

FERTILITY OF EGGS PRODUCED ON TERRITORIES OF VASECTOMIZED RED-WINGED BLACKBIRDS

OLIN E. BRAY, JAMES J. KENNELLY, AND JOSEPH L. GUARINO

One approach toward reducing populations of Red-winged Blackbirds (*Agelaius phoeniceus*), and thus the agricultural damage that they cause, is through a program of interfering with their ability to reproduce (Davis 1961), such as with a chemosterilant. Such a program in Red-wings might be best directed toward the male, because the species is polygynous (Allen 1914) and territorial males normally maintain a harem of 1 to 4 females. However, incidents of promiscuity have been reported in females (Allen 1914, Beer and Tibbitts 1950), and as a result of this or other factors, chemosterilization of only some fraction of the males in a local population might not result in a proportional reduction in fertile clutches. To explore the effects of a sterilized-male program, we conducted studies with breeding Red-wings in small marshes near Lakewood, Jefferson Co., Colorado, in 1971 and 1972. To circumvent the problems inherent in the development of an effective chemosterilant and to insure that each treated male was sterile, we surgically sterilized territorial males by vasectomy. The relative numbers of infertile and fertile clutches produced on territories of these males are presented here, along with discussions of their possible implications.

METHODS

Vasectomy study, 1971.—As techniques were still being developed, no formal sampling design was used in selecting the location or number of males that were vasectomized in the 2 cattail (*Typha* sp.) marshes studied (Red-wings were also present in other nearby habitats). From 19 to 26 May (after nesting had started), 8 territorial male Red-wings were captured, anesthetized with Metofane (Pitman-Moore, Inc., Fort Washington, Pa.), and bilaterally vasectomized. (Reference to trade names does not imply U.S. Government endorsement of commercial products.) To expose each vas deferens, a small incision was made on each side of the cloaca just anterior to the seminal sac. The distal segment of each vas deferens was ligated with 5-0 chromic cat gut about 3 to 5 mm from its entrance into the cloaca, and the segment between the ligature and cloaca was cut. After the incisions were closed, the birds were banded with both a U.S. Fish and Wildlife Service and a colored aluminum band. Then the birds were placed within their territories where they recovered rapidly and without any apparent ill effects.

We collected eggs from nests in the territories of vasectomized males to determine fertility and encourage reneating. As nesting had started before the males were vasectomized, their females could have been carrying viable sperm. Therefore, clutches started before vasectomy were not collected until 9 days after the operation; this allowed a period of approximately $\frac{3}{4}$ of the incubation period for retained sperm to lose viability before

females were induced to renest. Clutches started after vasectomy were collected about 2 days after the last egg was laid; Red-wing females lay one egg a day and start incubating the day the last egg is laid (Beer and Tibbitts 1950, Payne 1969). This reduced the chance of predation and allowed time for a greater number of renests.

Collected clutches were artificially incubated in a Jamesway Model 252 incubator. Eggs that did not hatch were opened and were considered infertile if no development was evident. Clutches were considered fertile if any of the eggs were fertile. Fertility was also determined for eggs in 45 clutches from territories of 10 normal males. These were incubated either naturally or artificially.

Sperm retention by females.—We could not locate references to the length of time that females of passerine species retain viable sperm, so in 1971 we arbitrarily chose a 9-day waiting period before collecting eggs to induce renesting. To investigate the sperm retention period of Red-wings, in 1971 we shot or trapped 30 females nesting outside the study marshes and examined the reproductive tracts for sperm. Females were obtained at 7 reproductive stages: nest-building; the day the second egg was laid; the first, sixth, and eleventh days after incubation started; and the sixteenth and twenty-first days after incubation started (females with young). We checked 17 females for sperm soon after they were shot or trapped (group I), and 13 females were held in captivity for 10 days before being examined (group II). The reproductive tract of each female was removed in toto and flushed with physiological saline; the flushings were then checked for motile sperm. As sperm are trapped in crypts in the infundibular and uterovaginal glands of some birds (Bohr et al. 1964), each tract was then tied at both ends and filled with saline until it was turgid; these flushings were then checked for sperm. Each tract was then fixed in Bouin's fluid, histological sections were prepared, and the relative abundance of sperm in each histological section was estimated.

