

KARYOTYPES OF SIX SPECIES OF NORTH AMERICAN BLACKBIRDS (ICTERIDAE: PASSERIFORMES)

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ABSTRACT.—Karyotypes of six species of blackbirds were prepared using in vivo techniques. Five species are karyotypically similar and may be more closely related than traditional morphological characters suggest. The Eastern Meadowlark (*Sturnella magna*), however, is very different in karyotype and may be more distantly related to the other species than has been previously thought. Karyotypic evidence is consistent with Beecher's hypothesis of a close relationship between emberizine finches and blackbirds. Received 25 June 1981, accepted 18 December 1981.

THE family Icteridae (Passeriformes) is a diverse group of predominantly tropical New World songbirds arranged by various investigators into 89–101 species in 25–36 genera (Storer 1960, 1971; Blake 1968; Short 1968, 1969; Clements 1978). Beecher (1951) believed that the radiation of the icterids was an "explosive" event, the family quickly filling all available niches. Later, Beecher (1953) described Icteridae as a terminal taxon, anatomically and behaviorally more complex than the group from which it arose.

Early phylogenetic studies of the icterids were confined to plumage coloration and gross morphological characters of the bill, skull, wing, and foot. In 1951, Beecher established the most recent phylogeny of the group, based on jaw and skull musculature and bill shape. He hypothesized that the icterids evolved from the emberizine finches, through the primitive genus *Molothrus*, in three phyletic lines: the Agelaiine, Quiscaline, and Cassicine. Based on myological and foraging studies, Raikow (1978) believed that Emberizinae and Icteridae may be sister groups.

Recently, comparative karyological studies have been used to discern patterns of chro-

mosomal variation that can reveal evolutionary and phylogenetic relationships. Among the karyotypically best known vertebrate groups are the mammalian orders Chiroptera, Primates, and Rodentia and the reptilian order Testudines. These groups show diverse patterns of variation in the chromosome complement (Capanna and Civitelli 1970, Gorman 1973, Matthey 1973, Wilson et al. 1975, Bush et al. 1977, Bickham and Baker 1979, Bickham 1981). The mammalian orders exhibit highly variable karyotypes, while lower vertebrate taxa usually are karyotypically conservative. Compared to the karyology of mammals and reptiles, avian karyology is relatively unexplored, with less than 3% of all species karyotyped (Takagi and Sasaki 1974, Shields in press). Many avian taxa show little or no variation in standard karyotypes, yet some avian genera (e.g. *Junco*) are composed of species that share identical chromosome polymorphisms (Shields 1973, 1976). Shields (in press) has reviewed the amount of variation in chromosomes of birds, and studies comparing the karyotypes of closely related bird species are rare. The availability of six species of icterids in our area provided an opportunity for comparative studies.

There are two basic strategies followed in karyotypic investigations. The first is to survey related forms throughout a taxon in an attempt to determine patterns of karyotype variation among subgroups. The second is to survey a large number of individuals from the range of a single species or species group to discern

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Fig. 1. Brown-headed Cowbird karyotype. A complete chromosome set from a male; inset are the sex chromosomes from a female.

what patterns of intraspecific variation and polymorphism are present in natural populations.

The purpose of this paper is to present the results of our karyotypic study of these six species representing five genera of Icteridae: the Brown-headed Cowbird (*Molothrus ater*), the Great-tailed Grackle (*Quiscalus mexicanus*), the Common Grackle (*Q. quiscula*), the Red-winged Blackbird (*Agelaius phoeniceus*), the Black-headed Oriole (*Icterus graduacauda*), and the Eastern Meadowlark (*Sturnella magna*). This represents the only cytogenetic information reported for most of these species, although DNA amounts were reported for *Agelaius phoeniceus* and *Sturnella magna* by Bachmann et al. (1972), and the karyotype of the former species was reported by Makino and Baldwin (1954).

METHODS

Somatic metaphase chromosomes were prepared from 27 icterids using in vivo bone marrow procedures. Individuals were injected intraperitoneally with a 0.05% solution of colchicine at 0.3–0.4 ml per 100 g body weight and were sacrificed after a period of 20–30 min. The femurs were removed and flushed with a hypotonic solution of 0.075 M KCl to obtain a suspension of bone marrow cells, which was incubated in the hypotonic solution at 37°C for 30 min. The cells were then fixed (3 parts methanol to 1 part acetic acid) for 10 min and resuspended two or three times in fresh fixative to ensure fixation. Slides were prepared by dropping three or four drops of cell suspension on cleaned glass slides and igniting the preparation. The slides were then stained for 5 min in a warm 10% Giemsa solution. Preparations were observed and photographed on a Leitz Dialux mi-

croscope. Chromosomes were arranged according to size; terminology for centromere position follows Patton (1967).

