and height, this should optically simulate 300 m of flight distance (Srinivasan et al., 2000). In the 'horizontal' tunnel, the same Julesz pattern covered its first 3.4 m, but the remaining 6.8 m were covered with a pattern of alternating 1cm black and white horizontal stripes oriented axially along the length of the tunnel (Figure 1, p. 9). Horizontal stripes provide little or no optic flow (Srinivasan et al., 1997; Srinivasan et al., 1998). Therefore, although it was the same actual length as the julesz tunnel, the horizontal tunnel optically simulated only 100 m of distance (100 m from the julesz section and 0 m from horizontal stripe section).

There were two reasons for providing a julesz pattern in the first segment of the horizontal tunnel. First, if bees measure their distance from the hive to be short, as would occur in a tunnel containing only horiaontal stripes, they perform round dances instead of waggle dances (von Frisch, 1967). Covering the first part of the horizontal tunnel with a Julesz pattern ensured that the bees would perform waggle dances, although ones that were to a shorter distance than those for the julesz tunnel. Second, we wanted the initial portion of both tunnels to look similar so that during training we were not preferentially recruiting different bees to different tunnels based on the appearance of the entrance.

The tunnels were aligned in opposite directions along the North-South axis of the hoop-house (see Figure 2, p. 10). The tunnels ran lengthwise under the center-line of the hoop-house in order to prevent honeybees from using different landmarks outside of the tunnels that might have influenced distance perception. The orientations of the two tunnels were reversed every time new bees were trained to avoid possible direction biases. The tunnel entrances were 1.5 m apart from each other. The hive was placed equidistant from the two tunnel entrances and slightly back towards the East edge of the hoop-house. Tunnel entrances were marked with cue cards to facilitate the bees finding them. A blue cue card was always used for the julesz tunnel and a yellow one was always used for the horizontal tunnel. Feeders used in the tunnels consisted of two painted vial caps covered with a wire mesh screen and placed upon a plastic spill tray that had a colored tape cross (see Figure 3, p. 11). Again, blue was used for the julesz tunnel, and yellow for horizontal tunnel. The tunnels were set on sawhorses 0.8 m above the ground.

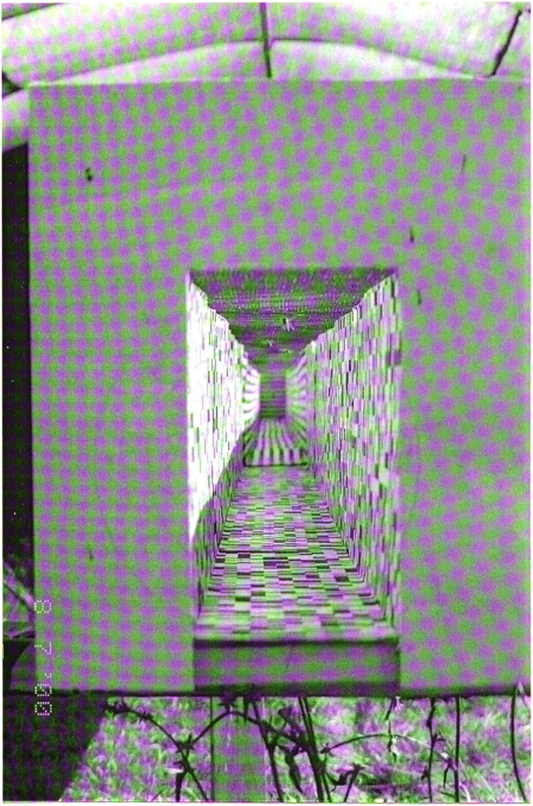

Figure 1 – Tunnel vision. This figure shows the view from the start to the end of the horizontal tunnel including the cue card on the front of the tunnel.

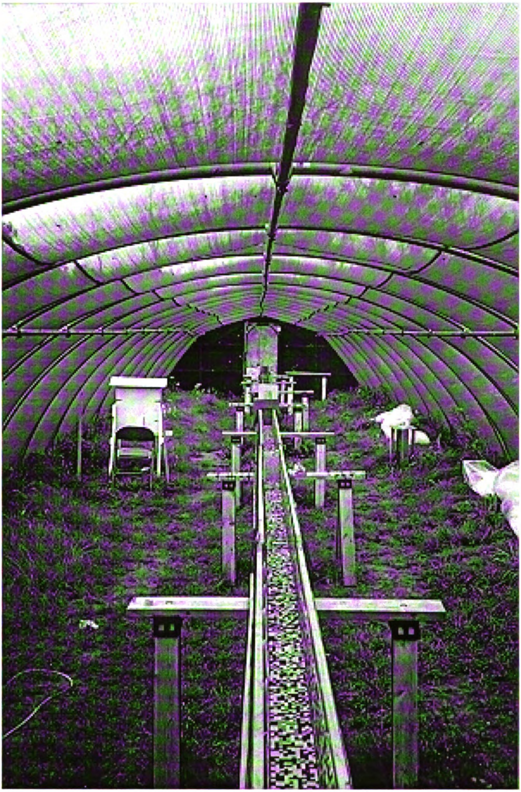

Figure 2 – Hoop-House setup. This is a complete view of the section of the hoop-house used for these experiments showing the location of the hive and both tunnels.

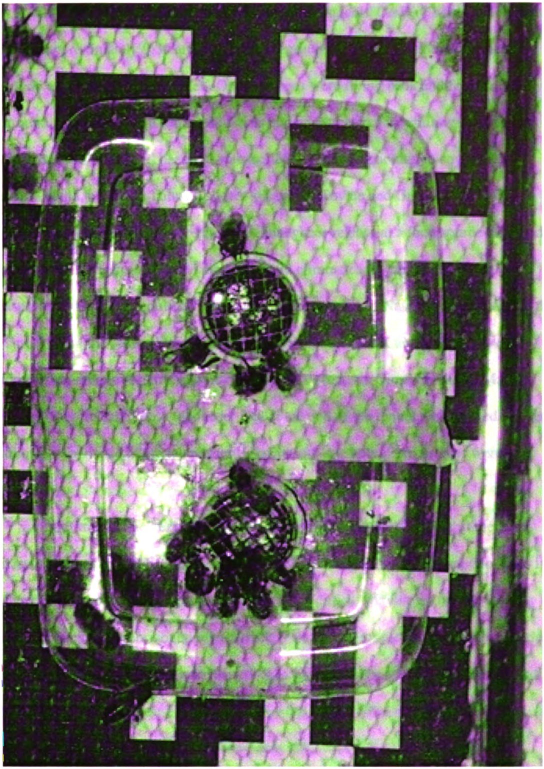

Figure 3 – Tunnel feeder design. This shows one of the tunnel feeders consisting of two vial caps, which were painted, with wire mesh inside them sitting upon a plastic spill tray with a cross of colored tape. This picture was taken from above the tunnel so you can also see the mesh that covers the tunnel to prevent bee escape.

