# How to Design Experiments in Animal Behaviour

## 1. How Wasps Find Their Nests

*Raghavendra Gadagkar*

**In this series of articles, I will introduce the reader to the science of ethology, somewhat indirectly by describing simple experiments, both old and new, designed to understand how and why animals behave the way they do. My emphasis will be on the design of the experiments and my goal will be to motivate readers not only to think about the design but also to come up with alternatives and improvements. Motivated readers can indeed replicate some of these experiments even if they end up replacing the study animal or the behaviours of interest with their own favourite choices. In the first part of the series, I describe how Niko Tinbergen – Nobel Laureate and one of the founding fathers of ethology (the science of animal behaviour) – designed remarkably simple experiments to successfully understand how digger wasps find their own nests in a complex habitat also consisting nests built by other wasps.**

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**Keywords**
Ethology, beewolf, digger wasps, visual cues, olfactory cues, nesting, orientation, experiment design.

### 1. Ethology

I study animal behaviour and I am technically called an 'ethologist'. Ethology, literally the study of 'ethos' or character, is not a very old discipline. Charles Darwin's *The Expression of Emotions in Man and Animals* (1872) [1] may be considered as the first modern treatment of the subject. Notwithstanding the award of the Nobel Prize to three of the founders of modern ethology, Niko Tinbergen, Konrad Lorenz and Karl von Frisch in 1973, and the popular appeal of its subject matter, ethology does not always enjoy the prestige it deserves in the academia. For an aspiring ethologist, and one desirous of elevating its prestige, it is inspiring to read Peter and Jeanne Medawar, in a remarkable book entitled *A Philosophical Dictionary of Biology* [2], describe ethology in the following words:

"The word 'ethology' is not merely an alternate designation for the science of behaviour: it is a term that stands for a genuine revolution in biological thought. Ethology is rooted in observation of animal behaviour, an activity that only simpletons think simple....observation is a difficult and sophisticated process calling upon all the intellectual virtues: attention, patience, heightened awareness, caution in coming to conclusions, courage in framing expectations."

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– Peter and Jeanne Medawar

## 2. Experiments

These words can, of course, be taken as praise, but budding ethologists would be better advised to rather take them as a challenge – to measure up to Medawars' expectations. Let us focus on the process of observation, so elegantly described by them. I believe that we often need to perform 'experiments' prior to observation, to match the rigour that is being demanded. In this series, I will describe several experiments in ethology, both new and old. My focus will be on the 'design' of the experiments while the ethology learned in the process will be a collateral benefit. I will deliberately pick simple experiments that almost anyone can perform without requiring much instrumentation or other research infrastructure. The goal will be to use reasoning and logic rather than technology and automation and will require a passion for animals rather than for machines. I encourage readers to attempt to repeat these experiments, modifying them in any way you wish, guided by necessity and creativity, swapping the animals used and even the questions asked, with your own personal favourites [3]. As a general introduction to performing simple and elegant experiments, I encourage readers to study *Darwin's Backyard: How Small Experiments Led to a Big Theory* by James Costa [4].

### 3. Wasps and Their Nests

Wasps are a diverse group belonging to the insect order Hymenoptera, along with ants and bees. Most wasps are solitary while a small number of them are social. All social wasps build nests to lay eggs and raise their offspring and are carnivorous, preying on other insects or spiders (or any other meat if they can get hold of – they are known to steal meat from butchers' shops), to feed their growing larvae. They themselves persist on the nectar and juices imbibed while masticating their prey, as they cannot ingest solid food on account of their characteristically narrow waists. Among the solitary wasps, many are egg parasitoids laying their own eggs in or on the eggs of other insects. Others lay their eggs on the larvae or adults of other insects (or spiders). Let us consider the life cycle of one such solitary wasp using the example of *Philanthus triangulum*, that was used in the experiments I will discuss in this article. The genus *Philanthus* consists of about 135 species that are often called the 'beewolf' because they usually hunt adult honeybees. Along with other wasps with similar habits, they are more generally called the 'digger wasps'. *Philanthus triangulum*, male and female, emerge from their underground nests to begin a new life cycle. Males mate and die while the females have to do more to pass on their genes to future generations. When it is warm enough, the female wasps painstakingly and with much trial and error, locate suitably soft patches of ground and dig tunnels at angles of about $30^{o}$ followed by several lateral branches that serve as brood chambers. Then they fly out to hunt adult honeybees and sting them, carefully maneuvering their posture so as not to be stung by the bees instead. They paralyze the bees with a neurotoxic venom and fill up the brood chambers with up to six honeybees per chamber, as food for their as yet unborn larvae, laying one egg per brood chamber. The brood chambers are built, stocked with prey, and supplied with an egg, sequentially so that a mother may be at work on a nest for several days. At the same time, other female *Philanthus triangulum* wasps are doing the same nearby and herein lies a problem. How does a wasp find her own unfinished nest among the many

