SOME EFFECTS OF X-IRRADIATION ON THE BREEDING BIOLOGY OF EASTERN BLUEBIRDS

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THE object of this study, which was conducted in the spring and summer of 1956, was to investigate certain aspects of the breeding biology of Eastern Bluebirds (*Sialia sialis*), with emphasis on effects of relatively heavy, but sublethal, whole-body X-irradiation on adult females, nestlings, and eggs. An underlying assumption was that marked departure from normal breeding behavior and/or normal production of viable eggs and healthy young would provide a rough index of the effects of radiation treatment on the nervous system, sex organs, and other parts of the body. Wild birds are deemed favorable material for the study of radiation effects in nature for several reasons: (1) Their breeding behavior involves a series of rather stereotyped behavior patterns, which tend to follow one another in a sort of chain reaction (one event releasing the next); (2) these patterns and their biological consequences, such as components of reproductive success, are well known in some species (including the bluebird) and hence abnormal behavior can readily be detected; and (3) by utilizing common species which will nest in artificial nest-boxes, one can easily capture individuals for treatment, observe breeding behavior, and follow the fates of eggs and nestlings. An advantage similar to that last mentioned was realized by Blair (1958) who, in a several-year, pioneer study of the ecology of a natural population of wild mice, subjected the adult males to heavy X-irradiation and determined the effects on litter size and other population characteristics.

PROCEDURE

General.—The population of bluebirds under study bred in nest-boxes placed on posts along roads in the Savannah River Plant (SRP) area, Aiken and Barnwell counties, South Carolina. As this species is two- to three-brooded, nesting activity began in late March and lasted through August. Study of the history of each nest required almost daily surveillance. About 70 trips or "rounds" were made to the boxes, which numbered 31, and many additional checks were made on particular boxes or families of birds. There were also limited observations of the Great Crested Flycatcher (*Myiarchus crinitus*) and Tufted Titmouse (*Parus bicolor*), which used four of the boxes in parts of the 1956 breeding season.

For the irradiation of birds and eggs, a Westinghouse "Quadrocondex" machine (228 kvp; 15 milliamperes; with unfiltered X rays) was employed. It was so adjusted as to deliver, in a horizontal beam, a relatively high dosage rate of 200 roentgens (r) in 8½ minutes (23.5 r per minute) at a target-field distance of 4 feet. This rate is deemed relatively high in view of Stearner's work with young chicks; for interpretations of high and low dosage rates, see Stearner (1949*a*, *b*), and quotations below (pp. 452–3). A Victoreen ionization chamber of 250-r capacity was used as a guide to dosages. A given delivery (say, 400 r) was usually accurate, as determined by the chamber reading, to within 3 or 4 r. Each 200-r exposure was continuous. Each 400-r exposure was fractionated into two 8½-minute periods separated by a 5-minute "rest interval," and each 600-r exposure was given in three 8½-minute periods separated by two such intervals. During an exposure a bird was kept in a resting state in a small container made of ½-inch mesh hardware cloth (with an additional cloth cover). It was believed that this large-mesh cage caused relatively little scatter of rays or, at any rate, modified only slightly the total amount of ionizing radiation intended for the bird. The several eggs exposed to radiation were wrapped in cotton and secured near the top of the cage, so that there was presumably even less scatter in rays that passed or penetrated these smaller targets.

Adult and young bluebirds could be identified as individuals by colored bands which were placed on their tarsi in different combinations. Eggs were numbered or otherwise marked with indelible pencils. Tiny nestlings were marked, where necessary, with colored threads tied rather loosely round their legs or with dye applied to their fluffy natal down.

Résumé of treatment of adult females.—Several of the non-irradiated bluebirds were "strict controls," being caught and taken to the laboratory but receiving no radiation. Most of them were "loose controls," being disturbed only where necessary for purposes of banding, examining nests, etc. Only one of the controls deserted her nest because of my activity. This bird was 6F, a strict control which was caught and handled after laying her third egg; she promptly disappeared without warming her apparently incomplete set or attempting to complete it.

