# How to Design Experiments in Animal Behaviour*

## 4. How Do Bees Estimate the Distance Flown?

*Raghavendra Gadagkar*

**This article describes simple experiments that show that honey bees estimate the distance they have flown, by means of 'optic flow', i.e., the extent of image motion experienced by their eyes. The estimated distance is then communicated to the bees at home through the tempo of their dance (number of dance circuits in 15 s) or the duration of the waggle phase in each circuit. The experiments also provide strong evidence against the previously held view that distance is estimated by the amount of energy consumed during the flight. These experiments illustrate how cutting-edge research is possible with little or no facilities, equipment or money, by asking the right questions, optimizing the design of the experiments and regarding previously fashionable theories with an appropriate degree of scepticism.**

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**Keywords**
Animal behaviour, experimental design, waggle dance, round dance, tempo of dance, optic flow.

### 1. Why are Social Insects Ideally Suited for Experiments in Animal Behaviour?

Insects, especially social insects, such as ants, bees and wasps, prominently feature in most of the experiments I will describe in this series. And there are very good reasons for this. Insects are everywhere, easy to observe and handle, and luckily, no one cares very much if you do some playful experiments with them. They are, therefore, ideally suited to fulfil the objectives of this series; doing simple, curiosity-driven experiments with little or no expense. Social insects are even better – they live in groups, often very large groups, facilitating experiments that require many trials and large samples. Besides, social insects construct elab-

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orate nests in which they spend most of their time, store food and rear their young. For this reason, they regularly travel to and away from their nests in a predictable manner – they are tellingly called 'central place foragers'. Not surprisingly, social insects have helped us understand how insects and other animals perceive the world around them and respond in adaptive ways. Honey bees and ants have been especially helpful in this regard, and in the next two articles, we will see how they have helped us understand how animals can estimate the distance they have travelled. In this article, we will see how simple experiments have helped us understand how honey bees estimate the distance they have flown, and in the next article, we will see how equally simple experiments have helped us understand how ants estimate the distance they have walked.

## 2. How do Honey Bees Assess How Far They Have Flown?

The better known aspect of the honey bee dance language is that the bees determine the direction of the food source by measuring the angle subtended at the hive, between the azimuth of the sun and the source of food, and convey this angle to bees at home as the angle at which they perform their waggle dance, relative to the line of gravity. The less-celebrated aspect of the dance-language is that the bees somehow estimate the distance they have flown and communicate that too, by the duration of their waggle dance.

We saw in the second article in this series that Karl von Frisch discovered that honey bees have colour vision. von Frisch is even more famous for discovering that by means of a dance-language, honey bees can communicate the distance and direction of the food source they have found to naïve bees back home. The better known aspect of the honey bee dance language is that the bees determine the direction of the food source by measuring the angle subtended at the hive, between the azimuth of the sun and the source of food, and convey this angle to bees at home as the angle at which they perform their waggle dance, relative to the line of gravity [1]. The less-celebrated aspect of the dance-language is that the bees somehow estimate the distance they have flown and communicate that too, by the duration of their waggle dance. von Frisch produced a classic graph plotting the distance from which he had trained his bees to seek a reward, against the waggle duration [2]. He measured the waggle duration (or the inverse of it) by simply counting the number of dance circuits the bees performed in a 15-second interval, a level of accuracy that was both feasible and adequate, at the time. Today, and as we shall see below, we can measure waggle duration much better and much more pre-

