TRACING VOLE MOVEMENTS BY RADIOACTIVE EXCRETORY PRODUCTS

By

LOTUS SIMON MILLER

Reprinted from *Ecology,* Vol. 38, No. 1, January, 1957

TRACING VOLE MOVEMENTS BY RADIOACTIVE EXCRETORY PRODUCTS[1]

LOTUS SIMON MILLER
Department of Zoology, University of Wisconsin[2]

The natural behavior of most species of small mammals in their native habitat is screened from the observer's eye by such hindrances as vegetation and darkness. Studies of movement and foraging range thus have been handicapped. With the exception of recent work by Godfrey (1953, 1954a, 1955), such studies have been accomplished mainly by live-trapping, which is time-consuming and limited in intensity by hazards to the life of the animal. This paper describes a new method for recording the paths of movement of small animals. The technique consists of injecting the animal with radioactive phosphorus and tracing its subsequent wanderings by detecting radioactive excretions on metal plates distributed in the field for this purpose. One field application to the meadow vole, *Microtus pennsylvanicus,* is described.

[1] Contribution from the University of Wisconsin Arboretum, Journal Paper No. 30. Sincere thanks are extended primarily to Dr. John T. Emlen, Dept. of Zoology, for enthusiastic encouragement and aid in this project, particularly in the planning and writing stages. Dr. John E. Willard, Dept. of Chemistry, gave valuable help in the selection and procurement of the isotope. The field meter and laboratory meter were made available by Dr. Donald F. Saunders, Dept. of Chemistry, and Dr. Folke Skoog, Dept. of Botany, respectively.

[2] Present address: c/o Serology Laboratory, University of California, Davis, California.

LITERATURE

Various investigators have already developed techniques for studying animal movement with the use of radioactive isotopes, both those which emit beta rays and those which emit the more penetrat-

ing gamma rays. The movements of insects underground were studied by Tomes and Brian (1946) and Brian (1948), who employed gamma-emitting Ra^{226} in elaterid beetles, and by Arnason *et al.* (1950), who used gamma-emitting Co^{60} in elaterid larvae (wireworms). Studies of mosquito range were made by Bugher and Taylor (1949) in Nigeria utilizing beta-emitting P^{32} and Sr^{89}, while Hassett and Jenkins (1949) and Jenkins and Hassett (1951) used P^{32} in subarctic mosquitoes for the same purpose. The latter authors (1950) recommended the use of beta-emitting isotopes, partly because of their relative safety, for ecological studies of insects. Subsequent studies using radioisotopes to trace insect movement have been ably reviewed by Lindquist (1952), Dahm (1953), and Jenkins (1954).

Nest attendance of an incubating bird tagged with gamma-emitting Zn^{65} was recorded with a stationary radiation meter by Griffin (1952), who reviewed the feasibility of radioisotopes in bird and mammal tracing work and maintained that only gamma-emitting isotopes were powerful enough to be useful. Godfrey (1953, 1954a), in an extensive study, tagged British field voles with leg bands containing gamma-emitting Co^{60} and traced their movements directly with a portable detection unit. Godfrey also studied the underground movement of moles by similar methods, using radioactive tail-rings (1955), and reviewed the use of isotopes, mainly gamma-emitters, in small-mammal ecology (1954b). All these investigators recorded the presence of the tagged animal itself. Detection of radioactive droppings to provide a record of movement in the field was suggested by Jenkins (pers. comm. 1950, see 1954) after he measured the radioactivity of the daily accumulations of feces of a lemming fed with beta-emitting P^{32} in the laboratory. The use of radioisotopes as markers in ecological studies of animals was reviewed by Pendleton (1956).

Procedure

A laboratory study was first conducted on two house mice (*Mus musculus*), each injected with 200 microcuries (μc) of radioactive phosphorus, to determine the feasibility of detecting small numbers of feces from a small mammal injected with P^{32}. Radioactive phosphorous was chosen because of its relative safety for use in a study area adjacent to a residential district, because of its convenient half-life (14.3 days), and because of its metabolic use, resulting in relatively gradual excretion from the body. Injection was selected in preference to oral administration because of longer retention in the body. For two weeks after injection, the total radioactivity of any group of five fresh droppings from either mouse elicited an unmistakable response from a Geiger-Müller field survey meter at a distance of five centimeters (unpublished).

The short distance necessary for detection of such radioactive droppings made the complete survey of any large field area impractical. Since the projected field study was part of an investigation into habitat preference and activity of a population of *Microtus pennsylvanicus* in a marsh, random search was discarded as being too destructive of this habitat. Instead, for the field study, definite points in one acre of a *Microtus*-inhabited grass-sedge marsh were selected to serve as stations for deposition of feces by the voles, and, therefore, for sampling of radioactivity of the feces.

Eadie pointed out that small mammals tend to concentrate their defecation on paper squares set out in the field (1948). Elaborating on this idea, Emlen, Hine, Fuller, and Alfonso (in press) developed the technique of laying squares of plywood and censusing small mammal populations by counting and identifying the droppings left on the boards.