Among the eight females that were irradiated, 5F, which received 600 r, was bringing in nest materials at the time of treatment. The disturbance caused her to abandon her nest. She disappeared within a few minutes and was not seen again. Among the seven females which received X-ray and laid eggs, 10F was given 600 r after she had laid two eggs; she laid three more but deserted soon after completing her set. Fortunately I was able to forestall other desertions by waiting until incubation was well under way before capturing females for treatment. As an extra precaution, I returned each female to her nest cavity, holding my hand over the entrance for a minute or more before withdrawing. In this way I made certain that each bird saw once again the interior of her nest chamber, whose contents had not been disturbed.

Of the remaining six birds, two, 25F and 26F, received 200 r each; 30F received 400 r; and 4F, 17F, and 28F each received 600 r. Each was taken to the laboratory only once. The mean dosage delivered to these birds together with 10F (all of which are involved in tables 1 and 2) was approximately 460 r.

ACKNOWLEDGMENTS

I am indebted to Marvin Dennis, Joe Halford, C. M. Patterson, and Charles Wright of the du Pont Company and to Goff H. Giboney and Karl E. Herde of the Atomic Energy Commission for their technical and administrative aid. It was through their efforts that routine usage of the X-ray unit (for calibration purposes) was rescheduled so that birds could be irradiated. Both Mr. Herde and Eugene P. Odum, in charge of the University of Georgia Ecological Studies in the SRP area, read the manuscript and provided helpful criticism. Dr. Odum also supplied ready-cut parts of nest-boxes, sawed by J. B. Gentry, which service enabled me to complete the boxes and place them strategically before the nesting season began. The study was carried out under Contract No. AT (07–2)–10 with the Division of Biology and Medicine, U. S. Atomic Energy Commission. In 1957 comparable studies of bluebirds, with emphasis on effects of whole-body irradiation of nestlings, were commenced by William K. Willard, working under the same contract.

RESULTS

Size of egg sets.—The bluebirds in the SRP population laid from 3 to 5 eggs. The eggs in a set were laid one a day, about 8:30 or 9 a.m. Ten sets produced by females in the irradiated group averaged 4.4 eggs; 16 sets laid by non-irradiated birds in a comparable seasonal period averaged 4.1 eggs. Among the non-irradiated birds there was the barest suggestion that smaller sets were laid in later parts of the breeding season. In some populations there is a strong tendency for bluebirds to produce smaller sets as the season advances (Laskey, 1940; Thomas, 1946). In the irradiated females for which two or three broods were recorded, the number of eggs per set was the same in first and subsequent nestings. Thus, 4F (irradiated April 16) completed laying sets of 4 on April 9 and June 9; 25F (irradiated April 20) completed sets of 5 on April 8, June 3, and July 16; and 30F (irradiated

April 25) completed sets of 5 on April 20 and June 12. These data, together with the lack of definitive evidence of reduced hatchability of eggs laid after irradiation of females, indicate that there is no reduction in the effective size of broods following radiation treatment of female parents. These results, preliminary though they are, stand in contrast to those obtained by Russell (1954:831), who states that "acute X-ray exposure of female mice with a dose of 150 r, or perhaps lower, results in permanent sterility. One, and occasionally two, litters can be obtained, even at much higher doses, before the sterility sets in. . . . The litter size in this temporary fertile period is reduced. . . ." Whether the female bluebirds eventually become sterile or less viable is an unanswered question; certainly there is no indication of diminished size of egg sets in the post-irradiation "fertile period."

In an extensive study of a natural population of deer-mice (*Peromyscus maniculatus*) in Texas, Blair (1958), through acute X-irradiation of sexually mature males, induced a significant reduction in litter size which was attributed to radiation-caused lethal mutations. It would be of interest to determine whether such irradiation of male bluebirds (in association with untreated females) would affect size of broods, viability of offspring, or overall productivity.