Vasectomy study, 1972.—Due to the fertile clutches that occurred on territories of males vasectomized in 1971, studies were conducted in 1972 to determine the effects of sterilizing different proportions of territorial males in different marshes.

To minimize the influence of territorial males outside the study marshes, these tests were conducted in 4 of the most isolated small cattail marshes. Marsh I was a control marsh; all 6 males there were sham-operated (they underwent surgery, but the vas deferens was neither ligated nor cut). All 3 males in marsh II were vasectomized, but the site served as a test area with only 30% of the males sterilized; this was because 7 normal territorial males were present within .3 km of the marsh, in a wet pasture and an alfalfa (*Medicago sativa*) field. In marsh III, 4 of the 8 males were vasectomized; the territories of the sterilized birds were grouped together. In marsh IV, all 8 males were vasectomized.

We operated on the males between 9 May (start of nesting) and 22 May. Anesthetic techniques were the same as in 1971, but surgical techniques differed in that 2 ligatures about 2 mm apart were tied around the most distal part of each vas deferens and the intervening segment was severed. After the incisions were closed, each male was marked with a colored aluminum leg band and 5 × 2.5 cm plastic leg streamer attached with a U.S. Fish and Wildlife Service band (Guarino 1968); the birds were then placed within their territories to recover.

Because of the results of the 1971 sperm retention tests, all clutches were left in the nests until the eleventh day of incubation; this was about the start of hatching. Clutches or broods were then destroyed to induce the females to renest. Fertility of clutches was determined by candling (Evans 1951) 2 or 3 days after the last egg was laid. Candling detected fertility in some eggs of some clutches on the day after the last egg was laid;

TABLE 1
FERTILITY OF CLUTCHES ON TERRITORIES OF 6 MALE RED-WINGS VASECTOMIZED IN 1971

Days after vasectomy	No. of clutches	No. of fertile clutches	Percent of fertile clutches
1-4	9	9	100
5-11	2	0	0
12-18	13	9	69
19-25	11	8	73
26-32	6	5	83
33-39	6	4	67
40-46	1	1	100
	—	—	
	48	36	

the detection of fertility improved to include all eggs of some clutches on the second day after laying was completed and all eggs of all clutches on the third day. As an additional check, eggs that had not hatched by the eleventh day were opened and were considered infertile if no development was evident. Predators destroyed about 20 clutches on the study areas before the eggs were candled; thus, some fertility information was lost.

As in 1971, we did not detect any change in aggressiveness or behavior of vasectomized males. Near the end of the nesting season, 5 vasectomized and 3 untreated males were shot and the testes and vasa deferentia were examined grossly and then microscopically in histological sections. In comparison with untreated birds, the vasectomized birds had very small testes but large and distended vasa deferentia; some of the vasa deferentia contained a cheesy material. Sperm were present in the vasa deferentia proximal to the ligatures, but none were observed in the distal portions.

RESULTS

Vasectomy study, 1971.—Infertile clutches were found on the territories of 6 of the 8 vasectomized males. The 2 males with no infertile clutches had been vasectomized early in the study, and necropsies revealed sperm in the cloaca of each. These were the only 2 males that were not successfully vasectomized during the 2 years of study.

The shortest interval between vasectomy and the start of an infertile clutch was 5 days (Table 1). Thus, the fifth day after sterilization was considered the earliest that infertile clutches could have been started. Of 39 clutches started 5 days or more after vasectomy on the territories of the 6 successfully sterilized males, 27 (69%) were fertile. All eggs were fertile in 26 of the 27 fertile clutches, and all eggs were infertile in the 12 infertile clutches. The percentage of fertile clutches did not decrease with time after vasectomy (Table 1).

The proportion of fertile clutches on each territory ranged from 44 to 86% (4 of 9 to 6 of 7). The distance between territories of sterilized males and the

closest fertile male's territory varied from 27 to 137 m. Sterilized males with more isolated territories had lower percentages of fertile clutches than those with territories close to fertile males. We did not detect any change in aggressiveness or behavior of the vasectomized males, as compared to normal, untreated males.

Of the 45 clutches checked for fertility on territories of normal males, 43 (96%) were fertile. The 2 infertile clutches came from the territory of a male with one female, and it appears that one member of the pair was sterile.

