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CHAPTER 1

Avoiding Asphyxia

Every journey starts somewhere and sometime. My scientific journey began in my boyhood home, which is a little valley called Ellis Hollow. It lies a few miles east of the small city of Ithaca, in New York State. The time was early June 1963, and I was not quite 11 years old. There were not many houses in Ellis Hollow back then, so not many people used the road that runs through this valley. I enjoyed walking along it slowly, looking and listening. The soft songs of hermit thrushes, veeries, and other forest birds floated out of the woods that sloped up Snyder Hill to the south. The bubbling chatters of bobolinks shot from the unused hayfields that tilted down toward Cascadilla Creek to the north.

One morning, as I approached the massive black walnut tree (*Juglans nigra*) that stands beside Ellis Hollow Road near my parents' house, I heard something strange: a steady, buzzy sort of hum coming from overhead. I looked up and saw thousands of insects flying every which way among the walnut tree's widely spread limbs. Cool! Even cooler was what I noticed next: hundreds of honey bees were landing on this tree's lowest limb, covering an area about the size of a cafeteria tray. Sunlight glinting off their wings had drawn my eyes to their landing zone, about 10 feet (3 meters) up. When I approached to get a better look, I saw that the bees were walking toward and disappearing into a knothole. They were moving in! This black walnut tree had long been special to me—for its immense trunk, deeply furrowed and dark-brown bark, sprawling limbs, yellow-green leaves shaped like fern fronds, and nuts inside aromatic hulls that stained my hands dark brown—but now it was super special. It was a bee tree. Ever since I was a little boy, and had studied the drawing of a bee tree in A. A. Milne's book *Winnie-the-Pooh*, I had hoped to find a real bee tree. At last, today, I had.

I figured this bee tree would be fun to watch, and indeed it was. I went to it often that summer, to see what I could learn about the bees by watching them at the entrance to their home. I saw bees standing around the knothole. Are they guards? I saw bees, presumably foragers, flying out of the knothole. Where are they going? I wondered about the bees' nest hidden inside the thick limb. Where exactly are the beeswax combs? What do they look like? How much honey is in them? One time, I lugged my father's heavy wooden stepladder to the tree to watch the bees close-up. I didn't have a beekeeper's veil, so I didn't dare get close enough to peer straight into the knothole. I did, though, get close enough to watch bees flying home with loads of pollen attached (somehow) to their hind legs. Other bees stood nearly still in the knothole, facing outward with their front legs and antennae raised. They looked extremely alert, so I guessed they were standing guard.

I remember wondering whether the knothole (Fig. 1.1) provided a big enough "breathing hole" for the thousands of bees living the tree cavity. How come they don't suffocate? A few days later, I saw something that gave me a clue: about a dozen worker bees stood side-by-side along the bottom of the knothole, with their heads pointing into it, their bodies hunched over, and their wings whirring so fast that they were nearly invisible. It was a beautiful sight. The bees' light-brown bodies stood out against the tree's dark-brown bark, and each bee's wings hummed steadily, as if they were battery powered. These little fanners were still going strong when I stopped watching them about 10 minutes later. I had no doubt that they were expelling air from their home, but I could only guess *why* they were doing this. To cool it? To ventilate it? Perhaps both?

My mother must have noticed that I enjoyed watching the bees living in the black walnut tree, because next Christmas my parents gave me a

FIG. 1.1. The knothole in the lowest limb of the big black walnut tree near my boyhood home, where I first enjoyed watching honey bees up close. This nest entrance is approximately 1.5 inches (4 cm) in diameter.

book titled *The Makers of Honey*, written by Mary Geisler Phillips, a professor at Cornell University. It is a lovely, 164-page book, and I greatly enjoyed the hours I spent poring over it. The writing was the right "speed" for me and the finely crafted, scratchboard drawings in each chapter, by Elizabeth Burckmyer, delighted me. (They still do.) In Chapter 7, titled "Odd Jobs for Young Workers," Professor Phillips explains that one of the jobs of young workers is to be a fanner, and that "these fanners are air-conditioners . . . who keep the hive ventilated and at the right temperature." This description of fanners as "air-conditioners" satisfied my curiosity at the time. I suppose it also primed me to explore the behavior of these bees more closely when I got older.