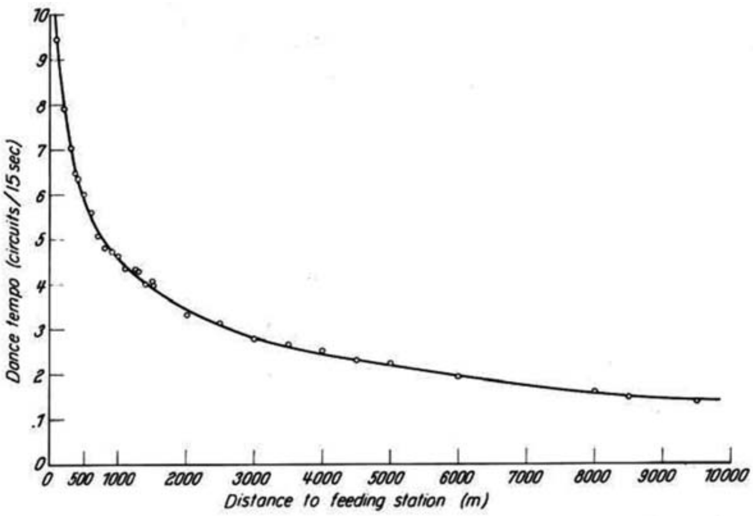


**Figure 1.** The tempo of bee dances decreases with increasing distance being conveyed. Reproduced with permission *The Dance Language and Orientation of Bees* by K von Frisch, translated by Leigh E. Chadwick, Cambridge, Mass.: The Belknap Press of Harvard University Press, Copyright 1967, 1993 by the President and Fellows of Harvard College.

cisely but as even Aristotle knew, "It is the mark of an instructed mind to rest satisfied with the degree of precision which the nature of the subject permits and not to seek an exactness where only an approximation of the truth is possible", or sufficient, I would add. von Frisch termed the number of dance circuits in 15 seconds as the 'tempo' of the dance and showed that the tempo of the dance decreased with the distance of the food source (*Figure* 1). This inverse relationship between the tempo and distance makes sense as it conveys a sense of urgency and greater enthusiasm for nearby sources of food.

But how do bees estimate the distance they have flown, in the first place? von Frisch observed that bees flying against the wind indicated longer distances, while those flying with the wind indicated shorter distances, and those flying on windless days indicated intermediate distances. He (and others) also observed that bees with lead weights glued to their backs indicated longer distances compared to the normal bees. From these observations, von Frisch proposed the 'energy hypothesis' suggesting that bees estimate the distance they have flown by the energy expended in flying to the source of food. Many subsequent experiments, however, have failed to uphold the energy hypothesis. Nobel laureates can also be wrong of course. We must remember that science is al-

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ways a work in progress and that all conclusions are provisional. Anyone, however great or small, can call to question, anything in science, using the scientific method, at any time. And so, others have called von Frisch's energy hypothesis into question and proposed and supported an alternate hypothesis, the so-called 'optic flow hypothesis'.

## 3. What is the Optic Flow Hypothesis and How Do We Know It Works?

The optic flow hypothesis states that bees estimate distance by the image motion experienced by them, i.e, by measuring the extent to which the image of the surrounding landscape has moved on their eyes.

The optic flow hypothesis states that bees estimate distance by the image motion experienced by them, i.e, by measuring the extent to which the image of the surrounding landscape has moved on their eyes. This is an attractive, almost common-sense idea. A good way to illustrate this is to recall what Sherlock Holmes told Watson in *Silver Blaze*, a remarkable tale of Holmesian deduction in the collection, *The Memoirs of Sherlock Holmes* by Sir Arthur Conan Doyle. Let me quote the relevant passage:

*"And so, it happened that an hour or so later I found myself in the corner of a first-class carriage flying along en route for Exeter, while Sherlock Holmes, with his sharp, eager face framed in his ear-flapped travelling-cap, dipped rapidly into the bundle of fresh papers which he had procured at Paddington. We had left Reading far behind us before he thrust the last one of them under the seat and offered me his cigar-case."*

*"We are going well," said he, looking out the window and glancing at his watch. "Our rate at present is fifty-three and a half miles an hour." "I have not observed the quarter-mile posts," said I. "Nor have I. But the telegraph posts upon this line are sixty yards apart, and the calculation is a simple one."*

The idea of estimating the distance from image motion has the added advantage of being independent of the speed of the flight. But do the bees actually do this? Can they do this? Interestingly, it is von Frisch who first seems to have considered 'optic flow' as a hypothesis. He observed that bees flying over a water surface which is unlikely to give as strong an optic flow as the ground

