CHAPTER 1

H. B. D. Kettlewell & the Peppered Moths

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INTRODUCTION

When he wrote his greatest book, *On the Origin of Species* (1859), Charles Darwin set out to accomplish two goals. First, he wanted to demonstrate that species change over time through the process of organic evolution. Second, he wanted to convince his readers that this evolutionary process occurs primarily as the result of natural selection. Eventually, both of these goals were achieved, but during his lifetime Darwin enjoyed only a partial victory. By the time of his death in 1881, virtually the entire scientific community had accepted the fact of evolution. But only a few biologists believed that natural selection was the primary cause of evolutionary change. For half a century after Darwin's death, biologists debated several alternative theories of evolution.

The eventual acceptance of natural selection depended heavily upon genetics, which provided a convincing explanation for the origin and spread of hereditary variations. Darwin realized the importance of heredity for natural selection, but he had no satisfactory explanation for it. By 1920 the basic principles of Mendelian genetics were well established. Combining the theory of natural selection with these new concepts of heredity, mathematical theorists demonstrated that evolution could occur as Darwin had claimed. This combination of Mendelism and Darwinism also caused the decline of alternative theories of evolution. By World War II, most biologists had rejected once-popular evolutionary ideas such as large-scale mutations or the inheritance of acquired traits. The Darwinian revolution was complete.

The evidence for natural selection was overwhelming, but most of it was indirect. The widespread acceptance of the theory, therefore, raised intriguing questions. Could biologists discover cases of populations evolving through natural selection? Darwin and many of his followers assumed that evolution was such a gradual process that it might take decades or centuries to detect changes in a population. On the other hand, some theoretical biologists predicted that populations could evolve very rapidly if natural selection was intense (see Chapter 15). Could suitable populations be found to test this prediction?

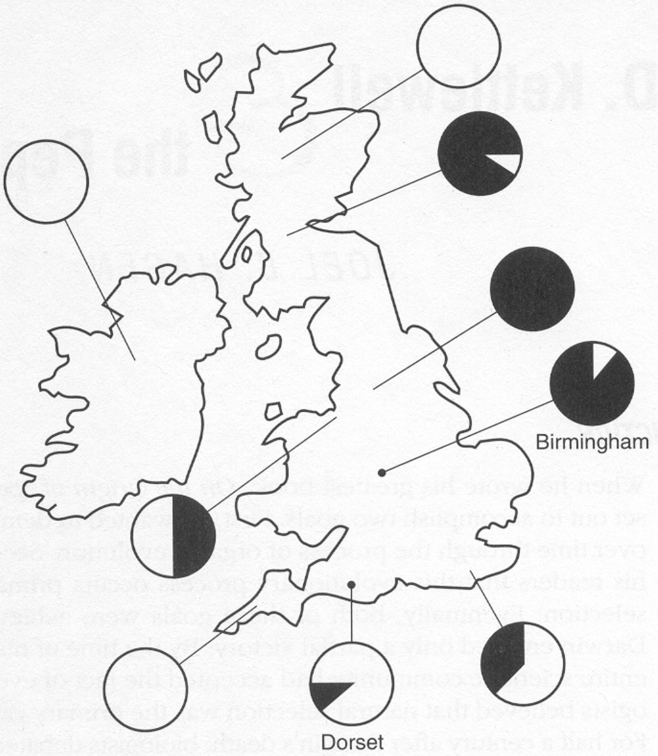

FIGURE 1.1 Some of the local populations of peppered moths in Britain. Pie diagrams show the relative frequency of dark- and light-winged individuals in each population. Birmingham and Dorset were the sites of H. B. D. Kettlewell's famous mark-release-recapture experiments.

THE PHENOMENON OF INDUSTRIAL MELANISM

Collecting insects is a popular hobby in England, and amateur naturalists were quick to record dramatic changes in wing coloration that occurred in several species of moths, notably the peppered moth (*Biston betularia*). This color change, which was due to a black pigment called melanin, seemed to occur most frequently in moths living near industrial cities (Figure 1.1). Populations made up almost entirely of light-winged individuals in 1800 had become mostly dark-winged a century later. Recognizing the evolutionary importance of this change, professional biologists also turned their attention to this phenomenon of **industrial melanism**.