My closer look at fanner bees came ten years later, in the summer of 1973. This was the summer following my third year as an undergraduate student at Dartmouth College, in New Hampshire. By then, I had taken courses in biology, chemistry, physics, and math, so I had picked up a fair amount of book knowledge and laboratory skills that I figured would be useful for what *really* interested me: studying honey bees. Also, by 1973 I had worked for three summers at the Dyce Lab for Honey Bee Studies at Cornell University (Fig. 1.2). The lab's director in those days was Professor Roger A. Morse. Everybody called him "Doc." Besides mowing the lawn, painting hives, and sometimes assisting Doc's graduate students and visiting scientists with their projects, I helped with the beekeeping. This was my favorite part of the job because it involved going to various apiaries and working with the bees. I continued to do laboratory chores in 1973, but that summer Doc said that I could devote some of my paid work-time to conducting a study of my own, on nest ventilation by fanner bees. Doc was a pretty gruff guy, but he was also supportive of his students . . . so long as they worked hard.

What spurred me to look closely at nest ventilation was something that I had seen back in September 1972, a few days before I would return to Dartmouth for the fall semester: rows of worker bees were fanning steadily at the entrances of my two hives *on a chilly evening of a rainy day*. This puzzled me because I figured that, given the conditions, these colonies didn't need fanners to cool the nest or to "ripen" fresh nectar into honey. (No colony has fresh nectar at the end of a rainy day.) I also figured that these fanners might, however, be ventilating their crowded home to avoid asphyxia. If so, then worker bees must be sensitive to either a lack of oxygen or an excess of carbon dioxide inside their nest.

Two days later, I did a simple experiment at Dyce Lab to see if a lack of oxygen stimulates worker bees to become fanners. The setup was easy. First, I moved a hive that housed a strong colony to a spot outside one of the laboratory's windows. Next, I drilled a quarter-inch diameter hole in the rear of this hive's upper box ("hive body") and inserted a glass tube through this hole so that that it poked into the center of the hive. Then, using a long rubber hose that snaked out the window, I connected the glass tube to a tank of compressed nitrogen inside the lab. The ex-

FIG. 1.2. The Dyce Laboratory for Honey Bee Studies at Cornell University, in summer 1998. Artist: Margaret C. Nelson.

periment started around 10:00 p.m. The air was cool, all the bees were at home, and I saw no fanners at the hive's entrance. I opened the tank's valve to send a gentle stream of nitrogen into the hive to displace the normal, oxygen-rich air inside. I figured that if worker bees are stimulated to start fanning by sensing a lack of oxygen, then what I was doing should elicit a strong fanning response. But it didn't. I neither saw nor heard any fanning bees. Displacing the oxygen from this colony's hive did, however, eventually narcotize the bees. I revived them by shutting off the gas and opening the hive. This experiment left me keen to find out how honey bees would respond if I sent a gentle stream of carbon dioxide into their home, but this follow-up experiment had to wait until the following summer.

What I could do in the meantime was find out what, if anything, previous bee researchers had reported on this subject. Back in the early 1970s, it was impossible to make a thorough search of the scientific literature—the launch of Google Scholar was 30 years in the future—but a kind librarian at Dartmouth helped me find two articles that contained information on the responsiveness of honey bees to carbon dioxide. The first was published in 1941 by a Dutchman, E. H. Hazelhoff. His report appeared in a Dutch beekeeping magazine, the *Maandschrift voor*

Bijenteelt [Monthly Journal for Beekeeping]. I could not locate a copy of this magazine and I could not read Dutch. So, all I knew about E. H. Hazelhoff's study was what a British entomologist, Dr. C. Ronald Ribbands, had written about it on page 212 in his 1953 book *The Behaviour and Social Life of Honeybees*: "Hazelhoff (1941) found that fanning commenced within one minute of the introduction of a stream of carbon dioxide in the hive." This finding intrigued me, for it was very different from what I had seen when I had introduced a stream of nitrogen. (Note: I remember thinking at the time [in November 1972] that E. H. Hazelhoff must not have done a rigorous study, for if he had then he would have published his report in a scientific journal, not a beekeeping magazine. At the end of this chapter, I will explain that I was *dead wrong* about the quality of Hazelhoff's study.)