Incubation period.—As in other birds—for example, the European representative of the Winter Wren, *Troglodytes troglodytes* (Armstrong, 1955)—in bluebirds there is some evidence of longer incubation periods in April and May and shorter ones in June and July. Table 1 indicates that this trend was apparent whether or not the female, which, in this species as in most songbirds, is the only parent that regularly incubates, was subjected to X-ray treatment. Among the various factors affecting length of incubation, the conditions in the nest, including the amount and temporal distribution of body heat applied to the eggs, are of no little importance. Consequently, if the incubating behavior of the female were changed significantly by the onset of radiation sickness, this change might well be reflected by a change in the length of the incubation period. Although the incubation periods of the non-irradiated females averaged slightly shorter than those of the irradiated birds, the differences shown in table 1 are not significant statistically (as determined by *t*-tests). The fact that the incubation period in bluebirds of the SRP area tends to average about 14 days is consonant with the findings of Laskey (1940) at Nashville, Tennessee, who found it to last 13 to 14 days, and of Thomas (1946) at North Little Rock, Arkansas, who found it to extend from 13 to 15 days, centering around 14. Like Smith (1937), I had one instance of a 16-day incubation period; this was a fairly early nest, wherein the set was completed on April 28.

TABLE 1

INCUBATION PERIOD IN RELATION TO SEASONAL CHANGE AND TREATMENT OF FEMALES

	Incubation Period Ending				*Total Records*	
	Apr. 25 to May 31		*June 1 to July 31*			
Treatment	*No.*	*Period in Days*	*No.*	*Period in Days*	*No.*	*Period in Days*
Irradiated*	4	14.9	3	13.8	7	14.4†
Not irradiated	5	14.3	9	13.7	14	13.9

* In most instances birds were irradiated early in the incubation period.
† Differences between incubation periods of irradiated and non-irradiated birds are not significant (P-values greater than .10).

An unusually long incubation period of 18 days (±1 day) was recorded at the first nest of 11F, an untreated bird whose first egg was irradiated and transferred to 7F's nest. It was because 11F failed to lay the next day after the first egg was irradiated that I transferred this egg; at the time I thought that she had deserted. But on the following days, April 30 and May 1, she proceeded to lay two more eggs. The protracted incubation period could have been due to 11F's laxity in the task of incubating, possibly because of the abnormally small set of two eggs. Or it could have been due to relative inviability of the young, both of which died shortly after hatching. The latter possibility is supported somewhat by the fact that 11F's second set, of which the fourth egg was laid June 11, failed to hatch. In all four eggs the embryos had died when about half developed. With this larger set, incubation was normal as attested by the parents' solicitude, as well as definite warmth of the eggs, for fully 16 days after the last had been laid.

Hatching success.—As brought out in table 2, there was a relatively small difference in hatching success of eggs under non-irradiated females as compared with irradiated ones. If two of the aforementioned birds, the control 6F and the irradiated 10F, are omitted from consideration (since they were all but "forced" to desert), then the difference between hatching-success values of non-irradiated and irradiated samples in a seasonally comparable period is so slight as to be non-significant if not negligible. These data would seem to provide further evidence that conditions in the nest, including the roles of the incubating females, were well nigh the same for treated and untreated birds.

Laskey (1952) has pointed out that the bluebird's hatching success is higher early in the breeding season and lower in the later, warmer part of the season. This trend is well exemplified by the data on hatching success of eggs of non-irradiated females (table 2), which ranged from about 86 per cent in spring and early summer (seasonal periods 1 and 2) to a lower, cumulative value of 70–71 per cent for the entire season. Such a trend is not evidenced in the irradiated

TABLE 2

HATCHING SUCCESS OF EGGS IN RELATION TO SEASONAL CHANGE AND TREATMENT OF FEMALES

	Cumulative Data on Hatching Success					
	Eggs Incubated by Non-Irradiated Females			*Eggs Incubated by Irradiated Females**		
Seasonal Period	*No. Laid*	*No. Hatched*	*Per Cent Hatched*	*No. Laid*	*No. Hatched*	*Per Cent Hatched*
(1) April 7–May 31	30 (7) †	30	100	30 (7)	22	73.4
(2) June 1–16	51 (12)	44	86.3‡	44 (10)	34	77.3
(3) June 17–30	64 (15)	53	82.8	(none)	(none)	—
(4) July 1–16	88 (22)	63	70.5	49 (11)	39	79.6‡
(5) July 17–30	96 (25)	68	70.8	(none)	(none)	—

* Birds were irradiated in seasonal period 1 when incubation of completed sets (with one exception) was under way; for irradiated females, data from periods 2 and 4 pertain to second broods of three individuals (at which time "delayed radiation effects" might have been operative).

