Some Studies of the Spermatozoa of Certain Species of the Icteridae (Blackbirds)

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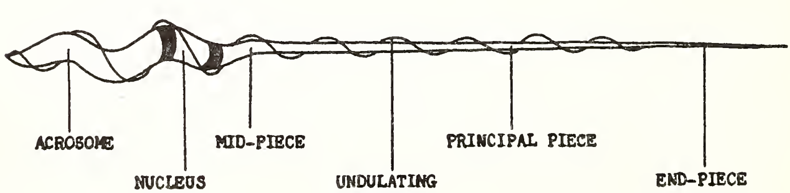
Abstract

This study was made to determine whether measurememnts of spermatozoa of some members of the family Icteridae (Blackbirds) could be used in a statistical analysis in distinguishing these species and subspecies from one another. The twelve Icterids studied were *Sturnella neglecta confluenta, Sturnella magna argutula, Quiscalus quiscula versicolor, Dolichonyx oryzivorus, Icterus parisorum, I. bullockii bullockii, I. spurius, Agelaius phoeniceus phoeniceus, A. p. mearnsi, A. p. nevadensis, A. p. floridanus,* and *Cassidix mexicanus major.*

The sperm cells could not be distinguished to species level by simple microscopic observations because they were so similar in apparance. Measurements of the sperm head, acrosome, nucleus, mid-piece. principal piece, end-piece, and total length of sperm were made. The date were analyzed by the One-Way analysis of variance and the Scheffe test of the comparison of measurement means by pairs. These tests provide criteria for distinguishing between most of the twelve blackbird forms studied.

There are few studies of the morphology of avian spermatozoa, and they are widely scattered in the literature. Wilson (3) suggested that avian species may be distinguished from one another by sperm characteristics. McFarlane (2) clearly demonstrated that the orders of birds could be separated on the basis of sperm anatomy. He made a general study of the gross morphology of the sperm of a wide sample of forms representing many orders, families, genera, and species. The present study was made to determine whether a statistical analysis of measurements of sperm parts of some members of the family Icteridae can be used as criteria in distinguishing these species and subspecies from one another.

FIGURE 1: PARTS OF TYPICAL ICTERID SPERM



An avian sperm cell (Figure 1) consists of two main structural divisions, the head, and the tail. The head is capped by a large, pointed anterior structure, the acrosome. The acrosome, originally derived from the Golgi Bodies, consists of an apical cap and a spine. The nucleus, very difficult to see with an ordinary microscope, lies just posterior to the acrosome. The nucleus has been found to be several times smaller than the acrosome in some of the passerine birds (2). The sperm tail consists of a mid-piece, and an axial filament. The mid-piece, which attaches to the nucleus posteriorly, surrounds the axial filament, and is originally derived from the mitochondria. The axial filament consists of an anterior principal piece that comprises most of the tail length and a terminal end-piece. The axial filament is a product of the centrioles of the developing spermatid. An undulating membrane is found over most of the length of the cell in the Icterid spermatozoa. This undulating membrane is found on the sperm of many passerine families. Birds in the order Paseriformes are considered phylogenetically the most advanced species.

Materials and Methods

Robert W. McFarlane collected the Icterid sperm in the field and preserved the entire seminal vesicle in 10% buffered formalin solution. Smears of spermatozoa were made and stained with hematoxylin. Observations were made with a Wild-Heerbrugg phase-contrast microscope, and measurements were taken with a calibrated Wild 15X optical micrometer at a magnification of 600X. Twenty measurements of each part of the sperm of twelve species and subspecies were made. The sperm cells to be measured were selected at random. The sperm parts measured were length of the acrosome, nucleus, mid-piece, sperm head, principal piece, end-piece, and total length of the sperm.

The twelve species and subspecies studied were the Bobolink (*Dolichonyx oryzivorus*), the Western Meadowlark (*Sturnella neglecta confluenta*), the Southern Meadowlark (*Sturnella magna argutula*), the Bronzed Grackle (*Quiscalus quiscula versicolor*), Bullock's Oriole (*Icterus bullockii bullockii*), the Orchard Oriole (*Icterus spurius*), Scott's Oriole (*Icterus parisorum*), the Eastern Red-Winged Blackbird (*Agelaius phoeniceus phoeniceus*), the Florida Red-Winged Blackbird (*Agelaius phoeniceus mearnsi*), the Grinnell Red-Winged Blackbird (*Agelaius phoeniceus nevadensis*), Maynard's Red-Winged Blackbird (*Agelaius phoeniceus floridanus*), and the Eastern Boat-tailed Grackle (*Cassidix mexicanus major*).