The second article that I found on carbon dioxide in relation to honey bees was published in 1964 by a neurobiologist, Dr. Veit Lacher. He had worked in the Department of Comparative Neurophysiology in the Max Planck Institute in Munich, and he had published his study in a highly respected scientific journal, the *Zeitschrift für vergleichende Physiologie* [Journal of Comparative Physiology]. Lacher had made a detailed study of the sensitivity of the olfactory cells on the antennae of worker honey bees, and one of his discoveries was that some of these cells are sensitive *specifically* to gaseous carbon dioxide (CO_2). He reported that the threshold concentration for the response of these CO_2-sensitive antennal cells is about 0.50% (5,000 ppm) (Fig. 1.3). This is far above the level found in the atmosphere. Back in the 1960s, the atmospheric concentration of CO_2 was about 0.03% (300 ppm); today it is about 0.04% (400 ppm). I figured, though, that the 0.50% response threshold of the bees' CO_2-sensitive antennal cells might be just right for monitoring the gaseous CO_2 level inside the crowded nest of a honey bee colony.

In addition, Lacher reported a curious feature of the CO_2-sensitive cells: they keep firing for as long as they are stimulated. In the lingo of neurobiologists, these sensory cells have a "tonic response." This told me these cells could function very nicely as detectors of a dangerously high

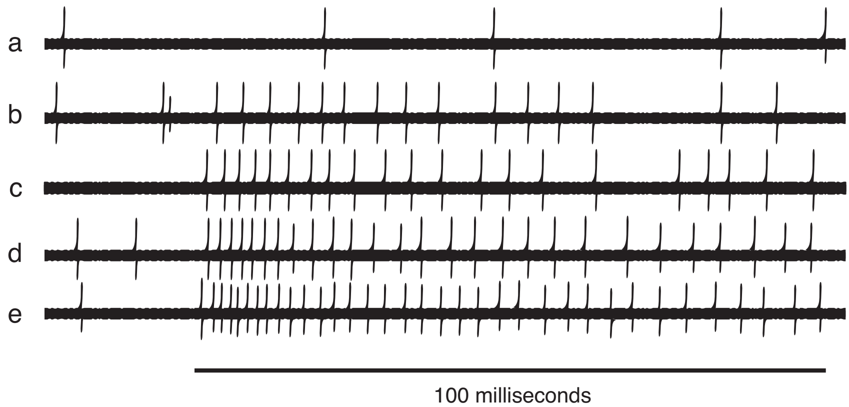

FIG. 1.3. Records of the electrical activity of an odor receptor cell (sensory neuron) in a worker bee's antenna that is sensitive specifically to CO_2. The top recording (a) shows the low rate of firing by this neuron when the antenna was exposed to air with 0.03% CO_2 (i.e., fresh air). The lower recordings (b–e) show how this odor-receptor cell started to fire more rapidly as soon as air with 0.5% CO_2 passed over it (line b) and increasingly so when exposed to air with 2%, 5%, and 10% CO_2 (lines c–e). In all cases, the cell fired steadily as long as air with elevated CO_2 passed over it. The bar at the bottom is a time scale.

level of CO_2 in the air inside a honey bee colony's home. As we all know regarding the smoke detectors in our homes, it is important that they keep sounding as long as there is danger.

Now I knew what I needed to do: (1) measure the gaseous CO_2 levels in bee hives, to understand what threats (if any) honey bees face from asphyxia by excessive CO_2; and (2) describe the response of worker bees to a high level of CO_2 inside their home. Would I see rises (and falls) in the gaseous CO_2 level in hives, and would I see corresponding rises (and falls) in the number of bees fanning their wings, as reported by Hazelhoff?