All experiments followed the same general protocol. On Day 1 the tunnels were set up by approximately 9:30 am, and separate groups of bees were trained in steps to find food at the end of each tunnel. Once the bees were flying back and forth smoothly, by around 1 pm, they were captured in vials and anaesthetized by chilling them in ice. A 50/50 mixture of rosin and beeswax was melted onto their abdomen to cover their Nasanov gland, a process known as scent-plugging (Towne and Gould, 1988). The Nasanov gland produces a pheromone that honeybees release at a food source they find profitable (von Frisch, 1967). Sealing the gland eliminates the possibility that a bee's measure of profitability could be influenced by the amount of Nasanov pheromone in the air surrounding the food. After scent-plugging the bees' thorax and abdomen were marked using unique patterns of colored paint dots (Testor's Gloss Enamel). Marked bees were then released onto the feeder tray at the end of the tunnel and allowed to gain additional experience in the tunnels until approximately 5 pm. Excess bees were captured.

At the start of each experiment we marked approximately 21 bees in the horizontal tunnel and 28 bees in the julesz tunnel. We used a smaller number of bees in the horizontal tunnel because they made faster round trips. Having an overall lower number of bees in the horizontal tunnel made the average number of bees at each feeder at any one time (5-10 bees) approximately equal in both tunnels. This was necessary for two reasons. First, each observer at the end of the tunnel could only keep track of a certain number of bees feeding simultaneously, and this limit was equal for both observers. Second, it is possible that bees use the number of other foragers at a food source as an indicator of profitability.

On Day 2 the tunnels were set up in the morning with the first sugar solution we planned on testing that day. The bees were allowed time to find the food (15 minutes to 4 hours, depending on the air temperature). One assistant was stationed at the end of each tunnel with a tape recorder and stopwatch. The assistants would record the exact time to the second of each bee's arrival and departure from the feeder. They were also responsible for maintaining abundant sugar solution in the feeder. I remained at the hive using a video camcorder (Canon E51) to record the arrivals and departures of the painted bees. A digital clock and thermometer were placed in view of the camera under the dance floor.

Once all observers were in place and there were at least 5 bees attending each feeder we would record flight times and dances for approximately 1 hour. Each recording session at a constant sucrose concentration is called a test period. Between test periods all observers would take a small break and choose another sucrose solution. Sucrose solutions were chosen from a set of sucrose solutions ranging from 0.50 mol/l to 1.00 mol/l in 0.25 mol/l increments. Occasionally higher or lower sugar concentrations were added to the set if the bees were particularly unmotivated or overly excited. This was necessary to avoid ceiling or floor effects in the bees' response to the two tunnels (Waddington & Gottlieb 1990). An assistant would haphazardly choose a solution and the previous solution was removed from the set of possible solutions to use during the remaining test periods that day. We continued recording test periods until either the day was over (approximately 5 pm) or the weather deteriorated. Overall we trained three groups of bees using this protocol (Bee Groups 1-3 in Table 1, p. 14), and recorded two to four test periods with each, which came to a total of nine test periods (Table 1, p. 14).

Table 1 - Test period conditions. This table shows the date, time, sugar concentration and bee group used for each test period.

Test Period	Date	Time	Sugar (mol/l)	Bee Group
A	10-Aug-00	13:59 - 14:30	1.00	1
B	10-Aug-00	15:04 - 15:31	0.75	1
C	10-Aug-00	16:13 - 16:40	0.50	1
D	14-Aug-00	11:57 - 12:27	0.50	2
E	14-Aug-00	13:10 - 14:01	0.75	2
F	14-Aug-00	14:38 - 15:06	1.00	2
G	14-Aug-00	15:42 - 16:16	1.25	2
H	1-Sep-00	13:52 - 14:35	0.75	3
I	1-Sep-00	15:10 - 16:30	0.50	3

By making the actual lengths of the two tunnels identical and offering identical sucrose solutions, we hoped to make the bees' metabolic measure of profitability equal. In order to check this we needed six variables: the flight time from the hive to the food, the time at the food, the flight time from the food to the hive, the time at the hive upon returning from foraging, unloaded bee mass, and stomach volume. We collected data on the four time segments during our initial experiments, but we performed a second experiment to obtain an estimate of stomach volume and unloaded mass for bees from the observation hive. For the second experiment we trained bees as in Day 1 of the previous protocol but without scent-plugging or marking them. At the end of Day 1 or on Day 2 we measured stomach volume and body mass of bees in each tunnel at three levels of sugar concentration, 0.5, 1.5, and 2.25mol/l. Bees were captured in vials immediately after they terminated imbibing sugar solution at the feeder. I would remove her head and draw her stomach contents from the esophagus into a capillary tube (Mettler P-100) as quickly as possible to avoid food consumption. The amount of solution in the capillary tube was measured in mm and converted into μl. The bee's body and head were weighed to the nearest 5 mg on a torsion balance.

