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## NEW METHOD FOR MARKING SMALL MAMMALS

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Various methods for marking animals with radioactive labels are finding increasing application in ecological field research. More than 20 radionuclides have already been approved, but only four of them – the radioactive isotopes of carbon, phosphorus, sulfur, and cobalt – have been used very frequently as internal labels (Progress in the Marking of Mammals, 1980). To mark animals, radioactive bait is used or a radioactive solution is injected into the esophagus or under the skin of previously trapped animals. The radiolabel activity corresponds to the sensitivity of the radiometric apparatus used. The presence of highly sensitive apparatus permits the use of very small indicator doses of radionuclides for marking, which have no influence on the behavior, life span, or fertility of the experimental animals. The risk of the radioactive contamination of the environment where the experiments are conducted is simultaneously eliminated. The relative difficulty of radionuclide-marking methods is compensated for by the potential for obtaining more complete, precise, and frequently unique information on the spatial and genetic relations of the studied animals and the structural dynamics of their populations in monitored regions of from several tens to several hundreds of hectares in area.

Studies on improving the methods for marking animals with radionuclides have been underway at the Institute of Plant and Animal Ecology, Urals Scientific Center, Academy of Sciences of the USSR, since 1979. One of the new, promising methods is prenatal marking of the young (Bazhenov et al., 1983). The goal of the present paper is to acquaint specialists with the experience of its practical application.

The potential for the prenatal marking with radioactive label of the young of not only small mammals, as claimed in the invention, but also other mammalian species is determined by a number of their physiological characteristics: firstly, by the reliable deposition of osteotropic radionuclides in the skeletons of mammalian females and their young during the growth and development period due to the stable fixation of radiolabel at an early age over a prolonged period, since osteotropic radionuclides are a "structural material" (Shvedov, 1968). For example, the strontium content in the second generation of rat pups 360 days after removal from the mother comprised 50% of the level on the 30th day after removal. Secondly, this is associated with the regular mobilization of label from the maternal skeleton during pregnancy and lactation, which ensures the entry of isotope across the placental barrier and with the milk of the nursing mother into the progeny of several successive litters (Kulikova, 1964, 1970; Ovcharenko, 1982).

The labeling procedure is carried out directly under field conditions by means of a single subcutaneous injection of the osteotropic radionuclides  $^{90}\text{Sr}$  and  $^{45}\text{Ca}$  in adult females of the species under study, trapped directly at the sites of reproduction, whence they immediately retreat after the injection of the radioactive label. Additional marking with a visually identifiable label is sometimes used, for example clipping the digits, to avoid a repeat injection of label. The best end results are achieved by injecting pregnant females with isotopes.

During the marking of small mammals, a one-time injection of 0.1–0.5 ml of a chloride salt (pH 4–5) of  $0.9 \cdot 10^4$  Bq  $^{90}\text{Sr}$  activity and  $1.11 \cdot 10^5$  Bq  $^{45}\text{Ca}$  ensures the vital marking of the female and all its future progeny up to the fourth generation. The experiments were initially conducted under laboratory conditions in monitored, caged groups of wood voles and laboratory white mice. The doses we recommend are minimum and do not cause radiation injuries in the animals and are also completely safe ecologically when experiments are conducted in the field. The difference in doses between strontium and calcium is due to the differences in physical half-lives and the more mild beta radiation for calcium.

The radiolabel activity fell from litter to litter, but at the tested doses the error of determination of the fourth labeled litter did not exceed 5% if the samples were ashed, and stationary radiometric apparatus of the Tesla type, VAV-100 and UMF-1500, were used to count the samples. Females and the first and second generation progeny marked by  $^{90}\text{Sr}$  can, owing to the presence of beta radiation, be directly detected during radiom-

TABLE 1. Dispersal Parameters of Wood Voles from Marking Zones in Reservations during Summer Season

Studied group	Regression equation	Regression coefficient and t criterion	Probability p	Maximum dispersal, m
Genus <i>Clethrionomys</i>	24,41—0,011R	—0,73 2,16	0,05	2133
sexually mature females	33,23—0,028R	—0,86 2,96	0,05	1172
sexually mature males	29,55—0,018R	—0,63 1,61	0,1	1633
immature females and males	22,40—0,0083R	—0,063 1,62	0,1	2712
<i>Cl. glareolus</i>	22,98—0,018R	—0,71 1,73	0,1	1290
<i>Cl. rutilus</i>	25,95—0,011R	—0,70 1,95	0,1	2291

etry of the animal carcasses in lead booths, while the females and first generation can be detected with portable radiometric apparatus during trapping under field conditions. In the final analysis, one radionuclide injection makes it possible to obtain 20–30 marked animals in a small-mammal marking zone.