For the present study, 121 squares of sheet aluminum, 4 in. x 4 in., were laid out at 20 foot intervals in a grid pattern on one acre of a marsh in the University of Wisconsin Arboretum at Madison. These squares were inspected for droppings each morning and then cleaned off. During the week before the experiment, about one-fifth of the total of 121 squares had *Microtus* droppings upon them each day. Droppings of the masked shrew, *Sorex cinereus,* were found, on the average, on about two squares a day, and represented apparently the only other small mammal resident on the acre.

Sixteen wooden box traps were set in the central region of the study area on the night of June 10, 1951. On the following morning, an adult male *Microtus* weighing 41 grams was selected from the eight meadow voles captured. One cc of a solution of radioactive phosphorus (H_3PO_4), with a total activity of 200 microcuries, was buffered to neutrality and injected subcutaneously into both flanks of the experimental vole. After ten minutes of apparently normal behavior in a cage, the animal was released at the point of capture, designated as X in Figure 1 at station 5.5 on the grid.

Testing of the acre for radioactivity began that afternoon with a field survey meter.[3] The microammeter dial was read before each use on the

[3] Model 2610, Nuclear Instrument and Chemical Corporation.

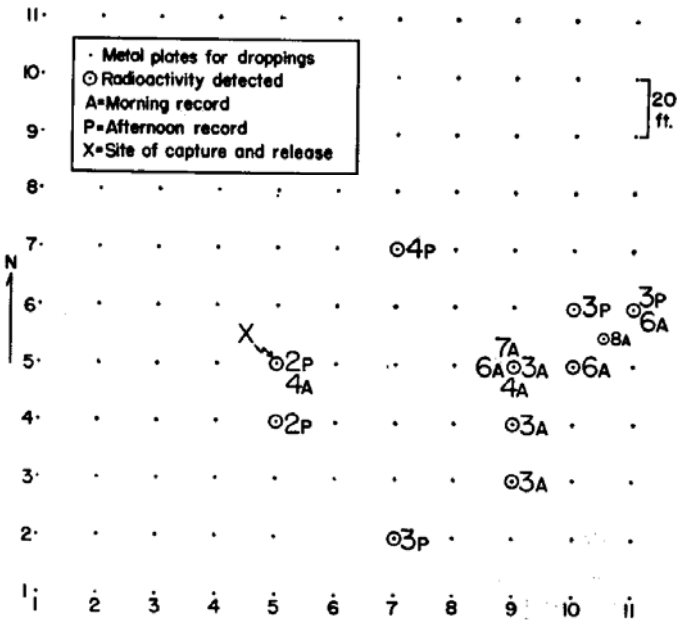

FIG. 1. Movements of one *Microtus* tagged with P^{32} in East Wingra Marsh, Univ. Wisconsin Arboretum during June 11-24, 1951.

Note: The records found on Day 3 AM ("3A") may have been produced any time between Day 1, PM and Day 3 AM. The record "8A" was found on one of 4 dropping plates placed around location 10.5 after a highly active dropping was found at 10.5 on Day 6.

squares to determine the "background" level of radiation normally impinging on the meter probe. Each square was then tested by holding the unsheathed probe of the meter close to its surface and watching the microammeter for a repeated upward swing of the needle to a level clearly above background level.

Improvements on the equipment used can be suggested. The field meter should be a model which is well protected against moisture, since high humidity proved to be enough to produce short circuits and, therefore, spurious momentary records of radioactivity. Headphones attached to the meter would increase the ability of the operator to detect radioactivity in the field.

For the first two days, the meter probe was repeatedly moved over each square at a distance of one to five cm for testing. After it was discovered that small amounts of radioactivity were associated with the surface of some squares with no droppings on them, further steps were taken. For testing in the field, the meter probe was held as close as possible (less than 1 cm) and each square which appeared to be even slightly radioactive was removed to the laboratory for further testing with a more sensitive meter. A scaler and time-clock attached to the laboratory meter made possible the recording of radioactivity in terms of disintegrations per minute (counts per minute). Determination of background again preceded testing of droppings and scrapings from the surface of the squares which were suspected of being radioactive. During most measuring periods, the background count was around 21 counts per minute, and an average of twenty counts or more per minute above background was assumed to be a clear indication of radioactivity. Each square removed from the field was replaced by a new clean square.

Difficulty was encountered in removing the radioactive material from the surface of the aluminum squares, for testing in the counting chamber of the laboratory meter. Chemical means (12.5% solution of Na_2HPO_4) proved unsuccessful, and it was necessary to scrape the surface of the aluminum with a razor blade in order to remove and test the radioactive material.

On days 1, 2, 3 and 4 after injection, an attempt was made to test all the squares in the field both morning and evening for radioactivity. From day 5 through 12, one check for radioactivity was made each day, and none thereafter. Unfortunately, the failure of the field meter to function properly during periods of high humidity made it impossible to complete the survey on the evening of day 1, and the morning and evening of day 2. None of the unchecked squares were radioactive during the survey following such a failure, with the exception of three adjacent squares (stations 9.3, 9.4 and 9.5), recorded on the morning of day 3. These three stations had not been checked for radioactivity since the morning of day 1 after injection.