Foraging trip time information, probability of dancing (O if no waggle runs, 1 if any waggle runs), and number of waggle runs performed were extracted from the audio and video tapes and entered into an Excel worksheet. The audio tapes contributed information on when each bee arrived and departed the feeder. The video tapes recorded the time each bee left the hive to go to the feeder, the time at which each bee returned to the hive from the feeder, how many waggle runs each bee completed after a trip to the feeder, and when each bee left the hive after dancing. If a bee could not be seen during its entire time in the hive after a foraging trip it was not included. This prevented underestimating number of waggle runs due to runs performed outside the view of the camera. Only data on the bees' activities after the sucrose concentration had been constant for 30 minutes were used for analyses. This was done to account for the possibility that bees take time to adjust their dance response to a new sucrose solution (Richter and Waddington, 1993; von Frisch, 1967).

To test the assumption that metabolically measured profitability was equal between the two tunnels energetic gain and cost were calculated. The amount of energy gained in J per foraging trip, gain (G), was calculated as

$$G = \text{Gain (J)} = \text{SV } (\mu\text{l}) \bullet \text{SC } (\mu\text{mol}/\mu\text{l}) \bullet 5.8 \text{ (J}/\mu\text{mol)}$$

where SV is stomach volume in μl and SC is sucrose concentration in μmol/μl. In this equation 5.8 J/μmol is the standard conversion for the energy content of sugar (Kleiber, 1961).

The metabolic measure of cost (C) was calculated using the equation

$$C = \text{Cost (J)} = \sum_{i=1}^{4} T_i \text{ (s)} \bullet MR_i \text{ (J/s)}$$

where T_i is the time in s of each of the four segments of a foraging trip, and MR_i is the metabolic rate in J/s for each of the four segments of a foraging trip. The numbering of the four segments of a foraging trip is the same for both the time and the metabolic rate: 1) the flight from the hive to the food source, 2) foraging at the food source, 3) the flight from the food source to the hive, and 4) visit at the hive upon returning from the food source. To determine the metabolic rate of each of the foraging trip sections I used an allometric equation for forward flight at 0.5 m/s for segments 1 and 3 and an allometric equation for walking or sitting bees for segments 2 and 4 (Wolf et al., 1989). I converted these equations to J/s by using a respiratory quotient of 1 for bees, therefore 1 ml O_2 = 21.117 J/ml (Nachtigall et al., 1989; Rothe and Nachtigall, 1989). The equations I used for each segment of the foraging trip are as follows:

$$MR_1 \text{ (J/s)} = 0.003015 \bullet UM \text{ (mg)}^{0.629}$$

$$MR_2 \text{ (J/s)} = 0.0026044 \bullet UM \text{ (mg)}^{0.492}$$

$$MR_3 \text{ (J/s)} = 0.003015 \bullet LM \text{ (mg)}^{0.629}$$

$$MR_4 \text{ (J/s)} = 0.0026044 \bullet LM \text{ (mg)}^{0.492}$$

where UM is the unloaded mass of the bee in mg, and LM is the mass of the bee plus the weight of its stomach contents in mg. To convert stomach volume in μl to the weight of the stomach contents in mg, I used the standard densities of different concentrations of sucrose solution in the CRC Handbook of Chemistry and Physics (Hodgman et al., 1958). I decided to use loaded mass for the last two segments of the foraging trip even though crop load does not directly affect metabolic rate (Balderrama et al., 1992; Moffatt, 2000) because flow rate does affect metabolic rate (Moffatt, 2000), and the feeders used in these experiments dispensed food ad libitum.

There are many other factors that could affect metabolic rate, but which do not affect the present study. Flight speed is known to affect metabolic rate, but not over the range of 0 to 4.3 m/s, which covers the flight speeds in my experiments (Table 2, p. 18) (Nachtigall et al., 1989). As mentioned above flow rate affects metabolic rate, but both tunnel feeders dispensed sugar solution ad libitum (Balderrama et al., 1992). Bees have also been found to increase their thoracic temperature with increasing sugar concentration (Waddington, 1990). Yet, in this study both tunnel feeders offered the same sugar concentration at the same time, so if this made the estimates of energy spent inaccurate it would have done so equally for both tunnels. Weight –Specific heat production has been shown to vary with age of the worker bees (Fahrenholz et al., 1992), but there is no reason to suspect that the foragers in one tunnel were systematically older than in the other tunnel on all three days testing took place. Malate dehydrogenase phenotype has been shown to affect flight metabolic rates (Harrison et al., 1996), but there is no reason to think that the bees in any one tunnel would have a different phenotype. These possibilities are one of the reasons we had the tunnel entrances designed to look identical at the start of training. This should have limited any possible bee preference.

Table 2 – Flight speeds. This table shows the average flight speeds and the minimum and maximum values of flight speed for the two tunnels. Average flight speeds were calculated by dividing the distance of the entire flight from the hive entrance (12.1m) by the average times calculated in Table 3, so values of N are the same as in Table 3.

	Horizontal Tunnel		Julesz Tunnel	
	Average	Min. and Max.	Average	Min. and Max.
Flight Speed to the Food (m/s)	0.20	0.02 - 0.81	0.07	0.02 – 1.10
Flight Speed from the Food (m/s)	0.16	0.003 – 0.48	0.12	0.01 – 0.58

RESULTS

Two lines of evidence suggest that the bees were interpreting the different optical patterns in the two tunnels as intended. First, I observed slower flight times in the optically longer julesz tunnel (Table 3, p. 20). This is consistent with previous studies showing that bees prefer to keep a constant rate of optic flow when they are flying, and will slow down or speed up to attain that preferred rate of optic flow (Srinivasan et al., 1996; Esch and Burns, 1996). The flight speed differences that I observed indicate that honeybees are noticing the difference in optic patterns. Second, I observed that bees imbibed more sugar solution in the optically longer julesz tunnel (Table 3). Previous studies suggest that honeybees tend to imbibe greater amounts of sugar solution with increasing distance (Nunez, 1982). Thus, my data suggest that bees interpreted the julesz tunnel as longer. To ensure that these differences were consistent across changes in sugar concentration I carried out this comparison at all sugar levels used in the experiments, and found the same general result (Tables 4, p. 21). The one exception to the trend was that at 1.00 mol/l bees flew from the food to the hive significantly faster in the optically longer julesz tunnel. Yet, the overall pattern of flight times and stomach volumes in Tables 4 indicates that bees were interpreting the tunnels as intended; so, further confirmation by investigating the distance signal of the waggle dance was unnecessary.