In a widely publicized series of experiments conducted during the 1920s, the British entomologist J. W. Heslop Harrison fed caterpillars leaves coated with toxic compounds commonly found in soot. For example, in one experiment, caterpillars captured in a nonpolluted forest were fed leaves coated with lead nitrate.

After pupation, 53 light-winged moths and 3 dark-winged moths emerged from cocoons. All of the caterpillars in the control group, which were fed unpolluted leaves, developed into light-winged moths.

Harrison concluded that these results were due to mutations induced by chemical pollutants. Because the dark wings were inherited by many descendants of his experimental moths, Harrison also claimed that he had documented a case of inheritance of acquired traits. Publishing his experimental results in the prestigious British journal, *Nature*, Harrison presented his theory as a clear-cut alternative to natural selection.

PROBLEM

In his *Nature* article, Harrison did not provide many details about his experiments. List alternative explanations (other than mutation) that could also account for the unexpectedly large number of dark-winged moths.

Later attempts to replicate Harrison's experiments failed, and his explanation for industrial melanism was criticized by Darwinians. For example, the prominent theoretical biologist R. A. Fisher pointed out that Harrison's explanation required a mutation rate much higher than any previously reported. Nonetheless, Harrison continued to argue for his Lamarckian theory. Because he was a distinguished member of the British scientific community, his ideas could not simply be ignored. Therefore, this controversy set the stage for later research on industrial melanism.

A decade after Harrison published the results of his experiments, the geneticist E. B. Ford presented an alternative explanation. According to Ford, industrial melanism could be explained by natural selection acting on rare mutations. According to Ford, random mutations had always produced a few melanic moths in light-winged populations, but the mutants were quickly eliminated by natural selection. However, in polluted areas melanism proved adaptive, gained a selective advantage, and rapidly spread through the population.

You should note the important differences between the hypotheses proposed by Harrison and Ford. Harrison claimed that mutations occurred as a direct result of pollution and that they occurred simultaneously in many members of the population. Ford claimed that the genetic changes were not directly caused by pollution and that these mutations had always occurred *in very small numbers* in the population. According to Harrison, evolution occurred because many members of the population simultaneously mutated to the dark form. According to Ford, evolution resulted from the higher survival rate and reproductive success of rare mutants compared to their more common light-winged relatives.

Ford did not conduct experiments to test his hypothesis, and his explanation left a number of important questions unanswered. What exactly was the advantage of melanism? Was it really camouflage, or was it some unrelated physiological advantage linked to wing coloration? Could predators actually distinguish between melanic and nonmelanic moths? Could natural selection account for such a rapid increase in melanic individuals in polluted areas? The stage was set for a dramatic experimental test when H. B. D. Kettlewell began studying peppered moths in 1951.

H. B. D. KETTLEWELL AND ECOLOGICAL GENETICS

Henry Bernard Davis Kettlewell was the son of a successful businessman. As a boy, he attended prestigious private schools (so-called "public schools" in Britain), and he later studied both zoology and medicine at Cambridge University. For several years, he happily practiced medicine in a small town in southern England. Apparently because he disapproved of the nationalization of health care in Britain after World War II, Kettlewell left medical practice in 1949. For a time he worked on a locust control project in South Africa, later returning to England to join E. B. Ford's laboratory at Oxford University.

Kettlewell was an avid field biologist who loved adventure. When he left the locust control project, he drove from South Africa to Egypt, certainly not a journey for the fainthearted. As his biographer recalled, "He was a big man, with a personality larger than life. Kind, charming, and irascible, he had a huge and infectious ebullience and energy, could be the life and soul of any party, and was much loved by his friends."

When Kettlewell arrived, Oxford University was a bustling center of activity in field biology. Aside from Ford, the university was home to a number of other internationally famous naturalists, including the ornithologist David Lack, the ecologist Charles Elton, and the ethologist Niko Tinbergen. This scientific community proved valuable; Tinbergen later helped Kettlewell demonstrate that birds choose their prey based upon differences in wing color.