To address the first topic, I would need a sensitive CO_2 analyzer. I did not have one, but I figured that I could build one using an electronic sensor—a thermal conductivity (TC) cell—of the sort found in a gas chromatograph. I knew from my courses in organic chemistry that a TC cell works well for measuring tiny amounts of chemical compounds in samples of gases and (vaporized) liquids. I knew, too, that TC cells respond especially strongly to CO_2. Furthermore, I knew that a former graduate student in the Department of Entomology at Cornell, Dr. Larry J. Edwards, had built a sensitive CO_2 gas analyzer with a TC cell to study how insects breathe, and that he was now an assistant professor of entomology at the University of Massachusetts at Amherst. So, I mailed Professor Edwards a letter in which I explained my interest in his studies and requested a meeting to get his advice for my project. He sent a letter back, telling me when I could visit him at his laboratory in Amherst, Massachusetts. This was only about 100 miles (160 kilometers) south of where I was in Hanover, New Hampshire. I did not have a car, and getting to Amherst by bus was complicated, so I visited Professor Edwards by hitchhiking down (and back) on Interstate 91 in mid-December 1972. We talked, and when he understood my project and saw that I was pursuing it seriously, he did something that amazed me: he handed me the TC cell that he had used for his own studies (a GOW-MAC 133 Thermistor Cell). I will never forget his spontaneous act of generosity.

The next step was to build the electronic circuit that would convert the outputs of the TC cell into a voltage that could be fed into a strip chart recorder to produce a hard copy readout of the amount of CO_2 in an air sample. I wrote to the manufacturer of the TC cell, in Bethlehem, Pennsylvania, for information, and somebody there was kind enough to send me the electronic circuit's design (a "Wheatstone bridge"). I still have those instructions. Then a friendly professor in the Department of Physics at Dartmouth, Dr. William (Bill) Doyle, helped me build the circuit. So, when I returned to the Dyce Lab the following June, I had with me a homemade, but sensitive CO_2 analyzer. It enabled me to measure, with 1-microliter precision, how much CO_2 was in an air sample. Given this sensitivity, and given that I would work with small (25-milliliter, or about 1.5-cubic-inch) samples of air drawn from my study colonies, I knew that my measurements of the CO_2 levels in my samples would have a precision of 0.004%. As we shall see, this level of precision was sufficient.

This became clear as soon as I began measuring the gaseous CO_2 levels inside the hives of bees. I knew, of course, that honey bees live crowded together in their homes—tree cavities and bee hives—so I expected to find inside those homes levels of CO_2 that were higher than the 0.03–0.04% level of CO_2 in normal air. I was astonished, however, when I made my first measurements and learned how incredibly "stuffy" it can get inside a bee hive. To make these measurements, I set up two colonies side-by-side outside the Dyce Lab. Each colony was housed in a 10-frame Langstroth hive. One colony was large, so that in the evening (when all of its bees were at home) its adult bees covered thickly all 10 frames of comb in its hive. This colony's hive also contained many thousands of immature bees; eggs, larvae, and pupae nearly filled the cells in 7 of its 10 frames of comb. The other colony was smaller. Its adult bees covered only 5 of its 10 frames of comb when everybody was at home, and it had only 3 frames of comb whose cells held brood. To collect air samples from the centers of these hives, I installed in each one a glass tube that extended to the hive's center and poked out its rear wall. I used a rubber suction bulb to pull air samples (held in small glass flasks) from inside these hives.

I made my first measurements of the CO_2 levels in these two hives in June 1973. I did so by extracting, and then immediately analyzing, an air sample from each hive once an hour, from 9:00 p.m. on June 21 to 9:00 a.m. the next day. I worked at night because I wanted to see how stuffy it gets inside a hive when the entire colony is at home. The results, shown in Figure 1.4, surprised me in two ways. First, I found that the average CO_2 levels inside the hives of the large and small colonies were 0.55% and 0.92%, respectively. These readings were approximately 20 and 30 times higher than in the fresh air outside the hives. Yikes! This showed that the homes of honey bees can be extremely stuffy, at least by human standards. Breathing air with a CO_2 level of just 0.50% can make us quite drowsy. (This is why 0.50% CO_2 is the recommended limit for workplaces in the United States.) Second, I found that the CO_2 level varied far less in the hive of the larger colony (range: 0.39%—0.72%) than in the hive of the smaller colony (range 0.33%—1.77%).

