The Chromosomes of the American blackbirds, *Agelaius phoeniceus* and *Xanthocephalus xanthocephalus* (Family Icteridae)

Sajiro Makino¹ and Paul H. Baldwin²

Received January 19, 1954

The study of the chromosomes of birds has been advanced recently by the extensive work of Yamashina (1943, 1944, 1951) with the cooperation of Makino. Passerine birds, however, have received less attention than most other major groups of bird. At present only sixteen species of passerine birds, including the two in this study, have been reported upon as to their chromosome complement, yet in five of these has the chromosome number not been determined accurately.

In view of the contribution the study of passerine chromosomes could make to the understanding of taxonomic relationship within and among the various families, it would be worth while to establish the chromosome complements of groups of related species. Hence, the chromosomes of common birds belonging to the American family Icteridae have come under study because of ease in obtaining material. In the present paper we record some results of a comparison of two morphologically and ecologically related icterids from Colorado, the red-winged blackbird (*Agelaius phoeniceus*) and the yellow-headed blackbird (*Xanthocephalus xanthocephalus*). The ecologic and plumage differences among the several diverging lines within the Icteridae are considerable, hence the comparison of chromosomes of additional members of the family should be of much interest from the standpoint of evolution. Comparison of chromosomes should provide an additional method of exploring phylogenetic relationships within the family to be used to contrast with studies in phylogeny based on other criteria, such as Beecher's work on muscular patterns of the Icteridae and other passerines (Beecher, 1953).

The work was done in the Zoological Laboratory of the Colorado A & M College, Fort Collins, Colorado, U.S.A., through the courtesy of Dr. W. Olsen and Dr. W. H. Leonard, to whom the authors are deeply grateful.

Material and methods

The most satisfactory material for avian chromosome study was provided by the developing gonads of the embryo. Eggs were collected from the nest by the authors in the vicinity of Fort Collins, Colorado, U.S.A., and the

¹ Zoological Institute, Faculty of Science, Hokkaido University, Sapporo.

² Department of Zoology, Colorado A & M College, Fort Collins.

embryos were removed and dissected. After removal of the surrounding viscera, the gonads, together with the kidneys, were fixed in Hermann's solution. Testicular tissue from adults of both species, and also from the Brewer's blackbird (*Euphagus cyanocephalus*), shot in the daytime were prepared for study, but spermatogonial and meiotic divisions were lacking or extremely rare in this material, perhaps because the season was too far advanced (June 27). The results reported here do not include data from the adult material.

The tissues were embedded in paraffin, and the sections were stained with Heidenhain's hematoxylin and counterstained with light green.

The sexes of the embryonic gonads were clearly evident from their histological characteristics. The embryonic testis was characterized by the presence of solid cords of primordial germ cells developing toward the medullary region, while the ovary showed a thick, elongated cortical layer of primordial germ cells.

Description of chromosomes

Agelaius phoeniceus

1. *Spermatogonial chromosomes*:—Several examples of metaphase plates of spermatogonial divisions were available for study. The number of chromosomes was found to be $2n=76$ in the male (Fig. 1). Morphologically the chromosomes can be described tentatively as V's, J's and rods, in accordance with the descriptions of Yamashina (1943, 1944, 1951). The macro-chromosomes seem to consist of from eight to nine pairs, and a formula for the macro-chromosomes based on their relative lengths could be designated as $aV + bJ + cJ + dj + er + fr + gr + hr$. The first and largest pair of macro-chromosomes is represented by chromosomes of a large V-shape, having dissimilar arms. The second and third largest pairs are in the form of a large J, each with one long arm and one short arm. The fourth pair is medium-sized and J-shaped. The fifth and all smaller pairs are rods of gradually reduced size. Since the macro-chromosomes become smaller gradually (Fig. 8), there is no sharp distinction between them and the micro-chromosome. When the chromosome pairs are arranged in a graded series, it is difficult to know where to draw the line between macro-chromosomes and micro-chromosomes. All of the micro-chromosomes are in the form of short rods reducing in size very gradually, many of the smaller ones being of spheroidal shape.