Ford and his associates were unusual geneticists because they preferred doing experiments in the field rather than in the laboratory. Working closely with the theoretical biologist R. A. Fisher, Ford had designed very sophisticated experimental methods for studying the genetics of natural populations, and unlike many naturalists at that time, he used statistics to analyze his experimental data. Partly influenced by Fisher's mathematical models, Ford believed that natural selection was the most important cause of evolution. According to Fisher and Ford, natural selection was often so intense that adaptations could quickly spread through a population if the environment changed. Ford's combination of experimental field studies, mathematical theorizing, and emphasis upon natural selection became known as "ecological genetics." It was an approach that Kettlewell skillfully applied to solve the problem of industrial melanism.

Kettlewell was convinced that wing coloration is an important adaptation in peppered moths. Females rarely fly; some spend their entire lives on a single branch. Males fly during the night and rest on tree trunks during the day. Both sexes rest with their wings open; therefore, camouflaged wings ought to provide some protection against predatory birds. Could this explain industrial melanism?

According to Kettlewell's hypothesis, light-colored wings provided camouflage when the moths rested on the lichens that often cover tree trunks in British forests. This cryptic coloration is so effective that moths are often invisible to humans standing only a few feet away (Figure 1.2). Melanic individuals are occasionally found in rural populations, but because their dark wings contrast with lichen-covered tree trunks, they are more likely to be eaten by predatory birds. As a result of the indus-

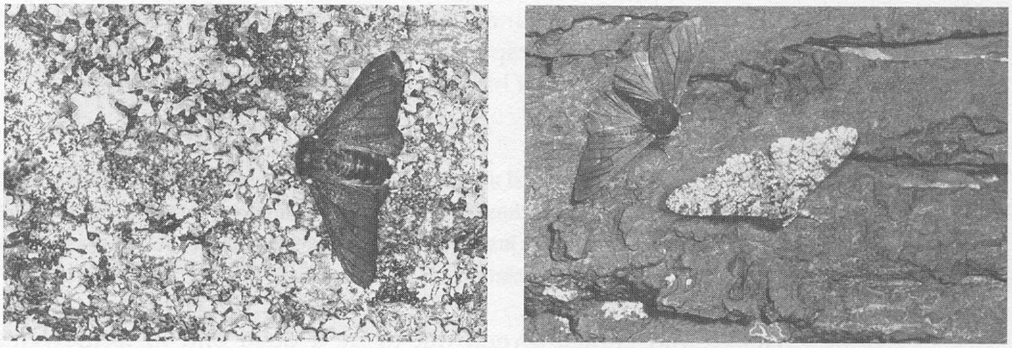

FIGURE 1.2 Dark- and light-winged varieties of the peppered moth resting on two contrasting backgrounds.
Source: © M. W. F. Tweedie/Photo Researchers, Inc.

trial revolution, many areas of Britain became heavily polluted. Smoke from burning coal killed the lichens and caused trees to darken with soot. Here the adaptive value of wing coloration was reversed. Against a dark background, melanic moths are camouflaged and light-colored moths are conspicuous. At least, that is what Kettlewell thought. Many questions remained, however. Were birds really fooled by this camouflage? Did wing color really provide a significant advantage to camouflaged individuals? Most importantly, could Kettlewell design convincing experiments to confirm his hypotheses?

KETTLEWELL'S EARLY LABORATORY EXPERIMENTS

One of the first questions that Kettlewell set out to answer was whether moths choose the background on which they rest. If they did not, what advantage would camouflaged wings confer? Failure to choose a correct background might lead to death, so Kettlewell believed that background choice must occur. Although skeptical of laboratory experiments, Kettlewell designed a simple test of his hypothesis. He lined a large cider barrel with overlapping strips of black and white cloth. In the evening he released equal numbers of dark- and light-winged moths in the barrel. The top of the barrel was then covered with a sheet of glass and a white cloth. In the morning, the resting position of each moth was recorded. Kettlewell obtained the following results:

	Dark-winged Moths	Light-winged Moths
Black Background	38	20
White Background	21	39

Analyzing the results statistically with a x^2 test, Kettlewell found that the differences in background choice were highly significant. He then attempted to further test his hypothesis by observing background choices made by moths in the field. To his disappointment, the field studies showed no statistically significant tendency for moths to choose correct backgrounds. Moths seemed to land randomly on correct and incorrect backgrounds. Despite these negative results, Kettlewell continued to believe in the importance of background choice.