Having confirmed the tunnels are in fact interpreted differently by the bees, the next step was to calculate the predictions of the metabolic odometer hypothesis. To complete this, I first had to use the equations described in the methods to determine values for gain and cost, and to do that I needed to determine correct estimates of stomach volume and unloaded mass for bees from the observation hive.

Table 3 – Tunnel flight time and stomach volume differences. This table shows average flight times and stomach volumes for the optically shorter horizontal tunnel and the optically longer julesz tunnel. Flight times were calculated by averaging each bee over all of her foraging trips and then averaging all the bees in each tunnel. Stomach volume was measured on a different group of bees that only made one foraging trip each. Shapiro-Wilkes tests revealed that all of the data were non-normal, so Wilcoxon rank sums tests were used to compare tunnels.

	Horizontal Tunnel		Julesz Tunnel		
	Mean ± SEM	N	Mean ± SEM	N	P-Value
Flight Time from the Hive to the Tunnel Feeder (s)	60.3 ± 6.1	40	182.1 ± 31.1	49	< 0.0001
Flight Time from the Tunnel Feeder to the Hive (s)	75.8 ± 14.2	40	97.6± 11.4	50	< 0.0001
Stomach Volume (μl)	39.4 ± 2.8	34	50.8 ± 2.6	22	< 0.01

Table 4 – Tunnel flight time and stomach volume differences at different levels of sugar concentration. Flight times were obtained by averaging each bee over all of her foraging trips at a particular sugar concentration and then by averaging all of those bee averages in each tunnel. Stomach volume data taken on bees that made one foraging trip to a feeder of 0.50mol/l solution were averaged. Shapiro-Wilkes tests revealed that all data were not normally distributed, except for stomach volume at 0.5 mol/l. Wilcoxon rank sums tests were performed to compare flight times in the two tunnels. Stomach volume was analyzed using a one-way ANOVA.

	Sugar Concentation (mol/l)	Horizontal Tunnel Mean ± SEM	N	Julesz Tunnel Mean ± SEM	N	P-Value
Foodward Flight (s)	0.50	60.9 ± 16.4	29	152.7 ± 24.2	28	< 0.0001
	0.75	62.4 ± 7.5	37	139.2 ± 26.3	35	< 0.001
	1.00	71.5 ± 10.0	25	183.8 ± 44.9	31	< 0.0001
	1.25	67.7 ± 6.0	6	195.0 ± 40.0	11	< 0.007
Homeward Flight (s)	0.50	64.4 ± 9.1	33	87.4 ± 19.0	30	< 0.02
	0.75	65.2 ± 10.5	38	87.9 ± 12.1	35	< 0.002
	1.00	92.9 ± 31.7	27	88.0 ± 9.8	35	< 0.0003
	1.25	62.0 ± 19.2	7	117.0 ± 22.6	13	< 0.02
Stomach Volume (μl)	0.50	34.7 ± 3.8	15	50.9 ± 3.5	6	< 0.03

To choose an appropriate estimate of stomach volume, I needed to determine whether the factors of tunnel and sugar concentration affected stomach volume. A Shapiro-Wilkes test for normality revealed that stomach volume was not normally distributed, so I used a Kruskal-Wallis rank sums tests to investigate the independent effects of sugar concentration and tunnel on stomach volume. These tests revealed that tunnel did significantly affect stomach volume ($p < 0.01$), but sugar concentration did not ($p > 0.07$) (Figure 4, p. 23). Previous studies have shown an increase in stomach volume with sugar concentration (Waddington, 1990); the fact that it was not quite significant in these experiments is probably due to small sample size in some of the sugar concentrations (0.50 mol/l N= 21, 1.50 mol/l N= 14, 2.25 mol/l N=21). Since I did not have estimates of stomach volume at every level of sugar concentration and the effect of sugar concentration was not significant, I used each tunnel's average of stomach volume for the energetic calculations of gain and cost.

To determine the appropriate estimate of unloaded mass for bees from the observation hive I needed to determine the effect of tunnel and sugar concentration. The data on unloaded mass were normally distributed (Shapiro-Wilkes probability $< W = 0.1183$), so I performed a two-way ANOVA to test for the effect of tunnel, sugar and their interaction on unloaded mass (Table 5, p. 24). There was no significant effect of tunnel or of the interaction between tunnel and sugar concentration, but there was a significant effect of sugar (Figure 5, p. 25). The cause of this significant effect of sugar could be due to an interaction effect of bee mass and feeder sugar concentration, so that bees of greater mass decided to stay in the hive and stop foraging after discovering that