2. *Oögonial chromosomes*:—The female diploid chromosome set was studied in embryonal ovaries. The number of chromosomes was determined to be $2n=75$ in the oögonial metaphase plates (Figs. 2-4). Thus the number of chromosomes in the female is one less than in male. This difference in number is due to the circumstance that in the female the

medium-sized, J-shaped chromosome designated as 'd' in the serial arrangement of macro-chromosomes (Fig. 9) remains unpaired. In respect to size and morphology, the other members of both the macro- and micro-chromosome sets of the female were identical with the corresponding chromosomes of the male previously described.

3. *Sex chromosome*:—Since the fourth largest chromosome, designated as 'd' in the serial alignment of macro-chromosomes (Figs. 8-9), is paired in the male diploid chromosome set but remains solitary in the female diploid

Figs. 1-4. Chromosomes of the red-winged blackbird (*Agelaius phoeniceus*). 1, spermatogonial metaphase, 76 chromosomes. 2-4, oögonial metaphases, 75 chromosomes in each. $\times 4500$.

set, it is evident that this is the sex chromosome (x). Thus the sex chromosome is a medium-sized, J-shaped element.

Xanthocephalus xanthocephalus

1. *Spermatogonial chromosomes*:—Metaphase plates as seen in spermatogonial divisions in embryonic testes provided the material for determination of the characteristics and the number of the chromosomes of the male yellow-headed blackbird. The diploid number of the chromosomes was found to be $2n=76$ for the male sex (Figs. 5-6). The shapes of the chromosomes could be described as V's, J's, and rods. When arranged in serial alignment the for-

mula for the macro-chromosomes appears as $aV + bJ + cJ + dj + er + fr + gr + hr$ (Fig. 10). The largest pair of supposedly homologous chromosomes, which are V-shaped with unequal arms, is designated as 'aV'. The second largest pair, which represents J-shaped chromosomes, is indicated by the symbol 'bJ', and so on through the eighth largest pair. The sixty chromosomes smaller than the eight largest pairs consist of short rods which are too similar in size and shape to permit matching into supposed homologous pairs.

Figs. 5-7. Chromosomes of the yellow-headed blackbird, *Xanthocephalus xanthocephalus*. 5-6, spermatogonial metaphases, 76 chromosomes in each. 7, oögonial metaphase, 75 chromosomes. $\times 4500$.

2. *Oögonial chromosomes*:—The female diploid chromosomes as observed in embryonic ovaries are 75 ($\text{♀}2n$) in number (Fig. 7). In the general morphology the oögonial chromosomes show no apparent difference from those of the spermatogonium, but the number of chromosomes is one fewer than in the male cell. Comparison of the chromosome complements between the male and female revealed that the fourth largest, J-shaped chromosome, designated as 'd' in the serial arrangement of the macro-chromosomes, has no mate in the female cell (Fig. 11).

The results of this investigation indicated that the number of the chromosomes of the red-winged blackbird (*Agelaius phoeniceus*) showed a complete agreement with those of the yellow-headed blackbird (*Xanthocephalus xanthocephalus*), and further that the morphological features of the chromosomes were also found to be nearly identical with each other.

3. *Sex chromosome*:—As described above, the medium-sized, J-shaped chromosome ranging in size to the fourth largest is present in the unpaired condition in the oögonial cell, while it has a corresponding mate in the

male. In the light of this finding, it is certain that the fourth largest, J-shaped element represents the sex chromosome in this species (Figs. 10-11, *x*).

Figs. 8-11. Serial alignments of macro-chromosomes. 8-9, *Agelaius phoeniceus*. 8: male. 9: female. 10-11, *Xanthocephalus xanthocephalus*. 10: male. 11: female.