Spending what might seem like idle time outdoors, being a keen observer curious about how and why the natural world is what it is, is the first part of being an ethologist. The second part requires the ability to ask questions, frame hypothetical answers, make predictions arising out of those answers and design simple experiments to test the predictions.

that do not belong to her? Watching many wasps rapidly fly in and out of their respective nest entrances made Tinbergen wonder. Spending what might seem like idle time outdoors and being a keen observer curious about how and why the natural world is what it is, forms the first part of being an ethologist. The second part requires the ability to ask questions, frame hypothetical answers, make predictions arising out of those answers and design simple experiments to test the predictions.

## 4. Niko Tinbergen

Niko Tinbergen, one of the founders of modern ethology and one of the recipients of the Nobel Prize in 1973 (as mentioned above), possessed all these traits. But how did he come to possess them and how did he come to put them to good use? You can read about Tinbergen's life and work in the accompanying article by Sindhu Radhakrishna. Here, I will quote a few passages from an essay by Tinbergen's first PhD student Gerard Baerends [5], indicating the environment that Tinbergen was born in:

"In the Netherlands, between 1930 and 1940, ethology grew from what was originally seen as a pleasant and harmless hobby, to a new biological discipline, recognized by the academic world....In the 1880s, coinciding with a growing awareness of the need for a more socially just society, cultural attitudes towards nature changed. Literature and the fine arts became increasingly interested in a realistic representation of nature. Writers and poets....and sculptors....began to deal with landscapes, plants and animals in a style that took as much care with the correctness of naturalistic details as with the emotional impressions felt by the observer. Entirely new methods were developed for the teaching of children in primary schools, aimed at making them aware of the life and work of people in different communities and professions, and with particular emphasis on informing urban children about rural life. Inspired by this atmosphere two schoolmasters....began writing a series of six popular books, each dealing with the life of plants and animals in a characteristic Dutch habitat and a field guide for identifying the more common animals and plants....As a consequence of the increasing interest in natural history, naturalist societies were formed all over the country....A unique feature of the Netherlands – and one that in my opinion was very important for the development of ethology in our own country – was that young naturalists, from 11 to 23 years old, formed societies of their own, quite separate from those of adults."

Tinbergen devised six simple outdoor experiments to understand how the wasps located their nests.

## 5. Tinbergen's Experiments

Now, I will briefly describe six simple outdoor experiments performed by Tinbergen for his PhD thesis, in order to understand how the wasps located their nests [6].

### *Experiment 1*

In the first experiment, Tinbergen tested the hypothesis that the wasps learned and remembered the visual landmarks around their nests to distinguish them from other nests. Between 8 and 10 a.m., he placed about 20 pine cones that were lying around in the general area around a wasp's nest. In the afternoon, by which time the wasp might have learned the new landmarks around its nest, he waited for the wasp to fly out on one of its hunting trips and moved the circle of cones about 30 cm away from the original nest and around a sham nest made by "imitating fairly accurately the sandy spot and the slight depression indicating the (covered) entrance", to use his own words (*Figure* 1).

