North American Bird Bander

Volume 9 | Issue 2 Article 1

1984

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Introduction and Objectives

Data obtained from individual birds in banding and in field studies often lack criteria or measures concerning sex and reproductive status. This situation of course varies with the particular species, age and time of year. I have been testing some approaches to the problem that appear to have not been presented or significantly utilized previously. A method that has merit and which I wish to present and evaluate here is cloacal lavage of sperm.

Cloacal lavage, a simple and harmless procedure, can provide important information about individual birds of many avian species during the breeding season. It can give objective proof of the timing of, and the individual participants in, breeding activity. These two parameters have depended hitherto on behavioral observations and indirect evidence, and have been influenced by some generalizations or assumptions that merit more critical study in relation to the biology of a number of species.

The present report has 3 objectives: (1) step-wise presentation of a basic and simple method for obtaining and processing permanent cloacal lavage specimens which can be part of the record for an individual banded bird; (2) demonstration of results of the procedure obtained during two years of banding, based upon 994 lavages or samplings obtained at localities in Texas, Missouri and California from 102 species; and (3) brief discussion of some of the probably useful variants in methodology, and of likely limitations and potential contributions of the method in providing the basis for new insights concerning some continuing questions about the reproductive biology of particular species.

Recommended Materials and Method

Materials and Equipment:

For obtaining and preserving lavage samples:

Microscope slides (1 × 3 inch size; supplied precleaned and ½ gross per box)
Disposable tapered plastic pipet tips
Small rubber squeeze bulbs ("medicine dropper type" to fit the above pipet tips)
Clean water (distilled or deionized) in a stock plastic wash (squeeze) bottle with tube outlet
Small beaker (10 to 25 ml volume) for dispensing water to pipet tips
Waste water receptacle (a large mouth bottle will do)
Diamond or carborundum glass-marking pencil
Slide boxes for 100 1 × 3 inch slides each

For optional staining of lavage slides:

Fixative and disinfectant solution (10% formalin or methyl alcohol in plastic wash (squeeze) bottle with tube outlet)
Distilled or deionized water in a plastic wash (squeeze) bottle with tube outlet
0.25% Basic Fuchsin (dye) in distilled or deionized water (= 250 mg/100 ml) in plastic wash (squeeze) bottle with tube outlet

For study of lavage slides:

Microscope with magnifications of about 100 and 400 ×

Method:

1. Draw water by bulb suction into a pipet tip to the appropriate volume. The volume should relate to the size of the bird and be less than what might cause traumatic distention of the cloacal chambers. Small passerines (band size 0) can accomodate 0.05 ml and the larger ones (band sizes 3 to 4) can handle 2.5 to 3.0 ml.
2. After banding, hold the bird with feet and tail restrained and then insert the prefilled pipet tip with bulb attached into the vent with a slow and gentle rotating motion, but without force. Penetration depth can be 1 to 6 mm, depending on bird size (band sizes 0 to 3 or 4 respectively).
3. Flush the pipet tip's water content out and in several times within the cloacal chambers. If excessive excreta are present, the first lavage sample may best be discarded. Presence of diverse cloacal contents in the lavages, however, does not hinder detection of sperm.