The One-Way analysis of variance was used for testing the null hypothesis, Ho : $u_1 = u_2 = u_3 = \ldots = u_{12}$, that the means of the measments taken for each sperm component of each species and subspecies were equal. The F′ value (tabled F value) was used to determine whether the null hypothesis was to be rejected or accepted. The F-distribution table (1) was used, and 0.01 was established as the level of significance. The F value was estimated to be 2.25. If F′ was less than 2.25, the means of measurements were considered to be equal, or nearly so, and no further calculations were made. If F′ was greater than, or equal to 2.25, the null hypothesis was rejected, and the means were considered unequal. The measurement means of these parts were then tested by Scheffe's method of analysis (1) to determine the actual separation level of significance of the comparison of means by pairs.

Scheffe's method of ranking measurement means from highest to lowest was employed to show which means were significantly different to allow for the separation of avian forms. A q^* value, or set number, was determined for each sperm part. A line drawn through table 2 for Scheffe's test separates sperm mean values above the q^* value from those below the q* value. All values above this line indicate that the means of these species and subspecies are significantly different from one another, and can be used as criteria for distinguishing these forms from one another. All values below this line are too similar in mean comparison to be used as effective criteria for species and subspecies distinction.

The linear model used in this study to analyze the data was:

$$X_{ij} = u + S_i + e_{ij}$$

where,

X_{ij} = the j^{th} observation on the sperm of i^{th} species of bird for each characteristic studied.

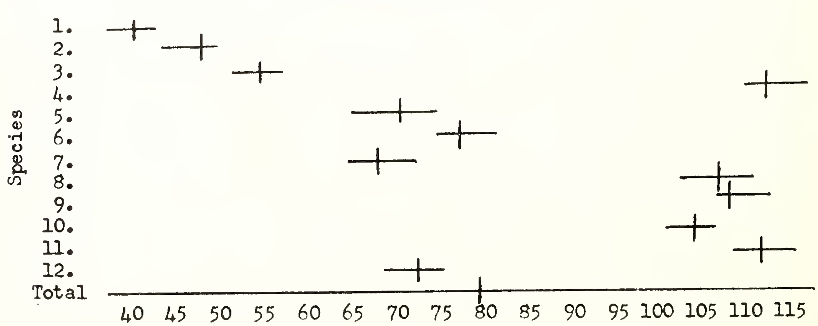
u = the overall mean of the given characteristic studied when all classes frequencies are equal.

S_i = the effect common to all observations for the i^{th} species of bird for a given sperm characteristic.

e_{ij} = the random error associated with the j^{th} observation of the i^{th} species of bird for a given characteristic and it is assumed to be NID $(0, \frac{2}{\Theta_e})$.

Results

Measurements of sperm parts for each avian form studied are summarized in table 1. All the Icterid sperm cells examined were basically similar in structure. One species could not be distinguished from another by simple observation of the sperm under the microscope. The sperm of all forms had a spiralled head and an undulating membrane over most of the length of the cell except for the end piece. The tail length made up more than three-fourths of the total length of the sperm in head-to-tail ratios in most cases, and the length of the acrosome was nearly double that of the nucleus. Acrosomal, nuclear, and mid-piece measurements varied little in length, with a close correlation among different species. A transparent undulating, or helical membrane, appeared to cover most of the length of the sperm cell, extending from the head to the point of origin of the end-piece; the end-piece is bare. Figure 2 is a graphic display of measurement means of spermatozoan principle pieces. The species listed in Figure 2 are assigned the code numbers designated on Table 1.



PRINCIPAL PIECE: Length in microns

Figure 2.