† Number of egg sets in cumulative arrangement are given in parentheses (these totaling 25 for non-irradiated birds, 11 for irradiated ones).

‡ On the basis of seasonal spread of laying, the cumulative hatching-success value, 86.3 per cent, should be compared with the value 79.6 per cent, as this subsample from the non-irradiated birds is seasonally comparable to the total sample from the irradiated birds (mean dates of completion of sets being May 18 and May 13, respectively).

birds, probably because here the sample is smaller and pertains chiefly to relatively early broods. Whereas in the SRP area hatching success of non-irradiated birds throughout the nesting season was about 71 per cent, that recorded in some other regions has been somewhat lower. Thus, Laskey's (*ibid.*) study of the 1952 population of bluebirds at Nashville revealed an overall hatching success of about 62 per cent. This value might have been higher, however, if the summer had not been extremely hot and dry.

Nestling period.—The average nestling period of seven broods of young bluebirds having non-irradiated female parents was 16.7 days (extremes 15.2—18.2); that of four broods having irradiated mothers was 16.4 days (extremes 15.2—17.1). This difference is not significant. There remains a strong indication that brooding and food providing by treated and untreated females is essentially the same. This has been borne out in some measure by observations of activities of females at and near their nests.

Comparable nestling periods of 14 to 17 days, commonly 16, have been reported from Tennessee (Laskey, 1940; Laskey, *in* Bent, 1949:244), and others of 17 and 18 days have been recorded in Arkansas (Thomas, 1946). In Vermont, Smith (1937) found the period spent by the young in the nest was usually 18 days. It seems likely that this period averages a day or two shorter in the southern United States than in the New England region.

General reproductive success.—Among 17 nesting attempts by pairs in which the females were not irradiated, 11 succeeded (in that one or more young were fledged) with a total of 42 young leaving the nests—an average of 2.4 fledglings per parent pair. Of 10 attempts by pairs in which the females were irradiated, 7 succeeded with a total of 30 young leaving the nests—an average of 3.0 fledglings per parent pair. These data are derived from all recorded nesting attempts of irradiated birds and from a majority of the recorded attempts of non-irradiated birds. In order to keep the samples seasonally comparable, some of the latest attempts—in late June and July—by untreated birds are omitted. As a consequence, the mean dates of nesting, specifically the times of laying of the last egg, for the treated and untreated birds here considered are May 13 and May 20, respectively. The establishment of seasonal comparability of samples is necessary, for Laskey (1939), among others, has noted that earlier nests of bluebirds have a higher percentage of success than later ones. It is likely that the above-indicated difference in which more young were fledged from nests of treated females than from those of untreated, is rather misleading and that a larger mass of data would reveal for the compared groups either no significant difference or possibly somewhat lessened reproductive success in the treated group. If any conclusion can be drawn, I believe it is best stated tentatively and negatively: there is no suggestion whatever that pairs in which females were irradiated were less successful in producing fledglings than were other pairs in which the females were not irradiated.

The fact that these quantitative aspects of the species' reproductive potential seemed essentially unchanged by the radiation treatment of female parents, suggests that one X-ray dosage strong enough to kill half the irradiated birds in, say, a period of 30 days (LD 50/ 30 days) would have to be comparatively high. Among mammals the LD 50 dosage (lethal dosage to 50 per cent) is about 200 r for guinea pigs, 400 to 600 r for different strains of laboratory mice, 700 r for hamsters, and 790 to 800 r for rabbits (Boche and Bishop, 1954; Bloom, 1948). These dosages could be either in one continuous treatment or fractionated. Because the general appearance and demeanor as well as the reproductive activity of the female bluebirds seemed to remain normal in spite of dosages of 200 to 600 r, it would seem that an LD 50 dosage for these birds would be higher than that of the laboratory mammals. One is tempted to conjecture that it might well exceed 1000 r, even with a high dosage rate.