4. Immediately eject the water suspension of cloacal contents onto the center of a clean slide. Radial outflow of the suspension can be controlled by pipetting the suspension into the center of an area ringed by a wax pencil or crayon. In quantitative studies it can be advantageous to make this circumscribed wax barrier around a cardboard or plastic plate or form of a standard size, such as 15 × 20 or 15 × 40 mm. Gentle mixing of the contents of the suspension on the slide with the pipet tip can provide a more even distribution of sperm. Combined use of known lavage volume, predetermined area of lavage sample on the slide, and standard microscopy for cell counts, enables an estimation of sperm concentration per ml of lavage.
5. Engrave a label on each slide with the diamond or carborundum pencil. The label can consist simply of the band number plus date, or a serial catalogue number that can be recorded and filed with the banding record. Labeling should be done immediately to avoid possible mix-ups. Labeling is easier when a standard (graphite-clay) pencil is used on a slide having a frosted area at one end. However, this kind of slide is more expensive, and the resulting label is vulnerable to smudging and loss during subsequent handling.
6. Air dry the slide while it is flat. One can expedite this in some climates by suspending a light bulb over the slide, or by using the flat surface of an automobile top or hood.
7. Store slides in slide boxes until study, or until staining and further processing before study. If kept dry and inaccessible to dust and vermin, the slide specimens are essentially permanent. For the best long-term preservation and some degree of disinfection the slides can be either inverted while moist over a shallow container of formalin or of paraformaldehyde, or flooded with 10% formalin or 80–100% methanol after drying, then drained and redried before further storage.
8. Dried slides can be studied microscopically either immediately or at any time later. Without further treatment the sperm on the slides are readily visible with phase contrast optics. A small microscope equipped with such optics would be advantageous in extended field studies. However, the slides can be stained at any time in order to make the sperm more easily seen microscopically either with or without phase contrast optics.
9. A simple and reliable staining method is as follows:
   a. Place dry slides flat on a sink edge or drainboard, and flood them with either 10% formalin or methyl alcohol if they were not so fixed or treated previously. Allow the slides to remain flat for 10 to 20 min. before decanting them and very briefly rinsing them with distilled or deionized water.
   b. With the slides again flat on a sink edge or drainboard, flood them with 0.25% Basic Fuchsin, and allow them to stain for 15 to 20 min. without draining or drying. Then drain and rinse them as in step (a).
   c. Air-dry the slides again. They can be studied immediately and/or stored in slide boxes.
   d. *Optional*: When dry, the stained slides can be covered with a mounting medium (such as Permount in xylene) and then a coverglass (#1 thickness) to enable photomicrography and/or study with an oil immersion objective.

Results

Identification of spermatozoa (sperm) in the cloacal lavage slides was rarely a problem (Fig. 1). This was true inspite of the great variety of other kinds of objects present, especially the residual fragments of ingested plant and animal materials. In comparison with miscellaneous other fibrillar or thread-like contents of the lavages, sperm in a particular sample or species had: (1) a relatively consistent size or length, (2) a usually tapered tail, (3) a darkly staining acrosome, corkscrew-like in passerines, and (4) variable swelling and/or disruption of the head and mid-piece (Fig. 2). Other kinds of disruption were seen also, such as fraying of the tail (Fig. 1B). Osmotic and other techniques have been used to create such disruption of fowl sperm for the purpose of better revealing their fine structure (Grigg and Hodge 1949). However, in my cloacal lavages 0.9% NaCl and distilled water appeared to be nearly equivalent in the results obtained. Since salt crystals left from the lavages made with 0.9% NaCl were themselves disruptive as well as an interference, I chose water alone for the standard lavage procedure.