Sperm retention by females.—Sperm were recovered in the saline flushings from only one female, a group I bird shot during nest-building. However, histological examination of reproductive tracts showed that most birds in both groups had sperm stored in the uterovaginal glands. The longest interval of sperm retention was by a group II female captured 16 days after incubation started. This female had 4 eggs, and if we assume that they were laid on consecutive mornings and that copulation ceased the day the third egg was laid (Beer and Tibbitts 1950, Payne 1969), she had retained sperm for about 26 days. However, the few sperm present were probably insufficient to fertilize a clutch.

The abundance of sperm observed in the uterovaginal glands appeared to be directly related to the stage of nesting. Sperm were abundant in the tracts of birds caught during nest-building or egg-laying, less abundant at the start of incubation, and few at hatching or shortly thereafter. It is not known if the sperm remaining in a female tract are capable of fertilizing eggs, but their presence indicates that it is possible. We therefore concluded that in future studies we should wait until the eggs were about ready to hatch before the clutches were collected to encourage females to renest.

Vasectomy study, 1972.—No infertile clutches were started less than 5 days after vasectomy. Thus, as in 1971, the fifth day after sterilization was considered the earliest that infertile clutches could have been started. The results of our candling of clutches, started 5 days or more after males were vasectomized or sham-operated, are summarized in Table 2. All clutches in marsh I (control marsh) were fertile, indicating that surgery did not stop copulation. In marshes II and III, 44 and 33%, respectively, of the clutches on territories of vasectomized birds were fertile. In marsh IV, 12% of the clutches were fertile.

At marsh II, one vasectomized male's territory was between those of the other 2 vasectomized birds, and his females had the lowest percentage of fertile clutches. At marsh III, fertile clutches were produced on only one vasectomized male's territory, which was between territories of both fertile and other vasectomized males. Thus, as in 1971, the most isolated males had the lowest percentage of fertile clutches.

TABLE 2
 FERTILITY OF CLUTCHES STARTED 5 DAYS OR MORE AFTER SURGERY IN TERRITORIES OF
 VASECTOMIZED AND FERTILE RED-WINGS IN 1972

Marsh	Status	Males		Clutches		
		No.	Percent	No.	No. of fertile clutches	Percent
I	Fertile ^a	6	100	8	8	100
	Sterile	0	0	—	—	—
	Total	6		8	8	100
II	Fertile	7	70	11	11	100
	Sterile	3	30	9	4	44
	Total	10		20	15	75
III	Fertile	4	50	5	5	100
	Sterile	4	50	6	2	33
	Total	8		11	7	64
IV	Fertile	0	0	—	—	—
	Sterile	8	100	17	2	12
	Total	8		17	2	12

^a Males were sham-operated (see Methods).

Only one egg was infertile in 8 clutches on territories of sham-operated males at marsh I, and only one was infertile in 16 clutches on territories of fertile males at marshes II and III. On territories of vasectomized males, all eggs were infertile in 24 clutches, and all eggs were fertile in 7 of 8 fertile clutches.

DISCUSSION

Fertile clutches.—As the vasectomized birds were apparently successfully sterilized, and we did not detect any change in their aggressiveness or behavior, the fertile clutches produced on territories of vasectomized males apparently were the result of sperm retention in the females from copulation before sterilization, replacement of females with new females carrying fertile sperm, or promiscuity by females. Given the choice of these explanations, we believe the fertile clutches may have been due to female promiscuity for the following reasons: the sperm retention study showed that few sperm were

retained after egg-laying was completed, the 1971 vasectomy study did not show a decrease in the percentage of fertile clutches with time after vasectomy (Table 1), and the most isolated males had the fewest fertile clutches.

All clutches on territories of fertile males near vasectomized birds were fertile. Thus, it appears that Red-wing females always copulate with their own males, as would be expected, but that they also copulate with other males—if the fertile clutches on territories of vasectomized males were indeed due to promiscuity.