**Figure 2.** Mandyan Veerambudi Srinivasan (1948–). Image Credit: M V Srinivasan.

or vegetation indicated a shorter than expected distance. Nevertheless, he favoured the energy hypothesis over the optic flow hypothesis but without actually ruling out the latter from playing at least ‘a modest part in estimation of the distance’, as he put it. The optic flow hypothesis was formally and more confidently proposed by Herold E Esch and John E Burns of the University of Notre Dame in 1996 [3]. An early experiment that supported the optic flow hypothesis showed that bees from hives placed on one tall building foraging from feeders placed on another tall building indicated much shorter distances than bees flying a similar distance at the ground level. Bees flying at a higher altitude would be expected to experience much less optic flow on account of the ground and vegetation being farther away. Esch and Burns [3] provide an interesting survey of various experiments performed over the years, to test the energy hypothesis, and explain the circumstances under which they proposed the optic flow hypothesis.

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## 4. Definitive Experiment

The definitive experiment to test the optic flow hypothesis was performed by Mandyan Srinivasan (*Figure* 2) and his colleagues at the Australian National University, Canberra. Mandyan Srini-


**Figure 3.** The simple and elegant experiment by Srinivasan *et.al* made it to the cover of *Science* magazine (see [4]).
Credit: Coverpage, *Science*, Vol.287, No.5454, 2000.

vasan, who studied electrical engineering for his BSc from Bangalore University and MSc from the Indian Institute of Science, Bangalore, has performed some of the simplest and most elegant experiments on honey bees, and the one I will describe below is exceptional even by his standards. Notice that the experiment in which the bees had to fly from one tall building to another, the optic flow was reduced resulting in their underestimation of the distance. Srinivasan and colleagues increased the optic flow by training their bees to fly through a narrow tunnel lined with a random visual texture and asked if the bees overestimated the distance. They constructed a wooden tunnel 6.4 m long, 11 cm wide and 20 cm high. While the floor and the two walls of the tunnel were lined with a random visual texture, the top of the tunnel was covered with a black insect-screen cloth so that the bees had a view of the sky, and the experimenters could observe the bees from the top. The far end of the tunnel was closed so that the bees could only enter and leave from the end closer to the hive. This is all the experimental apparatus they needed, and they performed just four experiments. For each experiment, they trained about 6 individually marked bees to fly into the tunnel, collect a reward of sugar solution and return to their hive (*Figure* 3).

In the first experiment, they placed the tunnel described above, 35 m away from the hive (see *Figure* 4 for a diagrammatic representation of the 4 experiments). Although the tunnel was 6.4 m long, the feeder from where the bees could collect their reward was placed right at the entrance to the tunnel. This was a control experiment because the bees essentially flew outside the tunnel and were rewarded immediately upon entering the tunnel. Thus, the bees performed a normal outdoor flight for 35 m. Bees are known to perform round dances if they have travelled about 50 m or less. As expected, the probability of a round dance, as opposed to a waggle dance (which the bees perform when they have flown more than about 50 m from the hive) was 85.2% in this experiment. In the second experiment, they placed the feeder at the far end of the tunnel, 6 m away from the entrance. Now the bees had to fly a total of 41 m (35 m outside and 6 m inside the tunnel) in order to get the reward. If the optic flow hypothesis is valid, the bees should overestimate the 6 m flown inside the tunnel because the random visual pattern on the walls and floor of the tunnel was much closer than the ground or vegetation would be during the outdoor flight. If the bees did not overestimate the distance flown inside the tunnel, then they should perform predominantly round dances to indicate their flight of 41 m. If the bees did overestimate the distance of their flight in the tunnel, then they should perform predominantly waggle dances. It turned out that in this experiment, the probability of waggle dance was 90%, clearly indicating that the bees had overestimated the distance of their flight inside the tunnel. So far, this is merely a reconfirmation of what Esch and Burns had found previously – when bees fly at a greater height, there is less optic flow, and they underestimate the distance flown, while when they fly in a narrow tunnel, there is greater optic flow and they overestimate the distance flown. What makes this study the definitive one, providing clinching evidence in favour of the optic flow hypothesis is the next experiment.