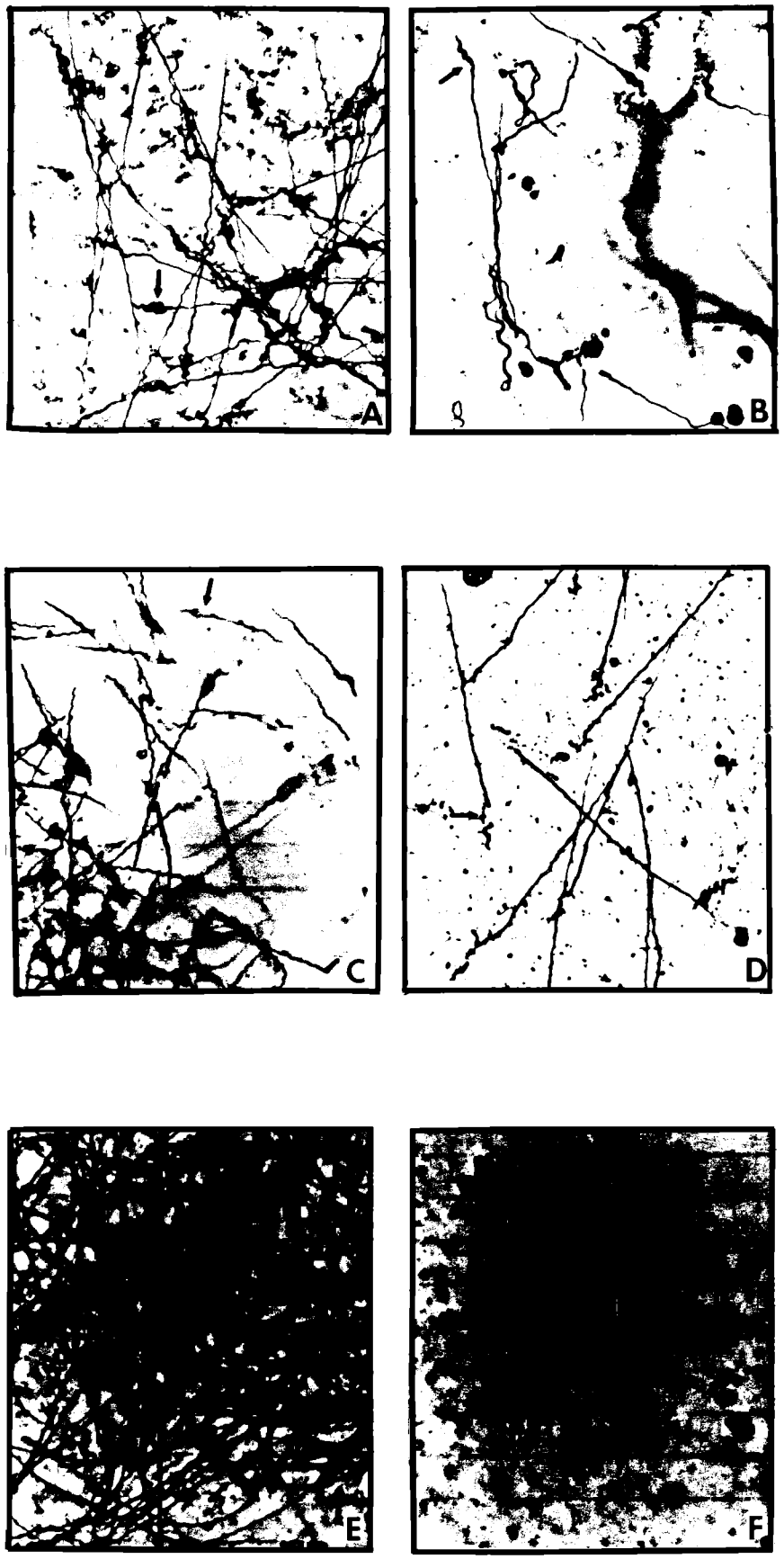

Figure 1.

Examples of sperm in cloacal lavage slides stained with Basic Fuchsin. A-D × 760; E and F × 800. Most lavage samples contained some sperm of normal appearance and others that were abnormal or partially disrupted. Swelling and disruption of the head and mid-piece were most common, and an example is pointed out (arrow) in each panel (A-F). A. SY Male Red-winged Blackbird #872–96025, Galveston, TX, April 26, 1982. B. ASY Male Bronzed Cowbird #872–96021, Galveston, TX, April 17, 1982. C. Mockingbird #941–79015, Galveston, TX, April 17, 1982. D. Male House Sparrow #1311–38682, Galveston, TX, March 17, 1982. E. sex uncertain Painted Bunting #960–73283, Galveston, TX, May 6, 1982. F. Male Black-capped Chickadee #1650–48023, Foley, MO, April 30, 1983.

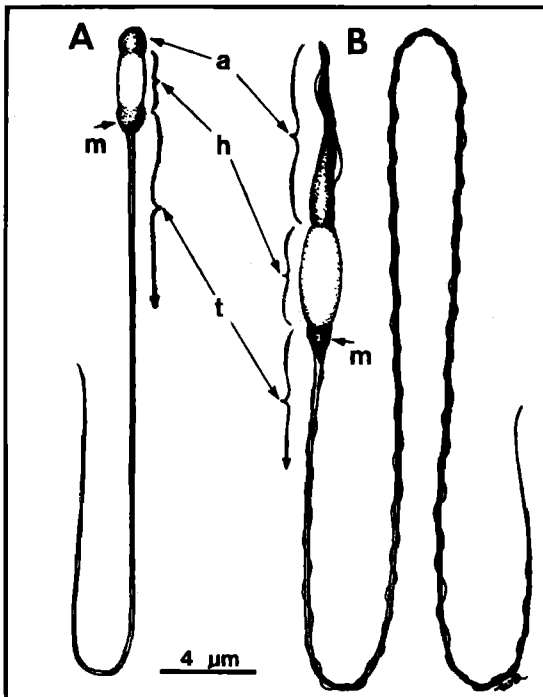

Figure 2.

Sperm morphology in two species, typifying the two general morphological sperm types among birds, and illustrating the three primary parts or regions (following the nomenclature of Lake 1981). A. Male Whip-poor-will #902–63129, Foley, MO, May 2, 1983. This is an example of the simpler, more primitive and widely occurring type of sperm seen in most nonpasseriform birds. B. Male House Sparrow #1321–23190, Foley, MO, May 4, 1983. This is an example of the more complex type of sperm morphology seen in most passeriforms. a = acrosome, h = head (the nuclear region), m = mid-piece (one of the four subregions of the tail), t = tail.