TABLE 1

SPECIES AND SUBSPECIES	SPERM HEAD	ACROSOME	MID-PIECE
	$\overline{X}_1$ = Std. Error	$\overline{X}_1$ = Std. Error	$\overline{X}_1$ = Std. Error
1. *Sturnella neglecta confluenta*	14.95 ± .0704	9.90 ± .0641	1.93 ± .0332
2. *Sturnella magna argutula*	15.73 ± .0491	10.39 ± .0837	2.00 ± .0232
3. *Quiscalus quiscula versicolor*	14.20 ± 0.688	9.55 ± .0896	2.13 ± .0194
4. *Dolichonyx oryzivorus*	14.18 ± .0701	9.23 ± .0872	2.24 ± .0289
5. *Icterus bullockii bullockii*	15.51 ± .0724	10.62 ± .1331	2.24 ± .0291
6. *Icterus spurius*	14.75 ± .0543	10.48 ± .0408	1.99 ± .0291
7. *Icterus parisorum*	14.54 ± .0683	9.35 ± .0456	2.31 ± .0311
8. *Agelaius phoeniceus phoeniceus*	16.11 ± .0407	10.75 ± .0763	2.13 ± .0272
9. *Agelaius phoeniceus mearnsi*	15.97 ± .0982	10.48 ± .0761	2.30 ± .0253
10. *Agelaius phoeniceus nevadensis*	16.12 ± .0970	11.09 ± 0.723	2.09 ± .0349
11. *Agelaius phoeniceus floridanus*	15.67 ± .0850	10.17 ± .0682	2.35 ± .0287
12. *Cassidix mexicanus major*	13.74 ± .0763	8.80 ± .0566	1.88 ± .0270
	TOTAL LENGTH	PRINCIPAL PIECE	END-PIECE
1. *Sturnella neglecta confluenta*	77.94 ± .1939	42.84 ± .2287	17.46 ± .1363
2. *Sturnella magna argutula*	85.97 ± .2461	49.11 ± .1035	18.57 ± .0946
3. *Quiscalus quiscula versicolor*	90.73 ± .3010	51.96 ± .2873	25.02 ± .1569
4. *Dolichonyx oryzivorus*	142.02 ± .3832	111.49 ± .2133	17.59 ± .1258
5. *Icterus bullockii bullockii*	110.45 ± .2974	70.47 ± .3224	23.59 ± .1632
6. *Icterus spurius*	105.92 ± .2295	75.07 ± .2030	17.75 ± .1289
7. *Icterus parisorum*	105.93 ± .2337	68.95 ± .1644	20.21 ± .0862
8. *Agelaius phoeniceus phoeniceus*	148.59 ± .4166	106.84 ± .2623	24.02 ± .1183
9. *Agelaius phoeniceus mearnsi*	146.48 ± .3623	107.85 ± .2326	23.24 ± .1233
10. *Agelaius phoeniceus nevadensis*	139.64 ± .3327	104.12 ± .2304	19.52 ± .1753
11. *Agelaius phoeniceus floridanus*	146.95 ± .3929	111.13 ± .2607	18.51 ± .1449
12. *Cassidix mexicanus major*	109.12 ± .3340	71.45 ± .2320	23.92 ± .1184

Key to the Tables on the Scheffe Test

The set number or value (q^*) is 3.92 for the sperm head, 6.17 for the acrosome, 16.62 for the total length of the sperm, 1.49 for the mid-piece, 12.27 for the principal piece, and 6.98 for the end-piece. The mean j ($\overline{X}_j$) is $\overline{X}_{10}$ for the sperm head, $\overline{X}_{10}$ for the acrosome, $\overline{X}_8$ for the total length of the sperm, $\overline{X}_{11}$ for the mid-piece, $\overline{X}_4$ for the principal piece, and $\overline{X}_3$ for the end-piece.