At least 10 metaphase cells of each species except *I. graduacauda* were examined to determine the diploid number. Both sexes were examined for each species except *I. graduacauda* (male only) and *Q. quiscula* (female only). *Sturnella magna* (Brazos County, Texas) and *I. graduacauda* (Webb County, Texas) were collected during the breeding season; all other individuals were trapped from wintering populations in Brazos County. Fifteen individuals of *M. ater* were examined, as were at least two each of the other species except *I. graduacauda*.

Voucher specimens deposited in the Texas Cooperative Wildlife Collection (TCWC) at Texas A&M University are: *M. ater* TCWC 11029, 11030, 11031, 11032, 10926; *A. phoeniceus* TCWC 11033, 11034; *Q. mexicanus* TCWC 11035, 11036; *Q. quiscula* TCWC 11028; and *I. graduacauda* TCWC 10910; 10 specimens of *M. ater*, 2 specimens of *S. magna*, 1 of *Q. mexicanus*, 2 of *Q. quiscula*, and 1 of *A. phoeniceus* are uncatalogued.

RESULTS

MOLOTHRUS ATER ($2n = 78-80$: Fig. 1), *QUISCALUS MEXICANUS* ($2n = 76-78$: Fig. 2A), *Q. QUISCULA* ($2n = 76$: Fig. 2B), *AGELAIUS PHOENICEUS* ($2n = 80$: Fig. 2C), *ICTERUS GRADUACAUDA* ($2n = 76-78$: Fig. 2D)

The chromosomal complements of these five species are similar. The six largest autosomal pairs (macrochromosomes) are distinguishable by size from the remaining 31–33 pairs (microchromosomes). The largest pair of macrochromosomes is submetacentric, the next four pairs are subtelocentric, and the sixth largest pair is acrocentric. Microchromosomes appear to be

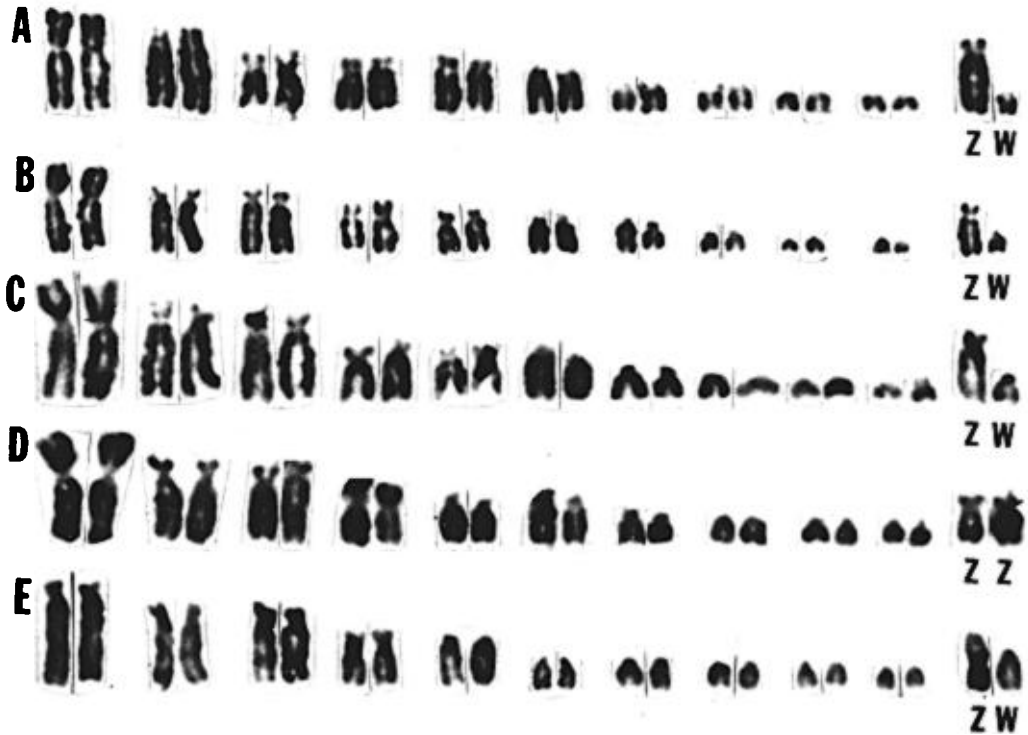


Fig. 2. Partial karyotypes of five icterids. Only the 10 largest autosomes and sex chromosomes are shown for A. Great-tailed Grackle, female; B. Common Grackle, female; C. Red-winged Blackbird, female; D. Black-headed Oriole, male; and E. Eastern Meadowlark, female.

acrocentric except for two pairs of biarmed chromosomes. Microchromosomes are figured only for *M. ater* (Fig. 1). The lack of resolution due to small size and apparent similarity among species limits the usefulness of microchromosomes for comparative studies. We have attempted only to enumerate them. The Z chromosome, which is about the fourth largest chromosome in size, is subtelocentric for all of these species. The W chromosome is probably an acrocentric microchromosome in all except *M. ater*, in which it probably is a small metacentric chromosome. Makino and Baldwin (1954) reported karyotypes for *A. phoeniceus* and *Xanthocephalus xanthocephalus* (the Yellow-headed Blackbird) with macrochromosomal complements identical to those reported in this study.