## 5. Clinching Evidence

At first, it might appear that the results of the experiments where bees flying above tall buildings underestimated the distance and

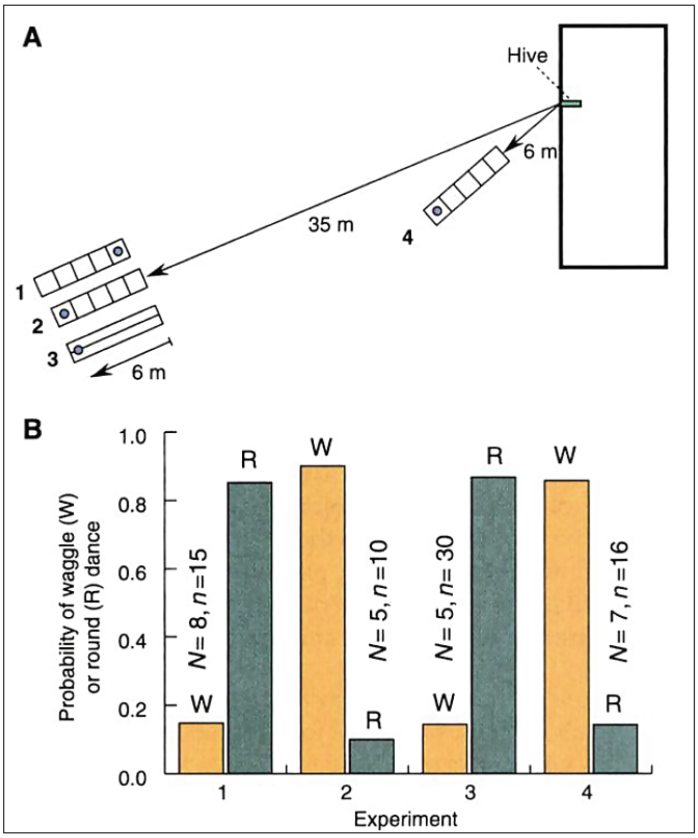


**Figure 4.** Four experiments using tunnels. **(A)** Layout for experiments using tunnels. Each tunnel represents a separate experiment (1, 2, 3, or 4). The dot in the tunnel shows the position of the feeder in each case. **(B)** Probability of waggle (W) round (R) dance for experiments 1 to 4. *N* and *n* represent the numbers of bees and dances analyzed, respectively, in each experiment. Reproduced with permission from Mandyan V. Srinivasan, Shaowu Zhang, Monika Altwein and Jürgen Tautz, Honeybee Navigation: Nature and Calibration of the Odometer, *Science*, The American Association for the Advancement of Science, Vol.287, No.5454, pp.851–853, 2000.

bees flying in a narrow tunnel overestimated the distance are adequate to uphold the optic flow hypothesis. It is not easy to imagine what else could explain these results. But here we should not rely on our ability or inability to imagine alternative explanations for the observed results. We should accept that any number of hard-to-imagine confounding factors might explain the result – flying at abnormal heights, in narrow tunnels, a mixture of outdoor and tunnel flights, who knows what else can confuse the bees? In the case of the tunnel experiment, at the very least, we should ascertain that the tunnel did not somehow cause the overestimation, independent of increased optic flow. If we can show these bees flying in the tunnel will not overestimate distance in the absence of increased optic flow, that would be clinching evidence for the role of optic flow, independent of the tunnel.