A second result was that sperm occurred about 10 times as frequently in males as in females (Table 1). This is not surprising, considering the prompt migration into, and prolonged survival of avian sperm in, the oviduct after a single mating (Lake 1975).

Table 1. Species[1] with sperm in cloacal lavages according to sex[2] and total number (N) of lavages per species[3].

Species[1]	Lavages with sperm	N
Whip-poor-will (*Caprimulgus vociferus*)	1 M	4
Northern Rough-winged Swallow (*Stelgidopteryx serripennis*)	1 M	2
Black-capped Chickadee (*Parus atricapillus*)	1 M,1 F	4
Tufted Titmouse (*Parus bicolor*)	1 M	4
Eastern Bluebird (*Sialia sialis*)	2 M	4
American Robin (*Turdus migratorius*)	1 M	2
Gray Catbird (*Dumetella carolinensis*)	2 U	22
Northern Mockingbird (*Mimus polyglottis*)	1 M	14
Brown Thrasher (*Toxostoma rufum*)	2 M	5
Loggerhead Shrike (*Lanius ludovicianus*)	1 U	5
European Starling (*Sturnus vulgaris*)	1 M	9
Red-eyed Vireo (*Vireo olivaceus*)	1 M	24
Nashville Warbler (*Vermivora ruficapilla*)	1 M	3
Kentucky Warbler (*Oporornis formosus*)	1 M	10
Common Yellowthroat (*Geothlypis trichas*)	1 M	14
Yellow-breasted Chat (*Icteria virens*)	1 M	4
Summer Tanager (*Piranga rubra*)	1 M	21
Northern Cardinal (*Cardinalis cardinalis*)	3 M,1 F	17
Rose-breasted Grosbeak (*Pheucticus ludovicianus*)	2 M	14
Indigo Bunting (*Passerina cyanea*)	5 M	42
Painted Bunting (*Passerina ciris*)	1 U	8
Rufous-sided Towhee (*Pipilo erythrophthalmus*)	1 M	1
Field Sparrow (*Spizella pusilla*)	1 M	2
Red-winged Blackbird (*Agelaius phoeniceus*)	10 M	20
Great-tailed Grackle (*Quiscalus mexicanus*)	1 M	15
Common Grackle (*Quiscalus quiscula*)	2 M	7
Bronzed Cowbird (*Molothrus aeneus*)	2 M	7
Northern Oriole (*Icterus galbula*)	1 M	8
House Sparrow (*Passer domesticus*)	21 M, 2 F	166
TOTALS: Species = 29	66 M, 4 F,4 U	456

[1]Names and sequence as in The A. O. U. Checklist of North American Birds, 6th ed..1983.
[2]M = males, F = females, U = sex not known.
[3]Species whose cloacal lavages lacked sperm are listed in the appendix.

Species of which one or more individuals showed sperm in cloacal lavages are listed in Table 1. Those not showing cloacal sperm are given in the Appendix. Several kinds of information are either given or suggested here. First, in any such sampling of both sexes and various ages at different times of the year, at best a rather small percentage of the birds would be expected to show sperm. In many instances where cloacal sperm were not found in any individual of a particular species, I have no basis for assuming that sperm would not have been found in another individual, or at another, more appropriate, age and/or time of year. Nevertheless, the lack of cloacal sperm in the Inca Doves, sampled in large numbers throughout the year within their breeding range, probably resulted from a difference in the handling or release of sperm by this species. A like situation may involve as well others of the species that failed to show cloacal sperm.

The primary result, illustrated by the data of Table 1, is the demonstration that cloacal lavages can be taken from birds of diverse sizes and habits, and without any apparent harm. I found no problems or signs of harm from the procedure in birds recaptured at various times after the intial or subsequent cloacal lavages. Signs that were looked for were unusual loss in body weight and indications of trauma, infection or inflammation in the cloacal region.

Discussion

Practice with the cloacal lavage technique, and with the microscopic identification of sperm, can be obtained most effectively with House Sparrows, before other species. Cloacal sperm are frequent during the extensive breeding season, and development of skills in cloacal lavage is less apt to cause problems in this hardy species.