The stage(s) in the nesting cycle when promiscuity occurs is unknown, as are the number and timing of copulations necessary to fertilize a clutch. Nero (1956) reported that Red-wings stop copulating before egg-laying begins, but R. B. Payne (pers. comm.) observed Red-wings copulating on approximately 10 occasions, and apparently all were during the female's egg-laying period. In our study, in 71 clutches laid 5 days or more after vasectomy on territories of vasectomized males in 1971 and 1972, 36 were completely infertile, 33 were completely fertile, and only 2 were partially fertile. If promiscuity occurred during egg-laying and contributed to fertilization of the clutch being laid, one would expect more partially fertile clutches than this. It is, of course, possible that some completely fertile clutches resulted from promiscuity throughout egg-laying, but it is hard to explain why steady promiscuity should occur at some times and no promiscuity at others. Perhaps copulation, including promiscuity, stopped before egg-laying began. We found no references indicating that passerine species do not require copulation during egg-laying to produce fertile eggs, but Lorenz (1959) obtained high egg fertility for 20 days after artificial insemination of Turkeys (*Meleagris gallopavo*). The infertile eggs in the 2 partially fertile clutches were probably not related to the territorial males being vasectomized. Occasional infertile eggs are not uncommon in the clutches of many species, and 2 of the 24 clutches on fertile males' territories also contained infertile eggs.

If fertile clutches on territories of vasectomized males were the result of promiscuity, the fact that the most isolated vasectomized males had the lowest percentages of fertile clutches suggests that females were promiscuous with nearby territorial males. However, some could have copulated with non-territorial adult males. Nero (1956) reported that territory-seeking Red-wing males commonly appeared on the breeding area throughout the breeding season. Orians (1961) and Holm (1973) obtained evidence that a non-breeding adult male population exists. The literature indicates that it is unlikely that females are promiscuous with one-year-old males, even though some are reproductively mature (Orians 1961, Payne 1969); Nero (1956) stated that female Red-wings usually are not receptive to one-year-old males,

and Wright and Wright (1944) said that one-year-old male Red-wings rarely copulate with females.

Factors Relevant to Male Chemosterilant Programs.—The effect of chemosterilants on Red-wings would probably vary by habitat type. In general, a larger proportion of fertile clutches should occur in habitats where there is more interchange among members of the population and more reneating. Holm (1973) reported that reneating was more likely in territories occupied by 3 or more females than in territories occupied by 1 or 2; and Case and Hewitt (1963), Dyer (1969), and our unpublished data have shown that Red-wing males in marshes tend to have more females than males in upland habitat. Jackson (1971) found that reneating was more common in marshes than in upland habitat. Upland nesters probably forage more within their own territories than marsh nesters (Jackson 1971), which would probably decrease the opportunity for promiscuity in upland areas. Thus, it appears that chemosterilants may be more effective on upland nesters than on marsh nesters.

It is possible that chemosterilants would somewhat reduce the number of young fledged per successful nest. Jackson (1971) found that clutches in reneats had significantly fewer eggs than initial clutches. Thus, even if a female that originally bred with a sterile male did reneat and breed with a fertile male, perhaps she would fledge fewer young than if her initial clutch had been fertile.

This study has shown that the number of fertile clutches produced on a Red-wing male's territory can be reduced by sterilizing the male, but that the extent of the reduction depends on the arrangement of neighboring fertile territorial males, and perhaps on the number of fertile nonterritorial males in the population. It appears likely that the results of chemosterilizing a certain proportion of the males in a marsh would be somewhat better than in our studies, where nests were systematically destroyed to encourage reneating. Studies with artificial eggs suggest that incubation of sterile eggs would be prolonged; Holcomb (1970) found that the incubation period of normalized artificial eggs averaged 19.4 days (range 1–26), and Jackson (1971) found that 3 female Red-wings incubated artificial eggs for 22–23 days. This would mean fewer reneating attempts, and thus fewer chances for a female who originally bred with a sterile male to encounter a fertile one.

SUMMARY

Six male Red-winged Blackbirds scattered throughout 2 marshes were vasectomized in 1971, and their females began laying infertile clutches in 5 days. However, of 39 clutches on their territories, 27 (69%) were fertile. In 1972, 30, 50, and 100% of the males in 3 small isolated marshes were vasectomized, and 44, 33, and 12% of the clutches on their territories were fertile, respectively. All clutches were fertile in a fourth marsh where

all males were sham-operated. All clutches checked were fertile on territories of fertile males adjacent to sterilized males. During the 2 years there were only 4 partially fertile clutches, 2 each on vasectomized and fertile males' territories.

Examination of reproductive tracts of female Red-wings from other marshes showed that sperm retained from copulation were abundant during nest-building or egg-laying but had dropped to a few by hatching.