Irradiation of eggs and nestlings.—From table 3 it is apparent that embryos are more vulnerable to X-irradiation than are adult birds. Yet it is of interest that three eggs, including two that were irradiated when incubation was under way, yielded nestlings which developed normally from all gross evidences and left the nest successfully.

TABLE 3

FATE OF IRRADIATED EGGS IN RELATION TO DOSAGE AND PROGRESS OF INCUBATION AT TIME OF EXPOSURE

Eggs Not Hatching			*Eggs Hatching But Nestlings Dying*			*Nestlings Successful in Fledging*	
Dosage (r)	*Progress of Incubation (Days)*	*Egg Contents*	*Dosage (r)*	*Progress of Incubation (Days)*	*Age of Nestling at Death (Days)*	*Dosage (r)*	*Progress of Incubation (Days)*
400	0–1*	6-mm. embryo	200	0–1	9 or 10	200	3–5
600	1–3	full-term embryo	400	0–1	9 or 10	400	8
600	5	full-term embryo†	400	9	3 to 7	600	0–1

* 0–1 refers to freshly laid egg.

† A teratological specimen (the limbs were poorly developed and the toes were mere nubbins).

Comparable data were obtained from two other bird species. Two fresh eggs of the Great Crested Flycatcher were each given 600 r. In one the embryo died when about 7 millimeters long; in the other it died when about full term, although it seemed unusually slender and showed signs of hemorrhage in the belly region. Two eggs of the

Tufted Titmouse, both of which had been incubated 9 or 10 days, were given 600 r and 200 r, respectively. Whereas the former failed to hatch and proved to have a dead embryo, the latter produced a nestling which, along with its four siblings, succeeded in fledging.

Although some of the heavily irradiated eggs resulted in nestlings and fledglings, it does not necessarily follow that all these young were to be vigorous, fertile, and otherwise successful in intra- and interspecific competition.

The oft-stated fact that young animals are more sensitive to radiation damage than older animals is borne out to some extent by the findings of Bloom (1948:751) who reported that "in . . . three-week chicks certain organs were extremely damaged while in older chickens and in the adult mammals they were [relatively] radioresistant." Similar differences, especially in the resistance of kidney tissues, have been reported by Stearner and Christian (1949–50) and by Stearner *et al.* (1955). In the four bluebird nestlings which were irradiated (table 4) there might have been considerable tissue damage, but there was no evidence of sickness in any of these birds. From all gross appearances they withstood the heavy dosages about as well as the adult females. Particularly interesting was the fact that the week-old, pin-feathered nestling given the massive dosage of 1200 r appeared to develop as normally as its siblings and was successful in fledging. At all the nests under observation, the immatures tended to disappear from the nest-box vicinity within a day or two after they left the nest. The post-fledging history of these young was not followed.

In one-week white leghorn chicks, Stearner (1949*a*) found that the LD 50 dosage (at both 4 and 14 days) was between 600 and 800 r where the dosage rate was 43 r per minute, and 900–1000 r where the rate was 6 r per minute. Newly hatched ducklings (mammoth white Pekins) "showed the same reaction to irradiation as was seen in

TABLE 4

IRRADIATION OF BLUEBIRD NESTLINGS

Nest-Box No.	*No. in Brood*	*Designations of Irradiated Individuals*	*Treatment* *Date*	*Age of Nestling (Days)*	*Dosage (r)**	*Results*
4	5	YY–G	May 10	13	400	Both fledged normally when 17 days old; no loss in weight
		BB–G	May 8 & 10	13 & 15	800	
31	5	B–O	May 29	7 (approx.)	600	Both developed normally, fledging 9 days after treatment
		O–R	May 28 & 29	7 (approx.)	1200	

* BB–G received 400 r each day; O–R, 600 r each day.