How do we delink the tunnel from increased optic flow? Actually, it is easy. Srinivasan and colleagues performed a third experiment in which they placed the feeder 6 m away from the entrance of the tunnel as before, but they provided axially oriented stripes (parallel to the flight direction of the bees), which would be expected to produce almost no optic flow. If image motion is the real cause of the distance overestimation in experiment two, then the bees in experiment three should not overestimate the distance and should show predominantly round dances. Indeed, they do – the probability of round dances in experiment three was 86.7%. They then performed a fourth experiment, which was not critical in the same sense that the third experiment was, but one which made the results of experiment two more dramatic while also providing a replicate. In the fourth experiment, they repeated experiment two, but placed the tunnel itself closer to the hive, at a distance of only 6 m, as compared to 35 m in experiments 1 to 3. Now the bees flew a total of 12 meters, 6 outdoors and 6 in the tunnel (with random visual texture). And yes, even though the bees had flown only 12 m, the probability of waggle dances in this experiment was 87.5%. Taken together, the results of the four experiments provide strong, indeed, clinching evidence in favour of the optic flow hypothesis. Nevertheless, their concluding statement is modest: "We conclude from these experiments that distance flown is inferred on a visual basis, the primary cue being the extent of image motion experienced by the eye". It is obvious that these experiments not only provide strong evidence in favour of the optic flow hypothesis but also strong evidence against the energy hypothesis. The phrase "distance flown is inferred on a visual basis" is meant to make that distinction and is a definitive statement. Note, however, that the second part of the conclusion namely, "the primary cue being the extent of image motion" is less definitive and leaves open the possibility other visual cues (other than image motion per se) may play, a role, even if a minor one. This reflects the previously mentioned idea that some other, as yet unimaginable, confounding factor may exist and cannot be completely ruled out.

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By any standard, this was an exciting outcome and enough to justify rushing to publication. But Srinivasan and colleagues had the patience to reflect a bit more and realise that by doing a simple calculation, they could extract much more information out of their data. Since they had video-recorded the dances of the bees back at the hive in order to determine the percentage of round and waggle dances, they could also measure the actual duration of the waggle phases of their dances. Thus, their data consisted not only of the percentage of round and waggle dances, which specifies near versus far but also the waggle durations which indicate the distance flown as a continuous variable.

## 6. Calibrating the Honey Bee Odometer

The video records indicated that bees in experiment 2 that had flown a distance of 41 m (35 outdoors and 6 in the tunnel) showed a waggle duration of 529 ms and the bees in experiment 4 that had flown a distance of 12 m (6 outdoors and 6 in the tunnel) showed a waggle duration of 441 ms.

The video records indicated that bees in experiment 2 that had flown a distance of 41 m (35 outdoors and 6 in the tunnel) showed a waggle duration of 529 ms and the bees in experiment 4 that had flown a distance of 12 m (6 outdoors and 6 in the tunnel) showed a waggle duration of 441 ms. To know what distances these waggle durations indicated to the bees, we need to know the waggle duration of bees flying outdoors, to known distances. Hence Srinivasan and colleagues performed a series of separate experiments with two colonies of honey bees, where they recorded the waggle durations of bees flying 60, 110, 150, 190, 225, 340, 350 m in an outdoor habitat. These data permitted them to produce a 'standard graph' with the distance plotted on the X-axis and waggle duration on the Y-axis (*Figure* 5). The function of a standard graph is that if we know any value of either the X or Y-axis, we can read off the expected value on the other axis. Thus, we can see from *Figure* 5 that the waggle durations of 529 ms (in experiments 2) corresponds to 230 m of outdoor flight. Since the bees in this experiment had flown 35 m outdoors and 6 m in the tunnel, they seem to have perceived a flight of 6 m in the tunnel as equivalent to $230 - 35 = 195$ m of outdoor flight. Similarly, the 441 ms waggle dances of bees in experiment 4 corresponds to 184 m of outdoor flight. Since these bees had flown for 6 m outdoors and 6 m indoors, they seem to have perceived a flight of 6 m in