PROBLEM

In what ways would the natural environment of a moth be different from Kettlewell's laboratory experiment? How might these factors explain the different results obtained in the two experiments? Was Kettlewell justified in holding his background choice hypothesis even though some of his experimental results were negative?

Background choice turned out to be a dead end in Kettlewell's research. Although he continued to believe in it, he could never convincingly demonstrate that it occurred. More important, as it turned out, was his claim that birds selectively prey upon conspicuous moths. In another early experiment Kettlewell released equal numbers of dark- and light-winged moths in a large, outdoor aviary containing light and dark tree trunks. After the moths had come to rest on the tree trunks, Kettlewell released a pair of insectivorous birds. During the first two hours no moths were eaten, even those resting on contrasting backgrounds. But once the birds learned to recognize the moths, they actively searched for conspicuous prey. Inconspicuous moths were less often eaten, although they, too, were sometimes killed, particularly if they happened to be resting near a conspicuous individual.

From this experiment, Kettlewell concluded that birds could act as selective agents, but predation was a learned behavior. The birds had to learn to recognize a specific type of food before they could effectively exploit it. Other biologists remained skeptical. Both ornithologists and entomologists denied that birds would actively search for moths on the basis of wing coloration. In response to this criticism Kettlewell enlisted the aid of the ethologist Niko Tinbergen (see Chapter 14), who took still photographs and motion picture films of predatory birds in the field. His observations revealed that birds captured conspicuous moths approximately three times more often than inconspicuous moths resting on the same tree trunk. This provided dramatic evidence to support Kettlewell's hypothesis.

KETTLEWELL'S FIELD EXPERIMENTS

Kettlewell provided even stronger evidence for natural selection with a series of mark-release-recapture experiments conducted in two different environments: a polluted forest near the industrial city of Birmingham and a pristine forest in rural Dorset. Following methods pioneered by Fisher and Ford, Kettlewell marked the undersides of the wings of male moths with dots of paint. Only males were used because females rarely fly and are, therefore, difficult to recapture. After marking, large numbers of light- and dark-winged moths were released at sundown. Every evening for the next week, males were recaptured using mercury vapor lamps and pheromone traps (i.e., traps containing virgin females). The following table summarizes the results of some of the experiments conducted in the two contrasting environments. The number of recaptures is expressed as a fraction of the total number of moths released.

	Environment	
Moths	**Polluted Woods (Birmingham)**	**Unpolluted Woods (Dorset)**
Light wings	18/137 (13%)	62/496 (12.5%)
Dark wings	136/493 (27.5%)	34/488 (7%)

This appears to be a classic example of a controlled experiment. Regardless of the environment, the camouflaged moths are approximately twice as likely to be recaptured in traps as the conspicuous moths. Kettlewell concluded that the missing conspicuous moths had been eaten by predatory birds. In retrospect, this clever set of experiments provides convincing evidence that industrial melanism is caused by natural selection. But how did Kettlewell's contemporaries respond to the experiments? The actual history of the case is more revealing than many textbook accounts would lead us to believe.

In 1952 and 1953, Kettlewell conducted the first series of mark-release-recapture experiments in the polluted forest near Birmingham. The comparable experiments in an unpolluted forest in rural Dorset were done several months later, and the results of the two sets of experiments were published separately. Readers of Kettlewell's first article encountered data from the Birmingham experiment, but they had no way of knowing about the results from the contrasting environment.

PROBLEM

Suppose that you have just read the report of Kettlewell's mark-release-recapture experiments near Birmingham. The results from Dorset have not been published, so you do not know about the second set of experiments. Considering only the Birmingham data, what alternative hypotheses (other than predation) might explain why dark-winged moths are captured more frequently than light-winged moths? How do the combined data from two environments make these alternative explanations unlikely?