RESULTS

During the twelve days after injection, 1,642 tests for radioactivity were made in the field. Radioactive traces were found first on the evening of the second day after injection, and last on the twelfth day, when the meter again failed and the surveys were discontinued. Sixty squares were either clearly radioactive or were suspected of being slightly radioactive. All of these squares were brought in to the laboratory for confirmation. Sixteen of these squares showed radioactivity definitely above the normal background count, four were only slightly above background, and forty were definitely not radioactive. Radioactivity on the sixteen squares was contained in the scrapings (presumably urine residues) in fourteen cases; clearly radioactive droppings were found on only two squares (Fig. 2).

The data obtained from records of the distribution of radioactive squares indicated that the experimental vole visited at least eleven stations one or more times during eight days and in that time traversed an area at least 120 feet across from

the species studied must be one which will deposit droppings or urine in easily detected locations (*e.g.* "dropping boards"). In Wisconsin, in certain habitats, *Microtus pennsylvanicus, Peromyscus leucopus noveboracensis, P. maniculatus bairdi, Blarina brevicauda,* and *Sorex cinereus* have been found to use dropping boards (Emlen *et al.*). A variety of small mammals in other regions also have been found to use them (Emlen, unpublished).

Summary

An adult male meadow vole was trapped from a population under study in a grass-sedge marsh in the University of Wisconsin Arboretum, injected with 200 microcuries of P^{32}, and released at the point of capture. Small aluminum squares, laid out over an acre in a grid pattern, were examined with a field meter each day for radioactive excretory products. During eight days after injection, radioactivity was detected a total of sixteen times, at eleven different stations which encompassed about one-fourth of an acre.

This technique appears to provide a method for recording spatial and temporal distribution of activity of small mammals in nature with a minimum of disturbance to the marked animal or its habitat. The P^{32} tracer is a short-lived beta-emitting isotope and therefore relatively safe to handle. The metal squares provide a convenient means of collecting data on a standardized basis and are applicable to a variety of small mammal species.

References

Arnason, A. P., R. A. Fuller, and J. W. T. Spinks. 1950. An electronic method of tracing the movements of soil-inhabiting insects. Science, **111**: 5-6.

Brian, M. V. 1948. On the ecology of beetles of the genus *Agriotes* with special reference to *A. obscurus*. J. Anim. Ecol., **16**: 210-224.

Bugher, John C., and Marjorie Taylor. 1949. Radiophosphorus and radiostrontium in mosquitoes. Preliminary report. Science, **110**: 146-147.

Dahm, Paul A. 1953. Studies of insects and insecticides with tracers. Paper **14**: 162-199 *in* A conference on the use of isotopes in plant and animal research. U. S. Atomic Energy Comm. TID 5098

Eadie, W. Robert. 1948. Shrew-mouse predation during low mouse abundance. Jour. Mamm., **29**: 35-37.

Emlen, John T., R. L. Hine, W. A. Fuller and Pablo Alfonso. The use of dropping boards for population studies of small mammals. Jour. Wild. Mgt. (in press).

Godfrey, Gillian K. 1953. A technique for finding *Microtus* nests. Jour. Mamm., **34**: 503-505.

———. 1954a. Tracing field voles (*Microtus agrestis*) with a Geiger-Müller counter. Ecology, **35**: 5-10.

———. 1954b. Use of radioactive isotopes in small-mammal ecology. Nature, Lond., **174**: 951-952.

———. 1955. A field study of the activity of the mole (*Talpa europaea*). Ecology, **36**: 678-685.

Griffin, Donald R. 1952. Radioactive tagging of animals under natural conditions. Ecology, **33**: 329-335.

Hassett, C. C., and D. W. Jenkins. 1949. Production of radioactive mosquitoes. Science, **110**: 109-110.

Hevesey, G., L. Hahn and O. Rebbe. 1939. Excretion of phosphorus. Kgl. Danske Videnskab. Selskab, Biol. Medd., **14**(3): 1-23.

Jenkins, Dale W. 1954. Advances in medical entomology using radioisotopes. Expl. Parasitol., **3**: 474-490.

———, **and Charles C. Hassett.** 1950. Radioisotopes in entomology. Nucleonics, **6**: 5-14.

———. 1951. Dispersal and flight range of subarctic mosquitoes marked with radiophosphorus. Can. J. Zool., **29**: 178-187.

Lindquist, Arthur W. 1952. Radioactive materials in entomological research. J. Econ. Entomol., **45**: 264-70.

Pendleton, R. C. 1956. Uses of marking animals in ecological studies: Labeling animals with radioisotopes. Ecology, **37**: 686-689.

Tomes, G. A. R. and M. V. Brian. 1946. An electronic method of tracing the movements of beetles in the field. Nature, Lond., **158**: 551.