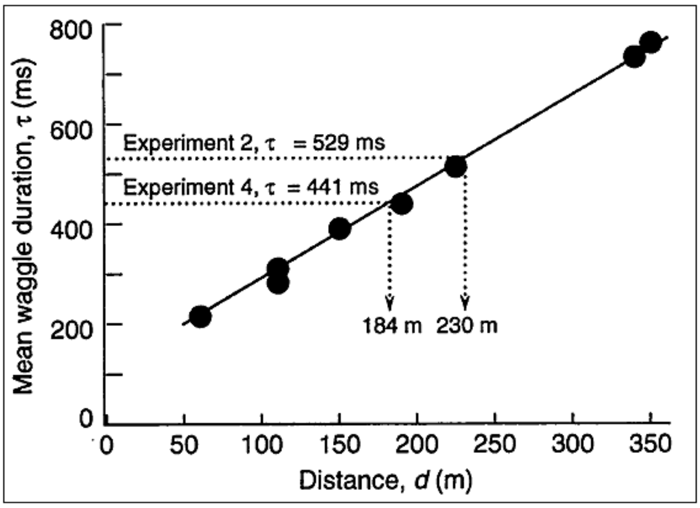


**Figure 5.** Distance–waggle duration calibration during outdoor flights. Mean waggle durations of dances elicited by outdoor feeders at various distances $d$. The straight line is a linear regression on the data, defined by the expression $\tau = 95.91 + 1.88d$. Also shown are the mean waggle durations measured in the tunnel experiments (experiments 2 and 4) and their equivalent outdoor flight distances as read off from the regression line.Reproduced with permission from Mandyan V. Srinivasan, Shaowu Zhang, Monika Altwein and Jürgen Tautz, Honeybee Navigation: Nature and Calibration of the Odometer, *Science*, The American Association for the Advancement of Science, Vol.287, No.5454, pp.851–853, 2000.

the tunnel as equivalent to 184 − 6 = 178 m of outdoor flight. Taking the average of these two overestimates, bees perceive a 6 m flight in the tunnel as equivalent to 186 m of outdoor flight, a 31-fold overestimate. Since bees are known to fly in the middle of the tunnel (think of how they might use optic flow to stay in the middle), the calibration of their odometer is a simple calculation, and I shall describe it more or less in the words of Srinivasan *et. al*.

- The distance to each wall is 5.5 cm. Thus, 1 cm of forward motion in the tunnel would cause the image of the wall to move backward by an angle of 10.3° in the lateral visual field.
- Therefore, 6 m of forward motion in the tunnel would generate 6180° of image motion.
- A 6 m flight in the tunnel corresponds to a flight of 186 m in natural outdoor environment as we have seen above.
- 186 m of outdoor flight is encoded by a waggle duration of 350 ms (*Figure* 5).
- Therefore, 1 ms of waggle in the dance encodes (6180/350) = 17.7° of image motion in the eye.

By means of a few simple experiments, Srinivasan and colleagues could support the optic flow hypothesis and disprove the energy hypothesis. They were also able to produce a definitive calibration of the honey bee odometer.

By means of a few simple experiments, Srinivasan and colleagues were not only able to support the optic flow hypothesis and disprove the energy hypothesis, but they were also able to produce a definitive calibration of the honey bee odometer. Today it is widely accepted that honey bees estimate distance flown by assessing the extent of image motion in their eyes [5, 6].

## 7. Reflections

The simplicity of these experiments and their use of inexpensive locally available material to do cutting-edge research admirably illustrate the theme of this series – no one should feel that they are incapable of doing great science due to the lack of facilities.

The simplicity of these experiments and their use of inexpensive locally available material to do cutting-edge research admirably illustrate the theme of this series – no one should feel that they are incapable of doing great science due to the lack of facilities. I cannot imagine any educational institution in India, whether it is a high school, undergraduate college or University, let alone a research institute, that is so impoverished of resources that these experiments could not have been done there and that the discovery that honey bees use optic flow to estimate distance flown, could not have been made there. We must realise that our institutions are not so much impoverished of resources as they are of an intellectual and academic environment that is conducive to free inquiry into the laws of nature unfettered by notions of immediate utility and so-called 'national importance'. We must also inculcate in ourselves and our students, the idea that science is always a work in progress and that even the ideas of Nobel laureates can and should be called into question.

## Suggested Reading


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