STURNELLA MAGNA ($2n = 78$; Fig. 2E)

The karyotype of *S. magna* differs from those described for the above five species in several respects. The largest autosomal element is subtelocentric, the fourth autosome is submetacentric,

and the fifth autosome is acrocentric; thus, there are four pairs of autosomal biarmed macrochromosomes as compared to five in the other species. The Z chromosome is a medium-sized metacentric, and the W is probably acrocentric. There are 32 pairs of microchromosomes.

DISCUSSION

Beecher (1951) hypothesized that the icterids evolved from the emberizine finches via the genus *Molothrus*. He described three phyletic lines of Icteridae descending from *Molothrus*: the Agelaiine, containing *Agelaius*, *Sturnella*, *Icterus*, and several genera not reported here; the Quiscaline, containing the subgenera *Quiscalus* (*Q. quiscula*) and *Cassidix* (*Q. mexicanus*) of the genus *Quiscalus* and several genera not reported here; and the Cassicine, none of which is reported here. Therefore, *Molothrus* might be expected to possess a primitive, or ancestral, icterid karyotype.

In general, the six species show little karyotypic variation. The diploid numbers range

from 76 to 80 due to interspecific differences in the number of microchromosomes. Five species appear to have identical macrochromosomal complements (Figs. 1 and 2A-D). *Sturnella magna* differs from these by centromere position in pairs 1, 4, 5, and the Z chromosome. *Molothrus ater* differs from all species studied by centromere position of the W chromosome (one of the larger microchromosomes). This difference in W chromosome morphology may be significant. The karyotypes of *Emberiza citrinella* (Hammar and Herlin 1975), *E. flaviventris* (Hirschi et al. 1972), and *E. bruniceps* (Misra and Srivastava 1978) are similar to the *M. ater* karyotype, differing in centromere position in three autosomal pairs. *Emberiza citrinella* shares the metacentric condition of the W chromosome with *M. ater*. Other emberizine species, however, are known to possess acrocentric W chromosomes, such as *Junco* (Shields 1973) and *Zonotrichia* (Thornycroft 1976).

We found no intraspecific polymorphism in the karyotypes of these species, although the limited sample sizes leave room for doubt. We have studied 15 specimens of *M. ater* from a Texas wintering population that has been shown to be made up of birds that breed throughout the Mississippi River drainage and beyond (Coon and Arnold 1977), so we may be justified in believing that karyotypic polymorphisms do not exist in this species.

The interspecific differences in microchromosome numbers are difficult to interpret, particularly in view of the difficulties of optical resolution of these very small chromosomes. Only the very best of karyotype preparations permit the microchromosomes to be seen well enough to be accurately counted. Use of microchromosome numbers and morphologies in cytosystematic studies awaits further improvement of resolution by advances in cytogenetic technique.

The close resemblance of emberizine and icterid karyotypes is indicative of a relatively close phylogenetic relationship. Likewise, the identical macrochromosomal sets of the icterids reported, except *S. magna*, indicate a close phylogenetic relationship. It appears that the chromosomal data are consistent with Beecher's hypothesis that icterids evolved from the emberizine finches. The primitive nature of *M. ater* is not well established on karyological grounds, because no strong case can be made

for a primitive biarmed W chromosome (the only character in which *M. ater* differs from most other icterids). Certain biochemical studies, however, indicate that Beecher was correct on this point also.

Smith and Zimmerman (1976) investigated the biochemical genetics of seven species of North American blackbirds representing the Agelaiine and Quiscaline groups. Of the Quiscaline species, *M. ater* was the most similar to the Agelaiine species, especially to *Agelaius*. This correlates well with Beecher's (1951) groupings, appearing to support the theory that *Molothrus* represents the primitive group that could have given rise to both the Quiscaline and Agelaiine radiations. Another biochemical study (Prager et al. 1974) showed a close relationship between *Molothrus* and *Agelaius*, which could also be interpreted to support Beecher's (1951) hypothesis.

It seems that morphologic, karyotypic, and biochemical data are consistent with the groupings of Beecher, with some reservation concerning *S. magna* based on the karyotypic data. Overall, the chromosomal variation noted could be attributed to pericentric inversions, as have been observed in the genus *Junco* (Shields 1973) and *Zonotrichia albicollis* (Thornycroft 1976), or to additions or deletions of heterochromatin. The demonstration of homologies and the rearrangements leading to variation is not possible without studying differentially stained chromosomes. These data seem to support Shields' (in press) contention that chromosomal changes do not play a major role in the speciation process of birds. Again, this is stated with reservation due to the differences noted for *S. magna*.

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