After I took each air sample, I looked (using a flashlight) for fanners in the entrance of each hive. I am glad I did, because these inspections

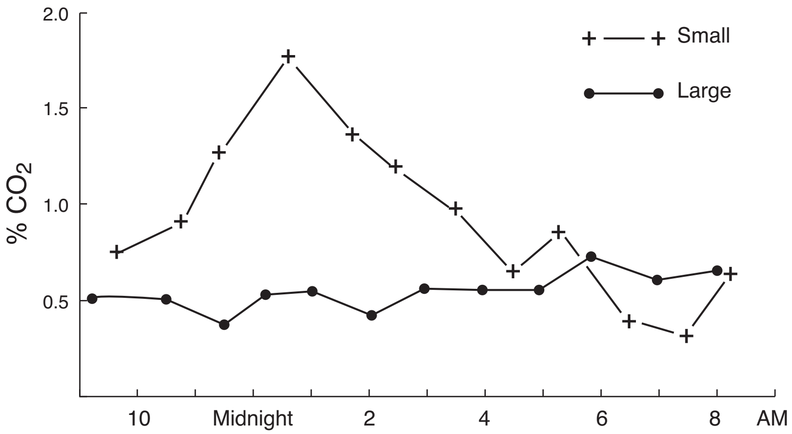

FIG. 1.4. Comparison of the levels of gaseous carbon dioxide in the hives of a small colony and a large colony throughout a cool night.

explained a lot about the difference in average CO_2 level between the two colonies. At the large (and crowded) colony, I *always* saw bees fanning at the entrance and heard the "roar" of more fanners working inside the hive. Two recent, high-tech studies of nest ventilation by honey bee colonies, by Jacob Peters and his colleagues at Harvard University, have shown that when workers stand at the nest entrance and ventilate their home, they use a special wing-fanning behavior (Fig. 1.5) that is different from the wing-flapping motions they use for flight. For example, the frequency and amplitude of the bees' wing movements are markedly different when they are fanning (174 Hz and 118°) than when they are flying (227 Hz and 87°). These studies also found that the stream of air that shoots from a hive's entrance can have a velocity of more than 10 feet per second (3 meters per second), which is 6.8 miles per hour (11 kilometers per hour). This is powerful ventilation!

At the smaller (and less crowded) colony, however, I *never* saw or heard fanners. I suspect that this colony was struggling to keep its brood warm, so was unable to keep its nest well ventilated throughout the cool night. This was certainly the situation between midnight and 2:00 a.m., for then its CO_2 level rose above 1.5%, which is high enough to make a human breathe fast and hard. I looked the next morning for dead bees in front

FIG. 1.5. Bees fanning their wings at their hive's entrance on a hot day.

of this hive, but I found only four—no more than usual. This showed me that honey bees can tolerate levels of CO_2 that are very stressful for human beings. This experiment also revealed something that, if you are a beekeeper, you may find surprising: when the outside air is cool, the air quality inside the hive of a small colony can be much poorer than that inside the hive of a large colony. This is because a small colony sometimes struggles to keep its home both properly warmed and suitably ventilated.

Besides the experiment just described (and various replicates of it), I did another experiment to check the accuracy of what the British biologist, C. Ronald Ribbands, had written when he summarized the work of E. H. Hazelhoff: "Hazelhoff (1941) found that fanning commenced within one minute of the introduction of a stream of carbon dioxide in a hive." For this study, I used a small observation hive that I built and then set up inside a heated room at Dyce Lab. I connected this hive to a tunnel through the building's wall, so the bees living in my hive could go outside and come back in as they wished. I stocked this hive with one frame of comb that contained brood, pollen, and honey, and that was covered with some 2,000 worker bees, one queen, and about a dozen drones. When I built this hive, I gave it two tubes, one at the top of the