The idea was to see whether the returning wasp would go to the real nest, now without the pine cones, or to the displaced circle of pine cones around the sham nest. If visual cues of the landmarks around the nest were guiding the homing behavior of the wasp, then she should return to the sham nest with the pine cones. But if some other cues were being used, then she should return to her own nest in spite of the missing pine cones. Since the returning wasps would make her choice only once and Tinbergen did not want to test any wasp more than once, he used a clever trick to increase his sample size. After the wasp had unambiguously

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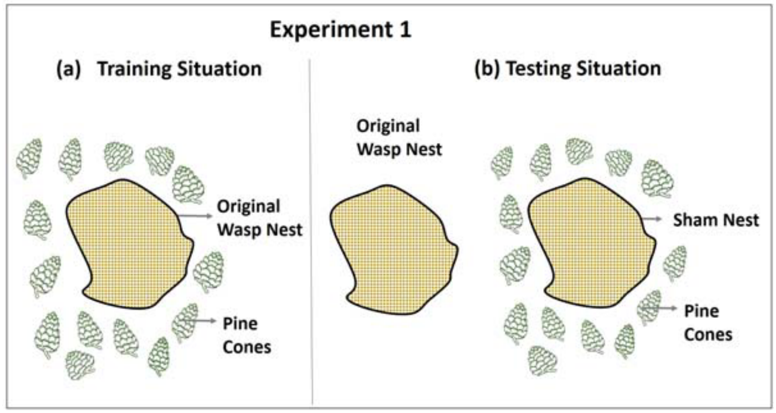


**Figure 1.** Cartoon depicting the arrangements in Tinbergen's first experiment. **(a)** Depicts the training situation during which Tinbergen had placed a circle of about 20 pine cones around the original nest in the morning. This allowed the wasp to learn these new landmarks for a few hours, during the course of its natural flights in and out of the nest. **(b)** Shows the test situation during which Tinbergen had moved the circle of pine cones from the original nest to a sham nest he had made about 30 cm away, leaving the original nest intact but without pine cones. Redrawn from Tinbergen (1932) (see [6]).

demonstrated her preference for the real or the sham nest but before she actually landed and dropped the bee she was carrying, he shooed her away gently, making her fly away some distance and try again. In this manner, he made the wasp demonstrate her preference at least five times. After shooing her away once more, he quickly relocated the circle of pine cones around the original nest and retested her preference, again several times. If visual cues were indeed involved, she should now switch her preference and go to her original nest, now with the pine cones returned. Tinbergen repeated the experiment with 17 different wasps, testing them five to twelve times each with pine cones around the sham nest (he called this the 'experiment'), and five to six times with the pine cones returned to the original nest (he called this the 'control'). His results (*Table* 1) could not have been more clear-cut. In 105 out of 105 trials, the wasps chose the sham nest when it had pine cones around it (experiment), and 86 out of 86 times, they chose the original nest when the pine cones were returned to it (control). This experiment showed clearly that the pine cones overwhelmingly decided the choice of the wasps. Tinbergen was well aware that this did not necessarily prove that the wasps had used visual cues, but only that they had used the pine cones.

| | **Results of Tinbergen's Control** *Training situation with the pine cones arranged around the original nest. See Figure 1(a)* | | **Results of Tinbergen's Experiment** *Training situation with the pine cones arranged around the sham nest. See Figure 1(b)* | |
|---|---|---|---|---|
| Wasp Number | No. of times the wasp returned to the original nest | No. of times the wasp returned to the sham nest | No. of times the wasp returned to the original nest | No. of times the wasp returned to the sham nest |
| 1 | 5 | 0 | 0 | 9 |
| 2 | 5 | 0 | 0 | 6 |
| 3 | 5 | 0 | 0 | 7 |
| 4 | 5 | 0 | 0 | 5 |
| 5 | 6 | 0 | 0 | 5 |
| 6 | 5 | 0 | 0 | 5 |
| 7 | 5 | 0 | 0 | 7 |
| 8 | 5 | 0 | 0 | 5 |
| 9 | 5 | 0 | 0 | 6 |
| 10 | 5 | 0 | 0 | 8 |
| 11 | 5 | 0 | 0 | 12 |
| 12 | 5 | 0 | 0 | 5 |
| 13 | 5 | 0 | 0 | 5 |
| 14 | 5 | 0 | 0 | 5 |
| 15 | 5 | 0 | 0 | 5 |
| 16 | 5 | 0 | 0 | 5 |
| 17 | 5 | 0 | 0 | 5 |
| Total | 86 | 0 | 0 | 105 |

**Table 1.** The results of Tinbergen's study showing the importance of pine cones in the choice of the wasps. The original data was published by Tinbergen in his 1932 publication [6].