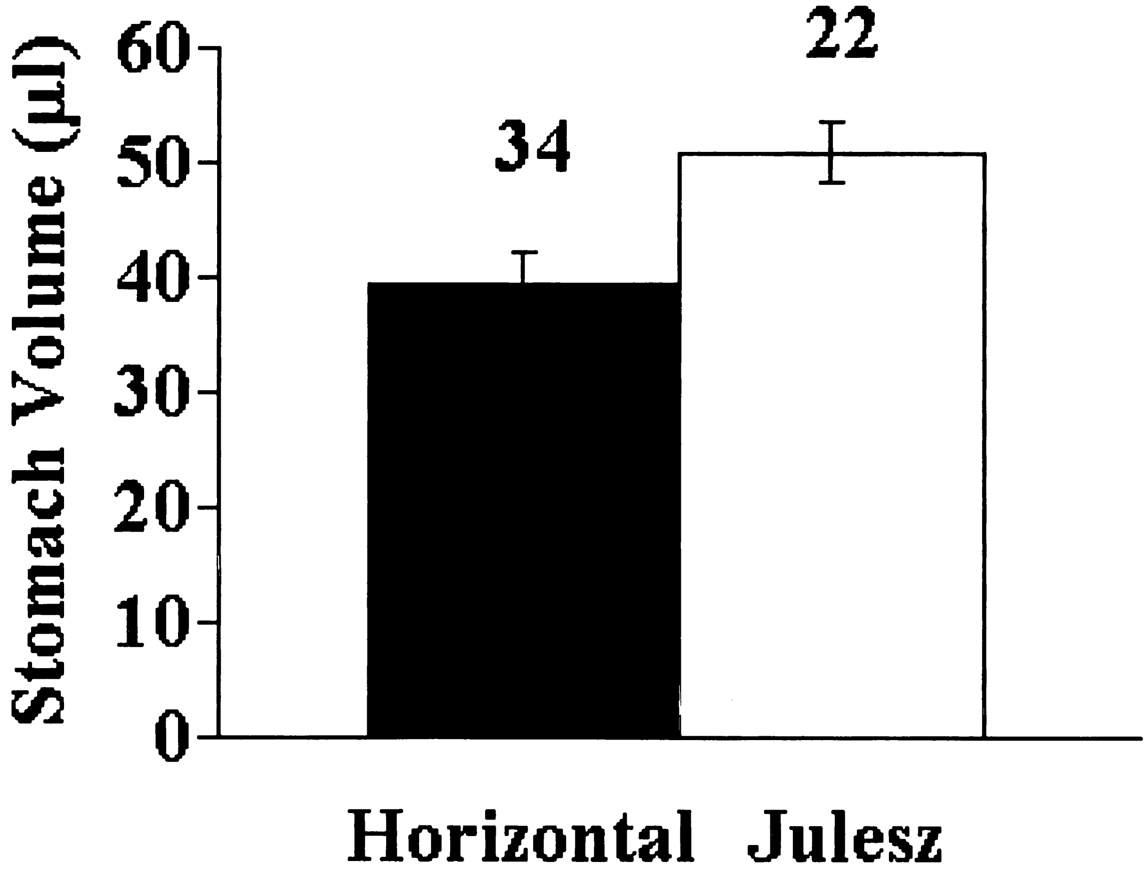

Figure 4 – Effect of tunnel on stomach volume. This figure shows the mean, SEM, and N of stomach volume in each tunnel.

the feeder was too low in concentration. Regardless of the cause, the best estimate of unloaded mass, given this effect, would be a separate estimate of weight for each of the sugar concentrations used. However, I did not have measurements of weight at all of the sugar concentration levels used; so, I decided that the least biased estimate of unloaded mass for bees from the observation hive was an overall average of unloaded mass.

This should not have biased the results for two reasons. First, all of my conclusions are drawn from comparisons of the two tunnels, and since there was no significant interaction between sugar concentration and tunnel, using an overall estimate of unloaded mass instead of a different value for each sugar concentration, would affect both tunnels equally. Second, the effect of sugar concentration is likely to be less in my experiments because the range of sugar concentrations over which this effect was discovered (0.5 to 2.25mol/l) was greater than the range of sugar concentrations used in the experiments (0.5 to 1.25mol/l). Therefore the overall estimate of unloaded mass is the best available estimate of unloaded mass and was used in the energetic equations.

Table 5 – Effect of tunnel and sugar concentration on unloaded mass. This table shows the results of the two-way ANOVA unloaded mass (mg) = tunnel + sugar concentration + tunnel * sugar concentration.

Source	Degrees of Freedom	Sum of Squares	F Ratio	Prob > F
Tunnel	1	1.8268	0.0166	> 0.9
Sugar Concentration	1	1233.1215	11.23666	< 0.002
Tunnel * Sugar Concentration	1	4.2701	0.0389	> 0.8