There are several plausible explanations for Kettlewell's decision to publish the two sets of data separately. Finding comparable woodlands in polluted and unpolluted areas of England was difficult, and at first he may not have thought it was necessary to duplicate the experiment in different environments. Conducting large field experiments is laborious, and Kettlewell usually had little help (his wife and son were often his only assistants). Traveling between two study sites, both many miles from Oxford, was time consuming. Breeding hundreds of moths to be released at the same time also posed practical problems. All of these factors prevented Kettlewell from conducting the complete set of experiments at the same time.

Whatever the reasons, Kettlewell's early paper gives little indication that it was written as a preliminary report. Nowhere in the paper did Kettlewell discuss the need for a parallel experiment in an unpolluted environment. It seems likely that he initially believed that the experiment in a polluted woods was sufficiently compelling to support his hypothesis. Perhaps he thought that every alternative explanation for his results had been effectively refuted. For example, he found no evidence to suggest that dark-winged moths were more likely to enter traps than light-winged moths. Nor did he find a greater tendency for light-winged moths to migrate out of the study area. Both types of moths seemed equally hearty; light-winged moths were no more likely than their dark-winged counterparts to die from causes other than predation. By doing these checks, Kettlewell believed that he had eliminated all possible variables other than the one he was testing.

Apparently other biologists found the initial experiment unconvincing. Kettlewell later recalled that the results of his Birmingham experiment encountered considerable skepticism from his contemporaries. Therefore, he felt compelled to repeat the experiment in a contrasting environment. The combined results, together with Tinbergen's films of birds selectively eating conspicuous moths, convinced most biologists that Kettlewell's explanation was correct. Natural selection, the result of selective predation by birds, was the most likely cause of industrial melanism.

RECONSIDERING KETTLEWELL'S EXPERIMENTS

Kettlewell's experiments are important for several reasons. It is often incorrectly assumed that evolutionary hypotheses cannot be tested by experiments. Kettlewell's research demonstrates how false this belief is. His large-scale field experiments were done with the degree of care and precision usually associated with laboratory science. As a result, we now know that intense natural selection can sometimes lead to rapid evolution in populations.

Like other great experiments, Kettlewell's work raised as many questions as it answered. If his explanation for industrial melanism was correct, what would happen if pollution was reversed? If smoke and soot were eliminated, would the selective advantage shift away from melanic moths and once more favor light-winged individuals? Biologists who have studied populations of peppered moths in industrial areas where pollution has been reduced find that light-winged individuals are once again on the increase.

Finally, in reconsidering Kettlewell's approach to doing biology it is important to remember that he used a variety of evidence to support his hypothesis. When he started, there were a number of possible explanations for industrial melanism. Natural selection through predation was a likely possibility, but it needed to be conclusively demonstrated. During the course of several years, Kettlewell combined several techniques to find supporting evidence for natural selection. Some of his approaches turned out to be dead ends and were abandoned. Others, like Tinbergen's movies, were dramatic, but only when combined with experimental evidence. The mark-release-recapture experiments began as a small part of Kettlewell's project but grew into the most important part. The combined experiments in two different environments turned out to be the key for solving the problem of industrial melanism.

☐ *EPILOGUE*

Kettlewell often referred to industrial melanism as Darwin's "missing evidence" for natural selection. Could this same process lead to speciation, as Darwin claimed, and could speciation occur rapidly enough for scientists to witness the origin of new species? In the case of the peppered moths this has not happened. There is no evidence that reproductive isolation has evolved in the moth populations. Dark- and light-winged individuals interbreed freely. Some evolutionary biologists believe that even with intense natural selection, speciation could not occur unless a geographical barrier prevented populations of light- and dark-winged moths from mating. Other evolutionary biologists question the need for such barriers and believe that speciation can sometimes occur even without geographical isolation. Perhaps it can happen quite rapidly.

