J. Field Ornithol., 59(4):389–394

SURVIVAL OF RED-COCKADED WOODPECKER NESTLINGS UNAFFECTED BY SAMPLING BLOOD AND FEATHER PULP FOR GENETIC STUDIES

PETER W. STANGEL
University of Georgia's Savannah River Ecology Laboratory
P.O. Drawer E
Aiken, South Carolina 29801 USA

MICHAEL R. LENNARTZ
U.S.D.A. Forest Service
Southeastern Forest Experiment Station
Department of Forestry
Clemson University
Clemson, South Carolina 29631 USA

Abstract.—Fragmentation and contraction of the Red-cockaded Woodpecker's (*Picoides borealis*) distribution suggests that small, isolated populations may be susceptible to loss of genetic variability. We examine the utility of non-destructive sampling techniques to obtain tissues from nestlings for genetic analysis. Removal of a small blood sample and a growing primary feather did not affect nestling survival. Feather pulp provided clearer resolution of enzymes than blood. Eighteen gene loci were resolved using standard electrophoretic techniques. Our results suggest that tissue samples for genetic analysis can be safely obtained from small birds.

SUPERVIVENCIA DE PICHONES DE *PICOIDES BOREALIS*, INAFECTADOS POR LA TOMA DE MUESTRAS DE SANGRE O PLUMAS PARA ESTUDIOS GENÉTICOS

Resumen.—La fragmentación y reducción de la distribución de *Picoides borealis*, sugiere que poblaciones pequeñas y aisladas son susceptibles a la perdida de variabilidad genética. Nosotros examinamos la utilidad de obtener muestras de tejidos de aves, para estudios genéticos, sin tener que destruir las mismas. La toma de una pequeña muestra de sangre y la remoción de una primaria en primeras etapas de crecimiento, no afectó la supervivencia de los pichones. La pulpa de las plumas, proveyó de una resolución más clara de las enzimas, que las obtenidas de las muestras sanguineas. Utilizando técnicas electroforéticas fueron identificados un total de 18 locus de genes. Los resultados sugieren que se pueden tomar muestras de tejidos para estudios genéticos en aves más pequeñas que pajaros carpinteros, sin que estas afecten la supervivencia de los mismos.

Genetic variability is commonly assessed with starch-gel electrophoresis, and enzyme activity is usually highest in muscle and internal organs. Destructive sampling, however, is unacceptable when working with endangered species or research requiring the continued survival of the individual sampled. The purpose of this study was to test a nondestructive sampling technique using blood and feather pulp for estimating genetic variability in Red-cockaded Woodpeckers (*Picoides borealis*). Our objectives were to determine (1) the effect of blood and feather pulp collection on survival of Red-cockaded Woodpeckers, and (2) the number of gene loci that could be analyzed with blood and feather samples. Our procedures and results should be applicable to other birds of similar size.

METHODS

Tissue collection.—This experiment was conducted in the Francis Marion National Forest, Berkeley County, South Carolina, during May and June of 1985. The Red-cockaded Woodpecker population here is one of the largest known (Lennartz et al. 1983) and has been under intensive study since 1976. Twenty-five nestling Red-cockaded Woodpeckers 9–12 d old were removed from cavities by noosing (Jackson 1977). By this age pinfeathers were 20–30 mm in length and sufficiently well-developed to provide an adequate tissue sample for electrophoretic analysis. Chicks were robust ($\bar{x}$ = 36.8 ± 3.8 g; range 30.5–45.0 g) and had obtained approximately 80% of adult mass ($\bar{x}$ = 44.5 ± 0.86 g). Beyond 12 d old, noosing must be attempted with extreme caution. At this age pin feathers were fully erupted and nestlings were sufficiently large that their removal from the cavity became difficult, and concern for damage to feathers precluded capture.

Laboratory studies on other bird species indicate that approximately 5% of the total blood volume can be removed without stress to the bird (Stangel 1986). Based on a mean body weight of 36.8 ± 3.8 g for chicks, we collected a sample of 250 μl from the brachial vein. Procedural details are in Stangel (1986). Blood samples were immediately placed on wet ice and centrifuged for 5 min at 15,000 rpm within 2 h. Plasma and hemolysate were stored in liquid nitrogen (−196 C) until analysis.

Two growing, centrally-located primary feathers, one from each wing, were collected from each individual. All primaries were at a similar developmental stage among birds. Feathers were grasped close to the follicle and pulled straight out to prevent breakage of the calamus inside the follicle. Bleeding from the follicle was observed in less than 10% of the nestlings. Feathers were placed in plastic vials and immediately frozen in liquid nitrogen. Time required for collection of blood and feathers averaged about 3 min per individual (n = 7). Nestlings were replaced in cavities; total time outside of the cavity depended on the number of chicks sampled, but was generally less than 15 min.