Significant morphological variation occurs in the spermatozoa of birds (Fig. 2). The most prominent variation resides in the differences between particular taxa. Ornithologists intending to use cloacal lavage for sperm in their studies will be aided by first referring to general descriptions of avian sperm structure (Sturkie 1976, Lake 1981), and then to review the range of comparative differences (Retzius 1909, Romanoff 1960, McFarlane 1963, Humphreys 1972). Different aspects of morphological sperm variations from environmental and physiological influences have been studied only rarely in birds outside of what has been compiled for domestic fowl. It is likely that in birds, just as demonstrated in mammals, variation in sperm morphology may provide a useful indicator of some kinds of harmful environmental factors.

Sperm found in cloacal lavages of females certainly represent a seminal residuum from one or more copulations. In studies with artificial insemination of domestic fowl it has been concluded that sperm are rapidly distributed throughout the oviduct after either artificial insemination or copulation, but that they disappear from the lumen within 24 hours (Kamar and Hafez 1975).

Nevertheless, the known duration of avian female fertility after a single insemination ranges from 7 to about 50 days depending upon the species and other circumstances (Howarth 1974). This extended fertility depends upon sperm storage in specific regions of the female reproductive tract, particularly the "utero-vaginal sperm-host glands" and sites within the infundibulum of the oviduct (Lake 1975). Along with this extended storage of sperm in the female tract, there is rapid excretion of the majority of the live and dead sperm by the female after insemination, as well as a massive leakage of sperm from the vagina to the cloaca (Kamar and Hafez 1975).

Sperm in cloacal lavages from male birds often may represent the remainders from recent ejaculation(s) prior to the lavage. However, further studies are needed to clearly distinguish this kind of cloacal sperm origin from other possibilities, such as ejaculation induced by handling during the processing and cloacal lavage. There are two reasons for believing that the latter kind of origin is unlikely: (1) My handling of the birds during processing and lavage was rapid, gentle and minimal. Artificial induction of ejaculation in non-domestic birds can occur in four kinds of circumstances: cooperative, massage, electroejaculation and use of a dummy female (Howell and Bartholomew 1952, Wolfson 1952 1960, Gee and Temple 1978). In instances where I obtained serial lavage samples from a male showing cloacal sperm, maximal or near maximal numbers of sperm occurred in the first lavage. After the first or second lavage sample, sperm numbers per sample decreased approximately exponentially in the subsequent ones.

A third possible origin of cloacal sperm in males is through "spontaneous ejaculation", or a continuous expulsion into the cloaca with final voiding with other cloacal contents. This kind of process has been suggested to occur in nonejaculated domestic cockerels (Reviers 1973), but definitive evidence for it is not available, and it has not been invoked in non-domestic species. If such a phenomenon occurs, it, as well as artificial induction of ejaculation, suggest methods by which the potential fertility of a male could be tested by means of cloacal lavage.

Acknowledgments

I am most grateful to Charlet Quay for continued help and understanding during this study, to William A. Knox and John Y.-K. Pun for generous hospitality during netting and banding operations on their properties, and to James Hendel for professional aid in photomicrography.