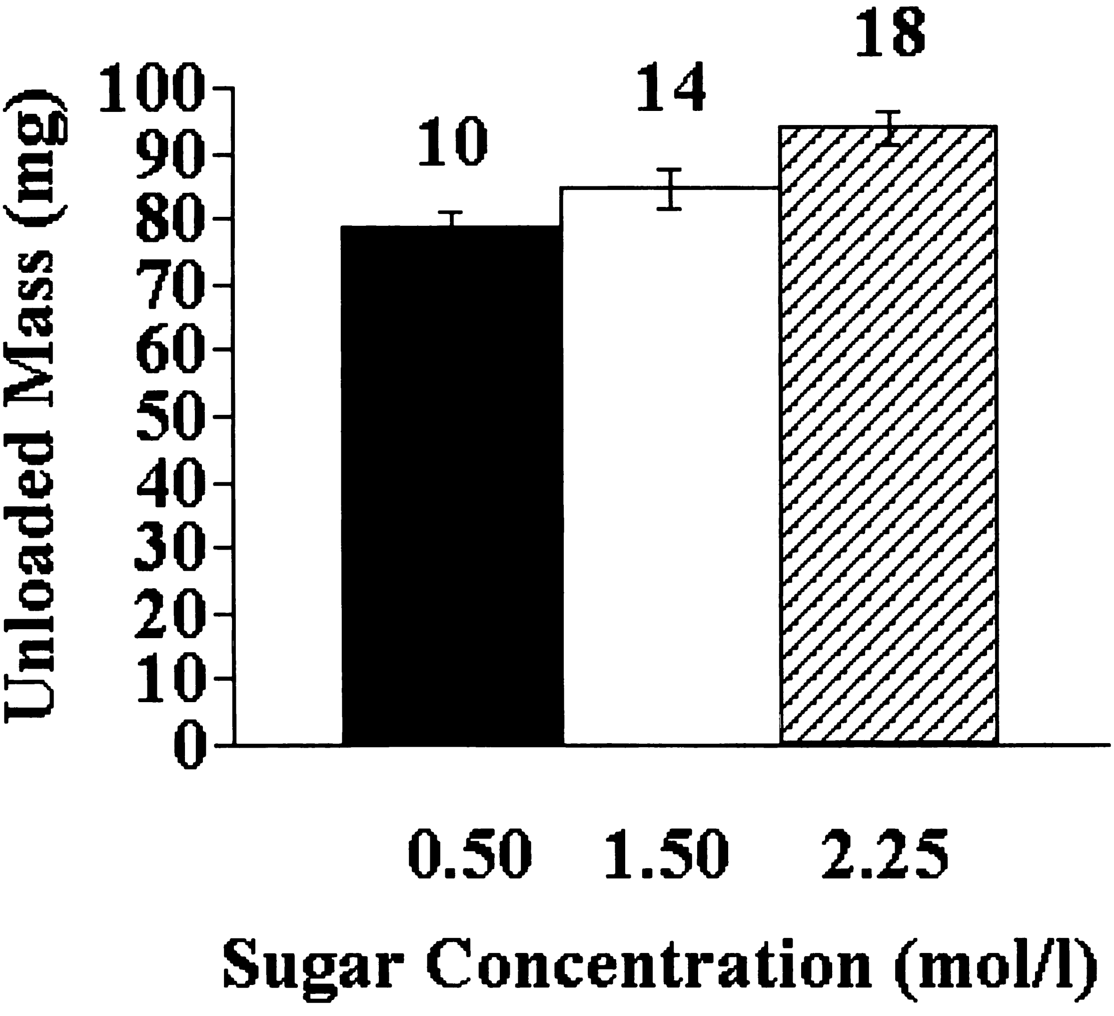

Figure 5 – Effect sugar concentration on unloaded mass. This graph shows the mean, SEM, and N of unloaded mass at each of the three sugar concentrations tested.

Once the estimates of stomach volume and unloaded mass were chosen, I used them to estimate metabolic Efficiency, as that is the currency bees most likely use to measure profitability (Schmid-Hempel et al., 1985; Kacelnik et al., 1986; Houston et al., 1988; Wolf and Schmid-Hempel, 1990; Seeley, 1994). To determine whether estimated metabolic Efficiency was equal between the two tunnels, I generated average values of Efficiency for each tunnel in each test period and performed a paired t-test (Figure 6, p. 27). Although the value was not significant, it was very near significance in the direction indicating the horizontal tunnel would be more profitable. Yet, there was no statistical difference between the two tunnels, so bees should judge the two tunnels about equal in profitability if bees use a metabolic odometer to estimate Efficiency.

Even though Efficiency is the most likely currency used by bees, I also examined what outcomes the metabolic odometer hypothesis would predict if bees were using the two other currencies. I calculated estimates of metabolic Net and metabolic Rate, analogous to the way described for Efficiency, and performed paired t-tests to compare te estimates for the two tunnels (Figure 7, p. 28; Figure 8, p. 29). For the currency Net, the values for the horizontal tunnel were significantly less than for the julesz tunnel. Thus, if bees were using a metabolic odometer to estimate this currency they should judge profitability to be higher in the julesz tunnel. For the currency Rate there was no significant difference between the horizontal and the julesz values. Thus, if bees were using a metabolic odometer to measure Rate they should have judged the profitability of the two tunnels to be equal. Table 6 (p. 30) summarizes these predictions of the metabolic odometer hypothesis and the predictions of the optic odometer hypothesis.

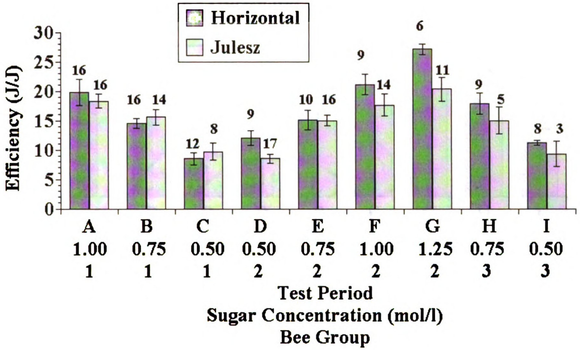

Figure 6 – Effect of tunnel within each test period on estimated metabolic Efficiency. This figure shows the average, SEM, and N of Efficiency in each tunnel in each test period. Average values were obtained by first averaging each bee and then averaging the bee averages. A Shapiro-Wilkes test for normality on the differences between the two tunnels generated a probability < W of 0.6427 indicating the distribution was normal. A paired t-test generated a two-sided $p > 0.05$ indicating there is no significant difference in Efficiency between the two tunnels.

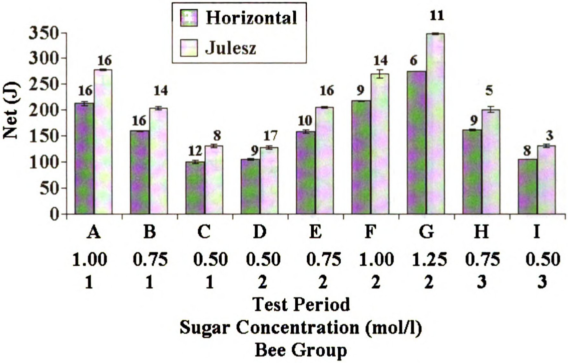

Figure 7 – Effect of tunnel within each test period on Net. This figure shows the average, SEM, and N of Net in each tunnel in each test period. Average values were obtained by first averaging each bee and then averaging the bee averages. A Shapiro-Wilkes test for normality on the difference between the two tunnels generated a probability < W of 0.7517 indicating the distribution was normal. A paired t-test generated two-sided p < 0.0001, indicating Net is greater in the optically longer julesz tunnel.

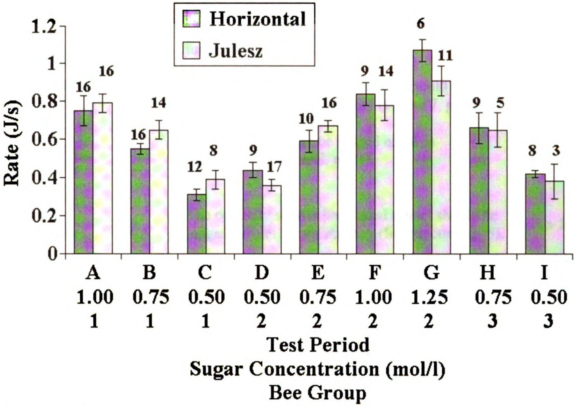

Figure 8 – Effect of tunnel within each test period on Rate. This figure shows the average, SEM, and N of Rate in each tunnel in each test period. Average values were obtained by first averaging each bee and then averaging the bee averages. A Shapiro-Wilkes test for normality on the differences between the two tunnels generated a probability < W of 0.5739, indicating the distribution was normal. A paired t-test generated a two-sided p > 0.85, indicating there is no significant difference in Rate between the two tunnels.