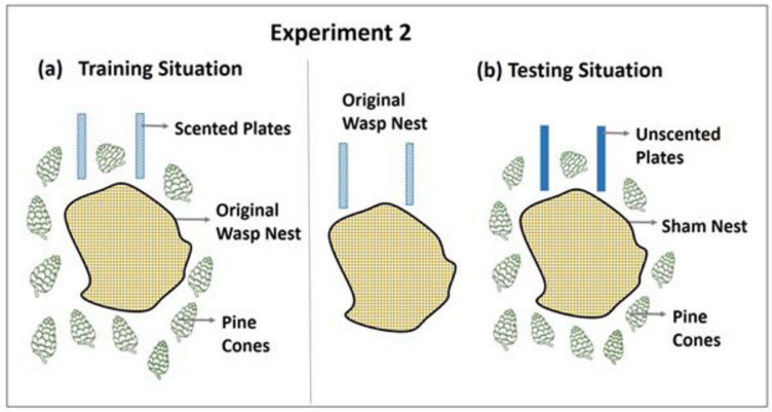


**Figure 2.** Cartoon depicting the arrangements in Tinbergen's second experiment. **(a)** Shows the training situation during which Tinbergen placed a circle of about 20 pine cones as well as a pair of scented plates around the original nest in the morning and allowed the wasp to learn these new landmarks (visual and olfactory), for a few hours, during the course of its natural flights in and out of the nest. **(b)** Shows the test situation during which Tinbergen had moved the circle of pine cones (visual landmarks) but not the scented plates (olfactory landmarks), from the original nest to a sham nest. To make the visual landmarks around the sham nest similar to those around the original nest during the training situation, he placed an identical pair of unscented plates around the sham nest. Redrawn from Tinbergen (1932) (see [6]).

***Experiment 2***

To rule out the possibility that the wasps had relied on the smell rather than the sight of the pine cones, Tinbergen did a second experiment. Now, during the training period, he placed along with the pine cones, two cardboard plates scented with pine-needle oil (*Oleum pini sylvestris*) that gives off smell characteristic of pine cones. The wasps could thus get accustomed to the sight of pine cones or the smell of pine cones around their nests, or both. During the test phase, he retained the scented plates around the original nest and moved only the pine cones to the sham nest. To mimic the visual cues of the scented cardboard plates he placed two identical cardboard plates but without scenting them, around the sham nest. Thus, the sham nest had all the visual cues and the original nest had the olfactory cues present during the training phase. After recording the choices of the returning wasps as in experiment 1, he created a control situation by interchanging the cues, i.e., he moved the pine cones and the unscented plates to the original nest and the scented plates to the sham nest and tested the wasps again (*Figure* 2). This time he used five different wasps and found that in 29 out of 29 trials, the wasps chose the sham nest when it had pine cones and unscented plates around it (experiment) and chose the original nest when it had the pine cones and unscented plates moved back to it (control) (*Table* 2).

Clearly, the pine cones won over the scented plates. However, Tinbergen was quite aware of a potential design flaw in the experiment. The scent from the scented plates may have been too strong leading to their rejection because the wasps might have been more accustomed to the smell of the real pine cones. Thus, the wasps may have been sensing the real pine cones by their smell after all. The real problem with this design was that Tinbergen had set up a competition between visual and olfactory stimuli rather than eliminate one of them altogether. In the third experiment, he set out to do the latter.

### *Experiment 3*

Tinbergen soaked the cones overnight in alcohol and dried them in the sun. Now he repeated experiment 1 taking care to train the wasps with fresh cones and test them with dried, presumably odourless cones. In 37 experimental trials and 30 control trials, the wasps never made a mistake – they always chose the cones. The operative phrase here is 'presumably odourless' which means he needed an even better experiment.