Electrophoresis.—Horizontal starch gel electrophoresis following the general procedures of Selander et al. (1971) and Harris and Hopkinson (1976) were used to screen for enzyme activity. Pulp was squeezed from the feather shaft and homogenized with 4–5 ml of 0.01 M Tris-0.001 M EDTA, pH 7.0 buffer solution. Serum and hemolysate were homogenized separately with 1–5 ml of buffer solution. Serum, hemolysate, and feather pulp were electrophoresed side by side to compare enzyme activity. We screened 31 commonly used enzymes on each of seven different buffer types. Enzyme resolution was given a score of 1–3 based on clarity. Details of electrophoretic conditions and scoring are available from the authors.

Selection of individuals and monitoring survivorship.—Samples were collected from nestlings in 18 nests. Treatment nestlings were haphazardly chosen: the first individual(s) noosed was sampled. In eight of 13 nests containing multiple nestlings, at least one chick was left unsampled (Table 1). Unsampled nestlings were not removed from the cavity or banded and

TABLE 1. Application of blood and feather sampling treatment to nestling Red-cockaded Woodpeckers to determine effect on survival to fledging.

Combinations of treatment and control nestlings within broods	Number of nests	Number of treatment nestlings	Number of within-brood control nestlings	Survival to fledging	
				Treatment	Control
1 Treatment	5	5	0	5	—
2 Treatment	5	10	0	10	—
1 Treatment-1 control	5	5	5	5	4
1 Treatment-2 control	1	1	2	1	2
2 Treatment-1 control	1	2	1	2	1
2 Treatment-2 control	1	2	2	2	1
Total	18	25	10	25	8

served as within-brood controls. Treatment nestlings were color-banded to permit individual identification. In this manner, 25 nestlings (10 males, 15 females) were sampled for blood and feather pulp, and 10 of these treatment chicks had non-sampled sibling controls.

Two checks were made at each nest to monitor survival of nestlings. The first check was made 5 d after sampling, when nestlings were 14–17 d old and still within the cavity. During the first check, drop lights and small mirrors were used to observe chicks within cavities. Leg bands could not be observed on the squatting chicks. Thus, this check provided only a nestling count, and was performed primarily to detect immediate mortality resulting from sampling.

The second check was conducted within 48 h of fledging, when chicks were 26–28 d old. Identification of fledglings was done by two observers using telescopes. Treatment fledglings were identified from color bands. Unbanded control fledglings were identified as such when fed by parents. One observer continuously monitored fledglings which had been positively identified while the second observer searched for the remaining fledglings. Fledgling checks were continued until all treatment and control birds were identified, which usually required less than 1 h. If any individuals could not be located after 2 h of continuous searching, a second visit was made to the location the following morning. If, after an additional 2 h of searching an individual bird could not be located, it was considered missing.

RESULTS

Electrophoresis.—Of the 31 enzymes screened, we found 14 encoding 18 presumptive gene loci that could be scored clearly on four buffer types: Tris-maleate pH 7.4—ICD-1,2(1.1.1.42), CK-1,2 (2.7.3.2), NSP-1,2 (2.4.2.1); Tris-citrate pH 8.0—MDH-1,2 (1.1.1.37), Es (β-naphylacetate substrate, 3.1.1.1), Pep (leucylglycylglycine, 3.4.11), GPI-1 (5.3.1.9); Tris-EDTA-citrate pH 7.1—GPI-2 (5.3.1.9), PGD (1.1.1.43), AK (2.7.4.3),

general protein; Tris-citrate pH 8.7 discontinuous—LDH (1.1.1.27), PGM (5.4.2.2), MPI (5.3.1.8). For all enzymes examined, feather pulp provided equal or better resolution than serum or hemolysate. Some enzymes showed good resolution on several buffer types. Where this occurred, only the buffer providing superior results (or number of loci) was reported. The amount of blood and feather pulp collected provided ample material for 10 electrophoretic runs.

Survivorship.—In 15 of 18 nests, the same number of chicks present during sampling were counted during the first check. Every nestling sampled was, however, positively identified following fledging, 12–15 d after treatment. Eight of 10 control birds were located. Survival of nestlings was not significantly different between groups (Contingency $\chi^2 = 0.455$, df = 1, $P = 0.5$).

DISCUSSION

The results of this study indicate that blood and growing feathers can be sampled from Red-cockaded Woodpecker nestlings without affecting survival or development. In birds, lost blood is replaced quickly (Jones and Johansen 1972). Red-cockaded Woodpeckers recaptured 15–20 d after treatment had regrown primaries to a length similar to that when plucked.

The loss of two control birds probably represents random mortality. In one case, the control nestling was observed during the first check, suggesting that mortality occurred at fledging or shortly thereafter. The second missing control could not be positively identified at the first check.

In some cases, our selection of treatment nestlings may have been biased toward heavier nestlings. Red-cockaded Woodpeckers hatch asynchronously. In two-chick broods ($n = 10$), early-hatched nestlings weighed significantly more than siblings (36.8 ± 3.9 g vs. 32.3 ± 6.0 g, respectively; t-test, $t = 1.96$, $P < 0.05$). Early-hatched nestlings may reach higher when begging and may thus be more likely to be noosed. If larger chicks were noosed, then the control group may consist of smaller individuals. If the smaller mass of younger chicks affects survival, this may contribute to mortality of smaller birds.