Discussion

Among the birds of the order Passeres for which the chromosome numbers have been previously reported upon in the literature eight families are represented. These are the Corvidae (crows, jays, magpies) with two species studied, the Turdidae (thrushes) with four species reported upon, the Laniidae (shrikes) with two species, the Ploceidae (weaverbirds) with one species, the Fringillidae (finches) with two species, the Alaudidae with one species, the Prunellidae with one species, and the Sylviidae with one species. Now the present report adds a ninth family, the Icteridae, with two species studied. Thus the total number of perching birds whose chromosome characteristics and numbers have been reported is no larger than sixteen species. The chromosome numbers for these birds are summarized in Table 1. Among the species in this list for which the chromosome number appears to have been determined with accuracy, four species have a greater diploid number than the two icterids studied, namely,

Pica pica serica —82 spermatogonial chrom's, 81 oögonial chrom's.

Turdus chrysolaus—84 " , 83 "

Prunella rubida —84 "

Emberiza spodocephala—83 oögonial chromosomes.

Three species had fewer chromosomes than the icterids, namely,

Lanius cristatus —72 spermatogonial chrom's, 71 oögonial chrom's.
L. tigrinus — "
Acrocephalus bistrigiceps— "

The icterids with 76 spermatogonial chromosomes and 75 oögonial chromosomes are intermediate.

Table 1. Comparison of chromosome numbers reported for sixteen species of passerine birds

Species	Number of chromosomes ¹	Observers
Family Corvidae		
<i>Coloeus monedula</i>	56-67♀, ♂m	Pogossianz ²
<i>Pica pica sericea</i>	82s, 81o	Suzuki (1949)
Family Turdidae		
<i>Turdus chrysolaus</i>	84s, 83o	Yamashina (1951)
<i>Turdus merula</i>	60-85s, o, ♀, ♂m	Unger (1936)
<i>Turdus pilaris</i>	74-81m	Pogossianz ²
<i>Luscinia calliope calliope</i>	80s	Udagawa (1952)
Family Laniidae		
<i>Lanius cristatus</i>	72s, 71o	Yamashina (1951)
<i>Lanius tigrinus</i>	72s	Udagawa (1952)
Family Ploceidae		
<i>Passer domesticus</i>	40-48♀, ♂m	Pogossianz ²
"	54-60s, ♀, ♂m	Riley (1938)
Family Fringillidae		
<i>Emberiza spodocephala</i>	83o	Yamashina (1951)
<i>Linota cannabina</i>	64-85o, ♂m	Unger (1936)
Family Alaudidae		
<i>Alauda arvensis japonica</i>	78s, 77o	Udagawa (1952)
Family Prunellidae		
<i>Prunella rubida rubida</i>	84s	Udagawa (1952)
Family Sylviidae		
<i>Acrocephalus bistrigiceps</i>	72s	Udagawa (1952)
Family Icteridae		
<i>Agelaius phoeniceus</i>	76s, 75o	Makino & Baldwin (this paper)
<i>Xanthocephalus xanthocephalus</i>	76s, 75o	

¹ s=spermatogonial chromosomes, o=oögonial chromosomes, s=somatic chromosomes.

Cited in Makino (1951, p. 250).

When the serial alignments of marco-chromosomes of these passerine species are compared (Table 2), it is seen that the number of V's, J's, and rods varies considerably. In most of these birds the two largest chromosomes are V- or J-shaped with the exception of *Luscinia calliope*, in which the largest pair is represented by rod-elements. In some of them the third

Table 2. Comparison of macro-chromosomes of passerine birds (Observers as in Table 1)