Table 6 – Predicted relative dance response of the horizontal (H) and julesz (J) tunnels under the metabolic and optic odometer hypotheses.

Hypothesis	Currency	Predicted relative dance response	
Metabolic Odometer	Efficiency	Both tunnels should be judged equal in profitability, with a non-significant trend of greater profitability in the optically shorter horizontal tunnel (Figure 6, P. 29).	H = J
Metabolic Odometer	Net	The optically longer julesz tunnel should be judged greater in profitability (Figure 7, p. 30).	H < J
Metabolic Odometer	Rate	Both tunnels should be judged equal in profitability (Figure 8, p. 31).	H = J
Optic Odometer	N/A	The optically shorter horizontal tunnel should be judged greater in profitability (Introduction, p. 5).	H > J

Having clarified the predictions of the metabolic and optic odometer hypotheses, I could determine how honeybees actually responded to the two tunnels. To capture each bee's measure of profitability I assessed two dance variables: number of waggle runs and probability of dancing (0 if no dances performed, 1 if any dances performed). I then assessed an average value of these variables for each bee and averaged the bee averages to obtain a value for each tunnel in each test period (Figure 9, p. 32; Figure 10, p. 33). Paired value tests revealed that both probability of dancing and number of waggle runs performed were significantly greater in the optically shorter horizontal tunnel.

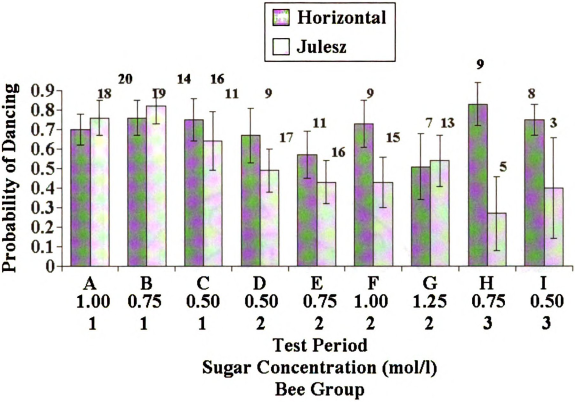

Figure 9 – Effect of tunnel within test period on observed probability of dancing. This figure shows the average, SEM, and N of observed probability of dancing in each tunnel in each test period. Average values were obtained by first averaging each bee and then averaging the bee averages. A Shapiro-Wilkes test for normality on the arcsin transformed differences between the two tunnel revealed a probability < W of .4051, indicating the distribution was normal. A paired t-test on the arrcsin transformed data generated a two-sided $p < 0.05$, indicating observed probability of dancing was significantly greater in the optically shorter horizontal tunnel.

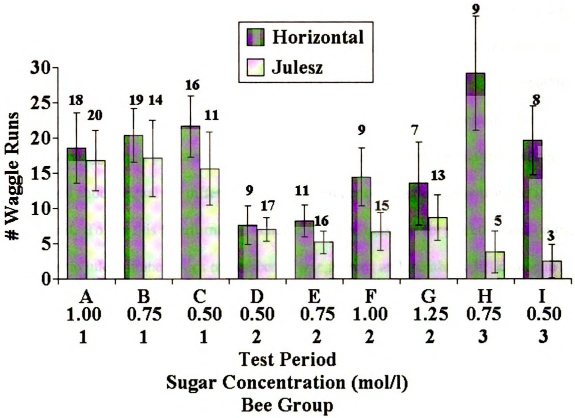

Figure 10 – Effect of tunnel within test period on number of waggle runs performed. This figure shows the average, SEM, and N of number of waggle runs performed in each tunnel in each test period. Average values were obtained by first averaging each bee and the averaging the bee averages. A Shapiro-Wilkes test for normality on the differences between the two tunnels generated a revealed a probability < W of 0.0181, indicating the distribution was non-normal. A Wilcoxon signed-rank test for paired values generated a two-sided $p < 0.005$, indicating the number of waggle runs was significantly greater in the optically shorter horizontal tunnel.

These results contradict the predictions of the metabolic odometer hypothesis (Table 6, p. 30). However, Efficiency was very near significantly predicting the optically shorter horizontal tunnel should be more profitable (Figure 6, p. 27), which would mean metabolic Efficiency and the optic odometer both predict the observed results. To clarify this situation, I took advantage of the linear relationship between number of waggle runs and Efficiency to test if the observed pattern of waggle runs is predicted by the metabolic odometer (Seeley, 1994). If bees were using metabolic Efficiency to determine the number of waggle runs performed, the difference between Efficiency measurements in each tunnel should equal the difference between number of waggle runs performed in each tunnel. To test this I performed a Wilcoxon signed rank test for paired values on the horizontal to julesz tunnel ratios of number of waggle runs and metabolic Efficiency in each test period (Figure 11, p. 34). The results show that the observed profitability difference between the two tunnels, as estimated by number of waggle runs, is greater than can be accounted for by metabolic Efficiency.

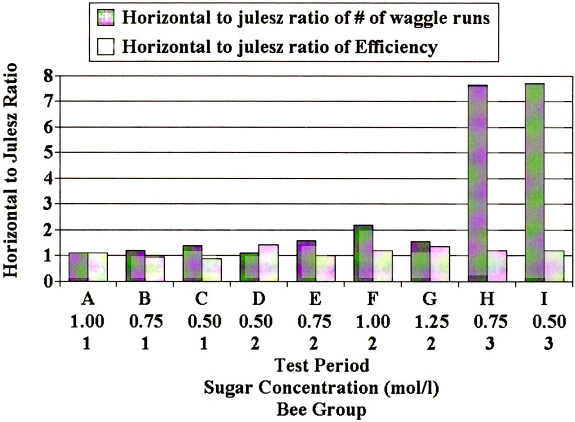

Figure 11 – Comparison of horizontal to julesz tunnel ratio of number of waggle runs performed to estimated metabolic Efficiency. Values were obtained by dividing the average horizontal tunnel value for each test period by the average julesz tunnel value (given in Tables 6 and 10 for estimated metabolic Efficiency and number of waggle runs performed respectively). Since ratios are non-normal, a Wilcoxon signed rank test for paired values was performed and generated a $p < 0.03$.