To study the patterns and parameters of the dispersal of small mammals after marking, the animals are trapped within and at a distance of 1–3 km from the marking zones. The site of capture of each animal is recorded on a schematic map. The carcasses or skeletal parts of the trapped animals are sealed in individual polyethylene bags with a label permitting the precise determination of the date and site of animal capture. All material is subjected to radiometric analysis under field-station conditions. During the field season of 1983, a method was developed for the direct fixation of a part of the skeleton with muscles in numbered standardized aluminum trays for automatic counting on field-station radiometric apparatus. The skeletal parts were first immersed for 2–3 sec in a 5–10% formalin solution and then dried in the trays; the samples can be stored in this form for several years, while the laboratory analysis of the material is greatly shortened.

Prenatal marking makes it possible to surmount many of the limitations of all previous methods based on the principle of postnatal marking. The mass marking of animals is done before their birth and is therefore independent of the individual response to bait or trap, which causes a variable capture probability in postnally marked animals, i.e., the character of prenatal marking is less selective. Furthermore, the mass character of marking makes it possible to obtain information that is better not only quantitatively but also qualitatively. The total marking of the progeny of specific females is achieved simultaneously on defined areas, which opens broad prospects for the study of the genetic relations of animals within a population and the action of natural selection directly under natural conditions. This question has already been considered (Tamarin, 1983).

Another advantage of the prenatal marking method is that the influence of the experimenter on the monitored population is minimized, while the animals are not traumatized.

We have used prenatal marking in field experiments since 1980. As an example we present the results of two experiments conducted on Iremel' Mountain (1586 m above sea level, Beloretsk Raion, Bashkir Autonomous SSR). On two regions of 3 and 6 ha area situated in the upper taiga subbelt, 99 female voles of the genus *Clethrionomys* were marked in early summer. At the end of summer snap-traps were set out on radial lines of 1000–1500 m with 100–150 traps per line at a spacing of 10 m. All 16 lines began at the centers of the marking areas. There were 6800 trap days, and 525 northern red-backed, 494 bank, and 128 grey red-backed voles were trapped. Of the 1147 voles trapped, 222 proved to be marked. A negative correlation was established between the fractions of marked animals (M) and the distance of the trap site (R) from the marking zone. This dependence is described by the regression equation  $M = 24.41 - 0.011 R$ ,  $r = -0.73$ ,  $t = 2.16$ ,  $P < 0.05$ . Hence one can calculate the maximum dispersal radius of wood voles as a whole, that of separate age–sex groups, or that of specific species.

The obtained results (Table 1) show that under the conditions of the Southern Urals northern red-backed voles disperse much farther during the summer than do bank voles. The maximum dispersal radius is seen in immature animals. It is important to note that the wood-vole dispersal recorded during the summer was limited by the radius of the maximum daily movements during a sedentary mode of life. One can calculate from the obtained data the average area that the emigrants from a single reservation colonize during a single reproductive season. For the bank vole this area is 500 ha with a dispersal radius of 1290 m per season; for the northern red-backed vole, no less than 1600 ha with a dispersal radius of 2290 m.

We believe that the spatial scale for the realization of the population patterns of the dynamics of numbers and structure of the population of widely distributed species of small mammals should as a minimum correspond to the size of the territory that emigrants from one reservation colonize during a single reproductive season. In this case the minimum spatial groupings corresponding to population rank will be the entire aggregate population of the species within a single reservation and within the dispersal radius of the young born in the reservation. In this connection the problem is raised concerning the suitability of many methods used in population ecology for the purposes of population analysis, since they frequently permit coverage of only a very small area. However, the use of methods adequate to the goal of population analysis opens broad prospects for the further improvement of the theoretical model of the mammalian population, underlying the population and evolutionary ecology of animals, and presents new facets in the long-standing discussion of the boundaries of populations situated within a continuous species distribution range.

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