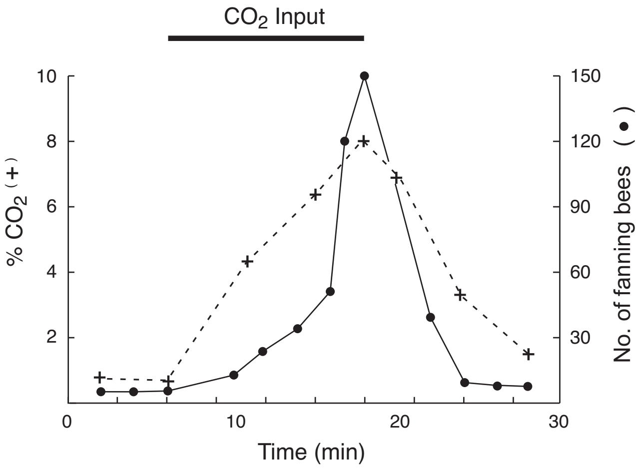

FIG. 1.6. Fanning response to induced rise in the carbon dioxide level in the air inside an observation hive. The numbers of fanning bees shown are based on counts of these bees inside the hive.

comb for introducing a gas (carbon dioxide or nitrogen), and the other halfway down the comb, for taking air samples from inside the hive. To perform a trial, I waited until it was night (when all the bees were at home); then I began extracting samples of the air in this hive and making counts of the bees in it that were fanning their wings. Once I had determined the baseline levels of CO_2 and of the bees' fanning, I introduced a light stream of either carbon dioxide (for about 10 minutes) or nitrogen (for about 30 minutes, long enough to displace the oxygen and narcotize the bees).

The results for the CO_2 trials—shown for one trial in Figure 1.6—confirmed Hazelhoff's report. Introducing a gentle stream of CO_2 stimulated strong fanning. But introducing a stream of nitrogen, even a strong one, did not stimulate fanning. This showed me that the bees' fanning response in the CO_2 trials was triggered by the CO_2 per se, not simply by the disturbance of introducing a gas.

I like very much the phrase "a canary in the coal mine," which refers to an early indicator of some danger or failure. Coal miners took caged canaries into mines as sentinels to tell them when the level of carbon monoxide or carbon dioxide had grown dangerously high (from the slow but steady oxidation of coal exposed to air). If the canaries stopped singing, then the miners knew that they needed to get out! In this chapter, we have seen that honey bees, like coal miners, face a danger of asphyxiation from carbon dioxide, and that they, too, have sentinels—the CO_2-sensitive olfactory cells on their antennae—to tell them when to take action to deal with the problem. We have seen, too, that for honey bees, as for coal miners, the best solution to this problem is to boost the ventilation of their workplace.

Postscript: In 2018, while writing my book *The Lives of Bees*, I discovered several important things about E. H. (Engel Hendrik) Hazelhoff. First, I learned that he was not a beekeeper, but rather a distinguished professor of zoology in the Department of Natural Sciences at Groningen University in the Netherlands. Second, I learned that he died unexpectedly and young (age 45 years) on 30 September 1945. Third, I learned that the study of bee hive ventilation that he reported in 1941, as a series of four articles in the Dutch beekeeping magazine *Maandschrift voor Bijenteelt*, was republished posthumously in English in 1954, as a 26-page paper in the scientific journal *Physiologica Comparata et Oecologia*. It shows that Hazelhoff made a meticulous study of what he called "the social regulation of the respiration of a honey bee colony." He built a sophisticated hive which he could cool or heat, monitor for internal temperature and gaseous carbon dioxide level, and ventilate at different levels by adjusting the rate of airflow through it. Using this hive, he conducted a rigorous study of the conditions inside a hive that stimulate ventilation fanning by bees: either a high temperature or a high concentration of gaseous CO_2. There is no doubt that Engel H. Hazelhoff was the first person to show that a high level of gaseous CO_2 in the home of a honey bee colony triggers fanning by its worker bees.

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