DISCUSSION

Research in foraging ecology has shown that a diverse array of species are sensitive to differences in food source profitability. Many studies have strived to discover which specific measure of profitability, or currency, an animal may use to make foraging decisions (for review see Stephens and Krebs, 1986). Unfortunately, the mechanisms by which animals perceive these currencies have remained largely inaccessible. My study attempted to determine which mechanism honeybees use to measure the cost associated with flight costs associated with of profitability.

Until recently, honeybees were believed to use a metabolic odometer to assess the flight distance reported in the waggle dance (von Frish, 1967). Yet, it has now been shown that honeybees use an optic odometer to measure the distance to a food source (for review see Esch and Burns, 1996). To determine whether bees use this optic odometer as part of their calculations for energetic profitability I constructed two tunnels of equal actual length, but differing in optically simulated length. I then observed bees' measure of profitability, indicated in their dance response, while they foraged at feeders of equal concentration in the two tunnels. The results show that bees measured the optically shorter tunnel to be more profitable. This result is consistent with the optic odometer hypothesis, and cannot be accounted for by the hypothesis that bees base their foraging decisions on a metabolic odometer. Thus, although we still cannot exclude the possibility that bees monitor metabolic expenditures in some circumstances, my results taken together with those of Esch and Burns (1996) and Srinivasan et al. (1996, 2000) suggest that there is no basis for assuming that bees measure flight distance by monitoring energy expenditure.

Note that my data do not exclude the possibility that bees use a time odometer. This is because their flight time is shorter in the tunnel deemed more profitable. However, a time based odometer has been excluded as a means of measuring to a food source (Srinivasan et al., 1996). Thus, such a mechanism seems unlikely to explain my results.

Not only do my data illuminate the process by which bees regulate recruitment to a food source, but they also may shed light on other foraging decisions. For examplem it is common for foraging honeybees to only partially fill their honey stomach (crop). The reason behind this has been a puzzle for several decades (Nunez, 1982; Kacelnik et al., 1986; Varju and Nunez, 1991; Schmid-Hempel; 1993; Schmid-Hempel et al, 1985; Moffatt, 2000). There are two main hypotheses as to why honeybees only partially fill their crops: informational exchange and maximization of Efficiency. The informational exchange hypothesis is that honeybees leave a food source with a partially filled crop in order to spread information about the food source to other bees in the hive (Nunez, 1982; Varju and Nunez, 1991; Moffatt, 2000). The Efficiency hypothesis is that honeybees partially fill their crops in order to maximize the Efficiency of the foraging trip (Kacelnik et al., 1986; Schmid-Hempel, 1993; Schmid-Hempel et al., 1995).

One of the arguments that has been used both to support (Wolf et al., 1989) and to cast doubt (Moffatt, 2000) on the maximizing Efficiency hypothesis is now made questionable by some of the findings in my study. The first premise of the argument is that in order for Efficiency [(G-C)/C] to predict variable crop loads, costs must increase with increasing crop load. The second premise is that costs are measured directly via metabolic rate. This argument has been used against the maximizing Efficiency hypothesis in studies that found metabolic rate (and hence cost) does not vary with crop load (Moffatt, 2000), and it has been used to support the maximizing Efficiency hypothesis in studies that found metabolic rate to vary with crop load (Wolf et al., 1989).

The results of my study undermine the second premise in this argument. In my experiments I induced bees to take on significantly different crop loads by exposing them to different amounts of optic flow on the flight to the feeder, but without a substantial difference in metabolic expenditure (Tables 3, p. 20, and 4, p. 21). This implies that honeybees are using optic flow instead of metabolic expenditure to judge the costs. If metabolic rate does not change with crop load it only implies that honeybees are not maximizing metabolically measured efficiency. They could still be maximizing their perceived efficiency if they were adding some aspect of crop load to their optical measure of costs for the flight home. Studies done with lead weights lend some support to this idea (Schifferer, 1952 as reported by von Frisch, 1967; Schmid-Hempel, 1986). By taking into account honeybee's optic measure of costs, future studies could clarify why honeybees only partially fill their crops.

The partial crop filling debate clearly shows the value of knowing the mechanisms underlying adaptive behavior. Without knowledge of these mechanisms one might be misled about decisions based on sources of information available to the animal. Furthermore, knowing the mechanism allows more accurate control of a variable during experiments. For example, to determine how precise honeybee profitability estimates are, one could decrease measurement error by precisely controlling the optic flow of the foraging flight. Also, my results suggest that studies of the energetic gains and costs involved in foraging decisions may have to deal with animals that use non-energetic modalities to measure gains and costs.

The discovery that honeybees' primary measure of distance related foraging costs is not a metabolic odometer also raises interesting questions about the neural mechanisms underlying the bees' estimation of profitability. My data taken together with previous work suggest that bees base their responses to food on a combination of optical and gustatory cues (sugar concentration and flow rate) (Waddington, 1990; Nunez, 1982). This raises the question of where and how in the brain this input is combined with the other components to modulate the profitability signal of the waggle dance.

In addition to the result that honeybees use an optic odometer to measure the cost of flight component of profitability, this study highlights the promise of using tunnels to answer previously inaccessible questions. Tunnels have previously been used almost exclusively to answer navigational questions (Srinivasan et al., 1996; Srinivasan et al., 1997; Srinivasan et al., 1998; Srinivasan et al., 2000; Esch et al., 2001). However, tunnels hold promise for investigating questions such as those raised in the partial crop filling debate as well as other areas of honeybee foraging ecology and energetics. Tunnels also show promise due to their logistical appeal. By simulating long distances within small enclosed areas, it is much easier to control for variables such as the amount of available forage, and often unnecessary to wait for days for the correct weather and flowering conditions as has been done previously (von Frisch, 1967; Seeley, 1995). Finally, as my results suggest, it may now be more feasible to study the sensory and integrative mechanisms underlying foraging decisions that are expressed in flight over many hundreds or thousands of meters.

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