In vasectomy studies, vasectomized males whose territories were the farthest from fertile males had the lowest percentage of fertile clutches. This and other evidence indicated that fertile clutches on territories of vasectomized males may have been due to females being promiscuous with fertile males.

The occurrence of fertile clutches on territories of vasectomized males indicates that sterilizing some of the males in a population (as in a chemosterilant program) would not result in a proportional decrease in fertile clutches. However, the decreases achieved (up to 88%), and the probability of better results in an actual program, indicate that male chemosterilization would be a feasible means of reducing Red-wing populations.

ACKNOWLEDGMENTS

We thank Brad E. Johns, Donald F. Mott, and Jerry D. Roberts of the Denver Wildlife Research Center for their help with laboratory and field work.

LITERATURE CITED

- ALLEN, A. A. 1914. The Red-winged Blackbird: a study in the ecology of a cattail marsh. Proc. Linn. Soc. New York 24-25:43-128.
- BEER, J. R., and D. TIBBITTS. 1950. Nesting behavior of the Red-winged Blackbird. Flicker 22:61-77.
- BOBR, L. W., F. W. LORENZ, and F. X. OGASAWARA. 1964. Distribution of spermatozoa in the oviduct and fertility in domestic birds. I. Resident sites of spermatozoa in fowl oviducts. J. Reprod. Fertil. 8:39-48.
- CASE, N. A., and O. H. HEWITT. 1963. Nesting and productivity of the Red-winged Blackbird in relation to habitat. Living Bird 2:7-20.
- DAVIS, D. E. 1961. Principles for population control by gametocides. Trans. N. Am. Wildl. Nat. Resour. Conf. 26:160-167.
- DYER, M. I. 1969. Blackbird and Starling research program. 1964-1968. Ontario Dept. Agric. and Food, Toronto.
- EVANS, C. D. 1951. A method of color-marking young waterfowl. J. Wildl. Manage. 15:101-103.
- GUARINO, J. L. 1968. Evaluation of a colored leg tag for Starlings and blackbirds. Bird-Banding 39:6-13.
- HOLCOMB, L. C. 1970. Prolonged incubation behavior of Red-winged Blackbirds incubating several egg sizes. Behaviour 35:74-83.
- HOLM, C. H. 1973. Breeding sex ratios, territoriality, and reproductive success in the Red-winged Blackbird (*Agelaius phoeniceus*). Ecology 54:356-365.
- JACKSON, J. J. 1971. Nesting ecology of the female Red-winged Blackbird. Ph.D. thesis, Ohio State Univ.
- LORENZ, F. W. 1959. Reproduction in domestic fowl, p. 569-608. In Reproduction in domestic animals (H. H. Cole and P. T. Cupps eds.). Academic Press, New York.
- NERO, R. W. 1956. A behavior study of the Red-winged Blackbird. Wilson Bull. 68: 5-37, 129-150.

- ORIAN, G. H. 1961. The ecology of blackbird (*Agelaius*) social systems. *Ecol. Monogr.* 31:285-312.
- PAYNE, R. B. 1969. Breeding seasons and reproductive physiology of Tricolored Blackbirds and Redwinged Blackbirds. *Univ. California Publ. Zool., Berkeley.* 90:1-115.
- WRIGHT, P. L., and M. H. WRIGHT. 1944. The reproductive cycle of the male Red-winged Blackbird. *Condor* 46:46-59.
- U.S. FISH AND WILDLIFE SERVICE, WILDLIFE RESEARCH CENTER, DENVER, CO 80225. (PRESENT ADDRESS JJK: MASSACHUSETTS COOPERATIVE WILDLIFE RESEARCH UNIT, HOLDSWORTH HALL, UNIV. OF MASSACHUSETTS, AMHERST 01002.) ACCEPTED 2 OCT. 1974.

NEW LIFE MEMBER



Dr. David E. Samuel is now a life member of the Wilson Ornithological Society. Dr. Samuel is an Associate Professor of Wildlife Biology in the Division of Forestry at West Virginia University. His principal interests in ornithology are with avian ecology and avian succession on strip-mined land. He has published a number of papers on Barn and Cliff swallows, and more recently he has been studying American Woodcock and Ruffed Grouse. In addition to his ornithological interests, Dr. Samuel enjoys hunting and fishing, backpacking, canoeing, and music.