### *Experiment 4*

With fine scissors and forceps, Tinbergen amputated both antennae of four wasps but of course only after the training period. To his delight, these antenna-less wasps flew about and performed their usual homing behaviour. In 20 experimental trials and 20 control trials, not one mistake! Wasps that had learned the presence of the cones when they possessed their antennae chose the cones even after they had lost their antennae. It was clear that visual stimuli were enabling correct orientation of the wasps. Never throwing caution and modesty to the winds even as a PhD student, Tinbergen imposed on himself two caveats. First, these experiments may have shown that visual stimuli were adequate and even dominant, but they did not prove that the wasps were entirely incapable of using olfactory stimuli. Second, visual cues may have worked in his experiments, but he had only studied the

**Table 2.** The results of Tinbergen's study after training and testing the wasps with pine cones and scented plates. The original data was published by Tinbergen in his 1932 publication [6].

| | **Results of Tinbergen's Control** *Training situation with pine cones and scented plates around the original nest and unscented plates around the sham nest. See Figure 2(a)* | | **Results of Tinbergen's Experiment** *Test situation with pine cones and unscented plates around the sham nest scented plates around and the original nest. See Figure 2(b)* | |
|---|---|---|---|---|
| Wasp Numer | No. of times the wasp returned to the original nest | No. of times the wasp returned to the sham nest instead of original nest | No. of times the wasp returned to the original nest | No. of times the wasp returned to the sham nest instead of original nest |
| 1 | 5 | 0 | 0 | 5 |
| 2 | 5 | 0 | 0 | 5 |
| 3 | 5 | 0 | 0 | 6 |
| 4 | 5 | 0 | 0 | 8 |
| 5 | 5 | 0 | 0 | 5 |
| Total | 25 | 0 | 0 | 29 |

role of visual stimuli in close proximity to the nests. How did the wasps get close enough to see the pine cones in the first place? Tirelessly, he set out to explore these caveats.

***Experiment 5***

To test whether the wasps could be trained to recognize their nest by odor alone, he first confirmed that the wasps could indeed smell the oil he was using. He did this by putting some oil near the nest entrance. He observed that the wasps reacted quite strikingly to the presence of oil, twitching their body and flying away for some time. Then he repeated his experiment 2 only with the scented and unscented plates and without the pine cones. During the training, he placed two scented odor plates at the original nest, and this time he trained the wasps for 2 to 3 days instead of 2 hours as before. During the experimental phase of the test he placed the scented plates around the sham nest and the unscented plates around the original nest. For the control phase, he interchanged the scented and unscented plates between the sham and original nests. He found that the wasps which appeared quite confident while landing on their nest of choice with the pine cones – be it sham or original – in all the previous experiments, now seemed a bit confused. In this experiment, the wasps, in spite of being trained with the scented plates, chose the original nest with the unscented plates and ignored the sham nest with scented plates 21 out of 24 times. In the control, they once again chose the original nest with the scented plates 19 out of 20 times. In other words, they chose their original nests, with or without the scented plates and were not distracted by the presence of the scented plates around the sham nest. Thus, it appeared that the wasps could not be trained to use odor for nest recognition.

***Experiment 6***

Tinbergen realized that his success in training the wasps to find their nest using visual cues is relevant only when the wasp is already close to the nest (proximate orientation) but it doesn't explain how the wasps find the general areas where their nest is located (distant orientation). Admitting that distant orientation was very difficult to study experimentally in the field, he tried to determine the point where the distant orientation ended, and the proximate orientation began. To do this, he trained wasps with pine cones around their original nests at 30 cm as before. But in the test, he placed the cones at increasing distances around the sham nests, making bigger circles of pine cones, varying the diameter of the circle of pine cones around the sham nest from a diameter of 50 cm to up to a diameter of 200 cm (he had already

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tried 30 cm with success). The idea was that if the circle of pine cones was too big for proximate orientation then the wasp will not be able to find the sham nest (let us say, will not be distracted by it) and should search harder for their real nests. Although he was able to test fewer and fewer wasps at longer distances (due to bad weather), he found that the wasps always chose the sham nest with pine cones when the diameter was 50, 70 or 100 cm. But a single wasp that he was able to test at a diameter of 200 cm could not find the sham nest and went to the original nest after a long search. Tinbergen concluded that proximate orientation operated up to about 100 to 200 cm only, cautioning of course that this value will vary depending on the physical features of the environment.