Species whose cloacal lavages lacked sperm are (number of individuals): Sharp-shinned Hawk (*Accipiter striatus*) (1), Sora (*Porzana carolina*) (1), White-winged Dove (*Zenaida asiatica*) (6), Mourning Dove (*Zenaida macroura*) (2), Inca Dove (*Columbina inca*) (209), Common Ground Dove (*Columbina Passerina*) (2), Yellow-billed Cuckoo (*Coccyzus americanus*) (3), Chuck-will's-widow (*Caprimulgus carolinensis*) (3), Belted Kingfisher (*Ceryle alcyon*) (1), Red-headed Woodpecker (*Melanerpes erythrocephalus*) (1), Red-bellied Woodpecker (*Melanerpes carolinus*) (4), Yellow-bellied Sapsucker (*Sphyrapicus varius*) (8), Downy Woodpecker (*Picoides pubescens*) (1), Northern Flicker (*Colaptes auratus*) (5), Eastern Wood-Pewee (*Contopus virens*) (5), Yellow-bellied Flycatcher (*Empidonax flaviventris*) (1), Acadian Flycatcher (*Empidonax virescens*) (2), Least Flycatcher (*Empidonax minimus*) (4), Eastern Phoebe (*Sayornis phoebe*) (1), Great Crested Flycatcher (*Myiarchus crinitus*) (3), Eastern Kingbird (*Tyrannus tyrannus*) (3), Purple Martin (*Progne subis*) (10), Tree Swallow (*Tachycineta bicolor*) (1), Blue Jay (*Cyanocitta cristata*) (18), Red-breasted Nuthatch (*Sitta canadensis*) (1), White-breasted Nuthatch (*Sitta carolinensis*) (4), Brown Creeper (*Certhia americana*) (1), Rock Wren (*Salpinctes obsoletus*) (2), Ruby-crowned Kinglet (*Regulus calendula*) (7), Blue-gray Gnatcatcher (*Polioptila caerulea*) (4), Veery (*Catharus fuscescens*) (8), Gray-cheeked Thrush (*Catharus minimus*) (13), Swainson's Thrush (*Catharus ustulatus*) (13), Wood Thrush (*Hylocichla mustelina*) (3), White-eyed Vireo (*Vireo griseus*) (3), Yellow-throated Vireo (*Vireo flavifrons*) (2), Philadelphia Vireo (*Vireo philadelphicus*) (3), Blue-winged Warbler (*Vermivora pinus*) (1), Tennessee Warbler (*Vermivora peregrina*) (18), Northern Parula (*Parula americana*) (1), Yellow Warbler (*Dendroica petechia*) (3), Chestnut-sided Warbler (*Dendroica pennsylvania*) (2), Magnolia Warbler (*Dendroica magnolia*) (4), Cape May Warbler (*Dendroica tigrina*) (2), Yellow-rumped [Myrtle] Warbler (*Dendroica coronata*) (23), Black-throated Green Warbler(*Dendroica virens*) (3), Blackburnian Warbler (*Dendroica fusca*) (1), Palm Warbler (*Dendroica palmarum*) (4), Bay-breasted Warbler (*Dendroica castanea*) (5), Blackpoll Warbler (*Dendroica striata*) (2), Black-and-white Warbler (*Mniotilla varia*) (8), American Redstart (*Setaphaga ruticilla*) (7), Prothonotary Warbler (*Protonotaria citrea*) (10), Worm-eating Warbler (*Helmotheros vermivorus*) (3), Swainson's Warbler (*Limnothlypis swainsonii*) (1), Ovenbird (*Seiurus aurocapillus*) (12), Northern

Waterthrush (*Seiurus noveboracensis*) (10), Louisiana Waterthrush (*Seiurus motacilla*) (3), Mourning Warbler (*Oporornis philadelphia*) (2), Hooded Warbler (*Wilsonia citrina*) (6), Canada Warbler (*Wilsonia canadensis*) (3), Scarlet Tanager (*Piranga olivacea*) (4), Black-headed Grosbeak (*Pheucticus melanocephalus*) (1), Blue Grosbeak (*Guiraca caerulea*) (3), Chipping Sparrow (*Spizella passerina*) (1), Song Sparrow (*Melospiza melodia*) (1), Swamp Sparrow (*Melospiza georgiana*) (1), White-throated Sparrow (*Zonotrichia albicollis*) (3), Brewer's Blackbird (*Euphagus cyanocephalus*) (1), Brown-headed Cowbird (*Molothrus ater*), (16), Orchard Oriole (*Icterus spurius*) (12), House Finch (*Carpodacus mexicanus*) (2), Lesser Goldfinch (*Carduelis psaltria*) (1). Totals without cloacal sperm: 73 species, represented by 538 individuals.

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A Brown Creeper Recovery Gives Evidence Of Slow Migration

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A Brown Creeper (Certhia famililaris) (1661–02070) that I banded on 11 October 1983 at Blue Island, Illinois, was recovered by me on 13 October 1983 at my Tinley Park, Illinois, Cook County Forest Preserve station. The two stations are eight miles apart, as the crow flies. The direction of flight is south-west.

The creeper was banded and released at Blue Island, as a HY U, about 10:30 A.M. It was then recovered at my Tinley Park station two days later at 3:30 P.M. at my last net check. The bird only moved eight miles in two and half days.

My home station in Blue Island, is one of only half a dozen tree and shrub clusters left in Blue Island. My yard is a cluster jungle of fruit, pine and wild black cherry trees. The yard also has some 30 poke berry (Phytolacca americana) and large clumps of wild asters.

Between the two stations (8 miles) there is only a slight sprinkling of trees, until the forest preserves begin.

My Tinley Park station is in the middle of two square miles of forest preserves.

I only use three mist nets in the middle of the two square miles of Forest Preserve, at the Tinley Park station. The creeper capture is most remarkable, due to the fact it had two square miles of oak trees to forage in.