One of the most intriguing candidates for such rapid speciation is the fruit fly, *Rhagoletis pomonella*, a common agricultural pest. Females lay eggs on apples and other related fruit, and the maggots ruin the fruit by feeding on it. Originally, the hosts for this parasite were hawthorns, small trees widely distributed throughout the eastern United States. Thanks to John Chapman ("Johnny Appleseed") and other pioneers, extensive orchards were planted throughout Ohio, Indiana, and Illinois during the early decades of the nineteenth century. *R. pomonella* rapidly colonized the new hosts: apple, cherry, and pear trees.

When evolutionary biologists began studying *R. pomonella* during the 1970s, they discovered that each host species seemed to harbor a genetically distinct population of the parasitic flies. These differences appeared to be maintained partly because fruit flies prefer the type of tree on which they hatch. Females raised on apple trees usually lay their eggs on apples, and females raised on hawthorns usually lay their eggs on the fruit of hawthorns. Males search for mates on the host where they hatch. Furthermore, maggots develop at different rates on the two hosts. Maggots living on apples develop in about 40 days, but those living on hawthorns take 55 to 60 days to develop. As a result, fruit flies on the two hosts become sexually mature at different times.

Despite these important behavioral and physiological differences, reproductive isolation is not complete in populations of *R. pomonella.* When flies from different hosts are brought together in the laboratory, they freely interbreed. Yet the populations have diverged in some important reproductive characteristics during a remarkably short period of time (about 100 fruit fly generations). Perhaps this is a case of speciation in action. Alternatively, reproductive isolation may never evolve completely, and despite their partial isolation the different populations may continue to form a single species. The problem continues to challenge evolutionary biologists.

Another unsolved problem in the case of *R. pomonella* is whether natural selection has altered populations. Apples and hawthorns provide two different habitats for fruit flies, and it stands to reason that selection might act differently in the two environments. But the genetic differences may also be due to chance. Discovering the cause of evolutionary change in fruit flies is another ongoing research problem for evolutionary biologists.

QUESTIONS AND ACTIVITIES

1. What does this case show about the following aspects of doing biology?
   — alternative interpretations of experimental data
   — difficulty of designing controlled field experiments
   — importance of experimental controls
   — different types of evidence used to support theories
2. Reconsider the early experiments on industrial melanism conducted by J. W. Heslop Harrison. If Harrison's hypothesis had been correct, what would be the rate of mutation in his experimental populations of moths? R. A. Fisher pointed out that most naturally occurring mutations appear in approximately 1 in 10,000 individuals. How might Harrison have responded to Fisher's criticism?
3. Kettlewell always believed that background choice was an important factor in the evolution of industrial melanism. How would background choice affect natural selection in peppered moths? Is background choice really necessary for natural selection to occur?
4. Kettlewell and other biologists found that industrial melanism is common in moths, but not in butterflies. What behavioral differences between these two related groups of insects might explain this observation?
5. Consider the data from Kettlewell's mark-release-recapture experiments in Birmingham and Dorset. In both experiments, camouflaged moths were about twice as likely to be recaptured as conspicuous moths. But the relative numbers (%) of recaptured moths were quite different in the two contrasting environments. How can you explain these overall differences in recapture rates?

SUGGESTED READING

Bishop, J. A., and D. P. Clark. 1980. "Industrial Melanism and the Urban Environment." *Advances in Evolutionary Studies* 11: 373–404.

Ford, E. B. 1980. "Some Recollections Pertaining to the Evolutionary Synthesis." In E. Mayr and W. B. Provine, eds., *The Evolutionary Synthesis: Perspectives on the Unification of Biology.* Cambridge, MA: Harvard University Press.

Kettlewell, H. B. D. 1973. *The Evolution of Melanism: The Study of a Recurring Necessity.* Oxford, England: Oxford University Press.

Provine, W. B. 1971. *The Origins of Theoretical Population Genetics.* Chicago: University of Chicago Press.

Ridley, M. 1993. *Evolution.* Boston: Blackwell.

Smocovitis, V. B. 1992. "Unifying Biology: The Evolutionary Synthesis and Evolutionary Biology." *Journal of the History of Biology* 25: 1–65.

Weiner, J. 1994. *The Beak of the Finch: A Story of Evolution in Our Time.* New York: Knopf.