Other recent field and laboratory studies provide additional evidence that tissues can be sampled with minimal stress to small birds. Stangel (1986) reported no mortality or change in body weight in three species sampled for blood and growing feathers. Westneat (1986) found that biopsy of a small section of breast muscle did not affect survival or condition of adult White-throated Sparrows (*Zonotrichia albicollis*). Similar muscle biopsies did not affect survival or breeding success of adult Indigo Buntings (*Passerina cyanea*), but first-year males were more likely to disappear following biopsy than were controls (Westneat et al. 1986). Muscle biopsy had a negative effect on Indigo Bunting nestling survival from fledging to return to the study area the following year (Westneat et al. 1986). Possible complications resulting from muscle biopsy suggest that more benign sampling techniques be employed for sensitive species.

Using blood and feather pulp for electrophoretic studies does have limitations. Compared to muscle and internal organs, blood and feather pulp apparently provide fewer gene loci for analysis. Most recent studies using muscle and internal organs from birds report resolution of 40 ± presumptive gene loci (Johnson et al. 1984). Studies using blood and feather pulp report resolution of only 15–25 loci (Marsden and May 1984, J. M. Novak, pers. comm.). This is apparently due to lower enzyme concentrations in these tissues.

Blood samples can probably be safely removed at any time of the year, but pulp from growing feathers can only be collected during the molt period. Although molt in many species lasts up to several months, this limits the time during which growing feathers can be collected. For studies in which birds are closely monitored for several weeks or months, feather growth could be induced by plucking mature feathers (Mengdon and Stock 1976).

Although blood and feather pulp do have some limitations for electrophoresis, they provide biological data that might otherwise be unobtainable. For example, preliminary results from our surveys of the Red-cockaded Woodpecker reveal loss of heterozygosity in some small, isolated populations (Stangel, unpubl. data). Reduced heterozygosity has been associated with a variety of negative effects on individual vigor (Allendorf and Leary 1986). If similar effects are recorded for Red-cockaded Woodpeckers, the loss of heterozygosity may be a contributing factor to population declines in small, isolated populations. Without genetic surveys this problem might have gone undetected, and the information necessary to implement management programs would not be available.

ACKNOWLEDGMENTS

Julia I. Smith, Mary Hunnicut, Ronald Sarno, and Adrianne Massey provided field assistance. Robert Hooper supplied information on colony location and status. Reviews from A. L. Bryan, G. R. Hepp, D. J. Stangel, and M. A. Walton improved earlier drafts of the manuscript. This research was supported by contract DE-AC09-76SROO-819 between the U.S. Dept. of Energy and the University of Georgia's Savannah River Ecology Laboratory.

LITERATURE CITED

ALLENDORF, F. W., AND R. F. LEARY. 1986. Heterozygosity and fitness in natural populations of animals. Pp. 57–76, *in* M. E. Soulé, ed. Conservation biology: the science of scarcity and diversity. Sinauer Assoc., Sunderland, MA.

HARRIS, H., AND D. A. HOPKINSON. 1976. Handbook of enzyme electrophoresis in human genetics. North Holland Publishing Co., Amsterdam.

JACKSON, J. A. 1977. Capturing woodpecker nestlings with a noose: a technique and its limitations. N. Am. Bird Bander 7:90–92.

JOHNSON, N. K., R. M. ZINK, G. F. BARROWCLOUGH, AND J. A. MARTEN. 1984. Suggested techniques for modern avian systematics. Wilson Bull. 96:543–560.

JONES, D. R., AND K. JOHANSEN. 1972. The blood vascular system of birds. Pp. 233–234, *in* D. S. Farner and J. R. King, eds. Avian biology vol. II. Academic Press, New York.

LENNARTZ, M. R., P. H. GEISSLER, R. F. HARLOW, R. C. LONG, K. M. CHITWOOD, AND J. A. JACKSON. 1983. Status of the Red-cockaded Woodpecker on federal lands in

the South. Pp. 7–12, *in* D. A. Wood, ed. Proc. Red-cockaded Woodpecker symp. II. Florida Game and Fresh Water Fish Comm. and U.S. Fish Wildl. Serv.

MARSDEN, J. E., AND B. MAY. 1984. Feather pulp: a non-destructive sampling technique for electrophoretic studies of birds. Auk 101:173–175.

MENGDON, G. A., AND A. D. STOCK. 1976. A preliminary report on the application of current cytological techniques to sexing birds. Intern. Zoo Yearbook 16:136–141.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON, AND J. B. GENTRY. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in Genetics VI. Univ. Texas Publ. 7103:49–90.

STANGEL, P. W. 1986. Lack of effects from sampling blood from small birds. Condor 88: 244–245.

WESTNEAT, D. F. 1986. The effects of muscle biopsy on survival and condition in White-throated Sparrows. Wilson Bull. 98:280–285.

———, R. B. PAYNE, AND S. M. DOEHLERT. 1986. Effects of muscle biopsy on survival and breeding success in Indigo Buntings. Condor 88:220–227.

Received 2 Nov. 1987; accepted 15 Jun. 1988.