Species	Chromosome formula	Sex chromosome
<i>Turdus chrysolaus</i>	aV + bJ + cJ + dJ + ev + fj + gj + hj	ev
<i>Turdus merula</i> ¹	aV + bJ + er + dv + er + fr + gr + hr	dv
<i>Luscinia calliope</i>	aR + bV + cJ + dR + ev + fr + gr + hr	—
<i>Lanius cristatus</i>	aV + bJ + cR + dv + ej + fr + gr + hr	ej
<i>Lanius tigrinus</i>	"	—
<i>Passer domesticus</i> ²	aV + bJ + cJ + dv + ev + fj + gj	dv
<i>Emberiza spodocephala</i>	aJ + bV + cJ + dJ + ev + fj + gj + hj	ev
<i>Linota cannabina</i> ¹	aJ + bJ + cv + dj + ev + fr + gr + hr	ev
<i>Pica pica sericea</i>		v
<i>Alauda arvensis japonica</i>	aV + bV + cR + dv + er + fr + gr + hr	dv
<i>Prunella rubida</i>	aV + bR + cR + dr + er + fr + gr + hr	—
<i>Acrocephalus bistrigiceps</i>	aJ + bJ + cJ + dj + er + fr + gr + hr	—
<i>Agelaius phoeniceus</i>	aV + bJ + cJ + dj + er + fr + gr + hr	dj
<i>Xanthocephalus xanthocephalus</i>	aV + bJ + cJ + dj + er + fr + gr + hr	dj

¹ Formulae based on figures of chromosomes in Unger (1936).

² Formula based on figure of chromosomes in Riley (1938).

largest chromosome is a rod, while in others the third is a V or J. There is similar variation in the rest of the macro-chromosomes. No trends or relationships are evident among these few families, perhaps because of the small number of species examined to date. The autosomes of the two American blackbirds appear to be rather similar to those of *Trudus merula*, but the sex chromosome differs in shape.

The sex chromosomes of these birds have been designated variously as either the fourth or fifth largest chromosome pair, and the shape appears to be a small V in the thrushes, finches, magpie, and house sparrow, but a small J in the shrike and American blackbirds.

In all respects the chromosomes of the two species of American blackbirds studied were identical in appearance. This great similarity may indicate a close genetic relationship between *Agelaius* and *Xanthocephalus*, especially since these two icterids are likewise similar in plumage characters, anatomy, and in ecological characteristics.

References cited

- Beecher, W. J. 1953. A phylogeny of the Oscines. *Auk* 70: 270-333.
 Makino, S. 1951. An Atlas of the Chromosome Numbers in Animals. Iowa State College Press, Ames. 290 pp.
 Riley, G. M. 1938. Cytological studies on spermatogenesis in the house sparrow *Passer domesticus*. *Cytologia* 9: 165-176.
 Suzuki, K. 1949. On the karyotype of the Korean magpie. *Jap. Jour. Genet.* 54: 90.
 Udagawa, T. 1952. Karyogram studies in birds, I. Chromosomes of five passers. *Cytologia* 17: 311-316.

- Unger, H. 1936. Beiträge zur Chromosomenforschung der Vögel. Zeits. Zellf. mikr. Anat. 25: 476-500.
- Yamashina, Y. 1943. Studies on sterility in hybrid birds. IV. Cytological researches on hybrids in the Family Phasianidae. Jour. Fac. Sci. Hokkaido Imp. Univ. Ser. VI, Zool. 8: (4): 307-386.
- 1944. Karyotype studies in birds. I. Comparative morphology of chromosomes in seventeen races of domestic fowl. Cytologia 13: 270-296.
- 1951. Studies on the chromosomes of twenty five species of birds. Papers from Coordinating Commit. Res. in Genetics 2: 27-38.

Addendum: Since this article went to press there has appeared a paper by T. Udagawa (Karyogram studies in birds, III. The chromosomes of five forms of birds. Ann. Zool. Japon. 27: 91-96, 1954) in which are reported the chromosome of *Emberiza elegans elegans* (Fringillidae, ♂ $2n$, 84) and *Acrocephalus arundinaceus orientalis* (Sylviidae, ♂ $2n$, 72).