chicks. . . ." (*ibid.*). As pointed out in a subsequent report (Stearner, 1949*b*), survival in chicks "following continuous and fractionated dosages at an average rate of 11.5 r per min. resembled that which follows low rate exposures, while survival after continuous or fractionated dosages at an average rate of 18.5 r per min. were characteristic of that seen after exposures at 43 r per min." For these reasons the dosage rate of 23.5 r per minute as applied to bluebirds is interpreted as "high" and as having effects comparable to that of 43 r per minute. Although the data from nestling bluebirds are scanty, they do raise the question whether, at a given age, the altricial young of wild songbirds are somewhat more resistant to ionizing radiation than are the precocial young of laboratory chicks and ducklings.

Notes on irradiated females of other wild birds.—Five pairs of Great Crested Flycatchers attempted to nest in bluebird boxes, but none of the attempts was successful. One female captured at box 13 at 9 a.m., May 25, was exposed to 600 r. Incubation had not yet begun (in fact, the bird laid her fifth egg in the cage while being carried in for treatment), and, perhaps for this reason, this individual deserted her nest soon after I returned her to the nest cavity. She was not seen again. Unfortunately I could not determine the fate of her eggs. Although I transferred them to a bluebird nest, they were punctured within a day or so—apparently by the bluebird, which seemed to reject these too-large, wrong-colored eggs.

Results with another irradiated Great Crested Flycatcher are somewhat more valuable. This female, in box 17, was given 600 r on May 31, one day after she had deposited her fifth and last egg. The incubation behavior that followed seemed relatively normal, for by June 13 three eggs, all non-irradiated, had hatched. Two other irradiated eggs, as mentioned in a preceding paragraph, did not hatch. About the time the three eggs hatched, the female disappeared, apparently deserting. The male did not stay around either, and the nestlings died. The untimely disappearance of this female which, like most of the bluebirds, had received X-ray treatment at the incubation stage, had no parallel among the bluebirds. Conceivably there are biologically significant differences in response to whole-body irradiation by different species of birds. Moreover, it is possible that the Great Crested Flycatcher, which belongs to the suborder Tyranni (tyrant flycatchers and allies), has relatively less resistance to radiation than the bluebird and Tufted Titmouse, which belong to the suborder Passeres (songbirds), following the classification of Wetmore (1951).

Nest-box 1 was used by a pair of Tufted Titmice. When the female was incubating six eggs on May 23, she was captured, color-banded, and given 600 r. On May 27 the eggs (excepting the above-mentioned, heavily irradiated one) hatched, and two days later both parents were watched as they fed their young. It was clear that both cared for the young throughout the nestling period. When I visited the box on June 8 in order to band the nestlings, both the male and female came fairly close and issued scolding notes. Three days later the young departed from the nest. Like the bluebirds, the female titmouse showed no signs of radiation sickness.

SUMMARY

In 1956 several aspects of the reproductive biology of Eastern Bluebirds were studied in the Savannah River Plant area of South Carolina. The birds bred in nest-boxes throughout the spring and summer. In April and May some of the adult females and nestlings were exposed to X-irradiation, color-banded, and returned to their nests. The dosage rate was high, 23.5 r per minute, and most of the individuals received from 200 to 600 r. Several eggs were also exposed. The breeding biology of irradiated and non-irradiated females was essentially the same in the following respects: size of sets of eggs, length of incubation, hatchability of eggs, nestling period, and general reproductive success. In both irradiated birds and controls there were records of second and third broods. Developing embryos were rather vulnerable to radiation. Among nine irradiated bluebird eggs (mean dosage about 420 r) the embryos died in three instances and the nestlings produced died in three instances; the nestlings hatching from the other three eggs succeeded in fledging. Four nestlings given 400 to 1200 r developed normally and fledged successfully. It is suspected that week-old songbirds might have greater radioresistance than laboratory chicks and ducklings of the same age. Limited data obtained from other box-nesting species, the Great Crested Flycatcher and Tufted Titmouse, suggested that the titmouse, at least, was similar to the bluebird in its resistance to radiation sickness.

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