Here, I will not describe the 4 other (rather inconclusive) experiments Tinbergen performed to test if the wasps used colour vision to find their nests.

In summary, Tinbergen concluded that "females of *Philanthus triangulum* are able to orient by means of visual landmarks once, through a yet unknown method of 'distant orientation', they have found the 'nest surroundings'. These occupy a roughly circular area of 1–2 m diameter, within which they can be misled by displacement of the landmarks in the immediate vicinity of the nest entrance."

## 6. Reflections

Let us now reflect on the set of six experiments as a whole. The first thing that comes to my mind is that Tinbergen vindicated Medawars' of any charge of exaggeration when they claimed that "observation is a difficult and sophisticated process calling upon all the intellectual virtues: attention, patience, heightened awareness, caution in coming to conclusions, courage in framing expectations." There are several useful lessons to be learned from these set of experiments. Where we can emulate Tinbergen, we must do so, and where we cannot, we must at least reflect on why we cannot. Scientists, especially during the early stages of their careers, are unsure about how to choose a problem to work on. Today, science has become such an 'industrial' and expensive activity that PhD students are not encouraged to and cannot afford to decide by themselves; their research problem is nearly always assigned by their thesis supervisors and research is almost always a collaboration between the student and the supervisor. But this was not how it always was and need not always be.

Tinbergen strolled around the woods and his curiosity about how the wasps managed to find their nest hole among so many others, was aroused. He framed hypotheses and proceeded to test them, designing the simplest possible experiments using what was readily available – a method that is sometimes affectionately called 'quick and dirty'. Tinbergen did not take detailed photographs of the nest surroundings and he did not try to reproduce the exact features of the nests around the sham nests. He did not apply for a big research grant nor did he try to make his research appear sophisticated. His experiments were not more sophisticated than absolutely necessary. They were literally playful. And yet, his experiments were designed very thoughtfully, with precision and imagination, yielding clear-cut results.

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Tinbergen's six experiments illustrate how we learn from our failures. When the wasps could not be trained with scented plates, he tried with de-scented cones, and when that failed he tried with antenna-less wasps. Even when he was successful with the circle of pine cones, he kept on increasing the diameter of the cone circles until he failed to train the wasps. Unfortunately, today it has become fashionable to discard negative results.

Tinbergen's modesty and caution come through clearly and are in stark contrast to the prevailing standards today. In his paper, he constantly refers to previous researchers, not just by way of introducing the subject but with a clear intention of giving credit where it is due. In his concluding remarks, he says less about what he has discovered and more about what he has not – "a yet unknown method of distant orientation", "did not succeed in demonstrating colour vision", "this in no way implies that *Philanthus* is unable to perceive colour", "wasps orient themselves to.....a complex of stimuli, which I have so far not analysed", "attempt to train the wasps to use olfactory stimuli was not successful" (rather than that the wasps cannot learn olfactory stimuli), "these results may not apply to other digging wasps", "my observations cannot decide whether *Philanthus* is able to register and remember the number of turns made on the way out".

I would like to recommend that readers reflect on the design of Tinbergen's six experiments, attempt to find flaws, come up with alternative designs which are just as good or perhaps better.

I would like to recommend that readers reflect on the design of Tinbergen's six experiments, attempt to find flaws, come up with alternative designs which are just as good or perhaps better. In addition to reflection, I encourage my young readers to try their hands at designing and carrying out simple experiments of their own, using animals and questions, driven by their own imagination and curiosity. In this article, wasps were the protagonists and they hunted and paralysed honeybees. In the next article in this series, I will restore the glory of the honeybees by making them the protagonists!

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## Suggested Reading

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