The key to the species and subspecies which correspond to the means ($\overline{X}_1$, $\overline{X}_2$, etc.) on Table 2 are listed below in numerical sequence:

$\overline{X}_1$ = *Sturnella neglecta confluenta*
$\overline{X}_2$ = *Sturnella magna argutula*
$\overline{X}_3$ = *Quiscalus quiscula versicolor*
$\overline{X}_4$ = *Dolichonyx oryzivorus*
$\overline{X}_5$ = *Icterus bullockii bullockii*
$\overline{X}_6$ = *Icterus spurius*
$\overline{X}_7$ = *Icterus parisorum*
$\overline{X}_8$ = *Agelaius phoeniceus phoeniceus*
$\overline{X}_9$ = *Agelaius phoeniceus mearnsi*
$\overline{X}_{10}$ = *Agelaius phoeniceus nevadensis*
$\overline{X}_{11}$ = *Agelaius phoeniceus floridanus*
$\overline{X}_{12}$ = *Cassidix mexicanus major*

TABLE 2

Scheffe test on principal piece

	$\overline{X}_j-\overline{X}_1$	$\overline{X}_j-\overline{X}_2$	$\overline{X}_j-\overline{X}_3$	$\overline{X}_j-\overline{X}_7$	$\overline{X}_j-\overline{X}_5$	$\overline{X}_j-\overline{X}_{12}$	$\overline{X}_j-\overline{X}_6$	$\overline{X}_j-\overline{X}_{10}$	$\overline{X}_j-\overline{X}_8$	$\overline{X}_j-\overline{X}_9$	$\overline{X}_j-\overline{X}_{11}$
$\overline{X}_4$ = 1081.45	665.95	605.05	577.45	412.60	397.85	388.35	353.25	71.50	45.15	35.30	3.50
$\overline{X}_{11}$ = 1077.95	662.45	601.55	573.95	409.10	394.35	384.85	349.75	68.00	41.65	31.80	
$\overline{X}_9$ = 1046.15	630.65	569.75	542.15	377.30	362.55	353.05	317.95	36.20	9.85		
$\overline{X}_8$ = 1036.30	620.80	559.90	532.30	367.45	352.70	343.20	308.10	26.35			
$\overline{X}_{10}$ = 1009.95	594.45	533.55	505.95	341.10	326.35	316.85	281.75				
$\overline{X}_6$ = 728.20	312.70	251.80	224.20	59.35	44.60	35.10					
$\overline{X}_{12}$ = 693.10	277.60	216.70	189.10	24.25	9.50						
$\overline{X}_5$ = 683.60	268.10	207.20	179.60	14.75							
$\overline{X}_7$ = 668.85	253.35	192.45	164.85								
$\overline{X}_3$ = 504.00	88.50	27.60									
$\overline{X}_2$ = 476.40	60.90										
$\overline{X}_1$ = 415.50											

Discussion

A comparison of ranges and the differences within each range for each part of the sperm reveals that a significant difference exists for the total length of the sperm and for the principal piece. These two sperm components also gave the best results for distinguishing species by comparison of their means. The difference in the ranges were relatively small for the end-piece, sperm head, acrosome, and mid-piece, but the results of the One-Way analysis and Scheffe's test were significant. Acrosomal analysis was of least significance in distinguishing the species by comparison of the means.

In the acrosome, nearly 53% of the pairs of means compared gave significant results (35 out of 66 cases above the q* value). The table below lists significance values for each sperm part:

Sperm Component	*Value—Percent*
1. Principal piece	95% (63 out of 66)
2. Total length of sperm	92% (61 out of 66)
3. End-piece	85% (56 out of 66)
4. Sperm head	77% (51 out of 66)
5. Mid-piece	55% (36 out of 66)
6. Acrosome	53% (35 out of 66)

Icterid species and subspecies which had four means of no significance (measurement means too similar to separate species), and the sperm parts involved (S.H. = Sperm head; Acro. = Acrosome; M.P. = Mid-piece; T.L. = Total length; P.P. = Principal piece; and E.P. = End-piece) include: *S. neglecta confluenta-I. spurius* (S.H., Acro., M.P., E.P.); *A. p. mearnsi-A. p. floridanus* (S.H., Acro., T.L., M.P.).

The forms with three means of no significance include: *S. magna argutula-A. p. phoeniceus* (S.H., Arco., M.P.); *S. magna argutula-A. p. floridanus* (S.H., Acro., E.P.); *I. b. bullockii-A. p. phoeniceus* (Acro., M.P., E.P.); *I. b. bullockii-A. p. mearnsi* (Acro., M.P., E.P.); *I. b. bullockii-A. p. floridanus* (S.H., Acro., M.P.); *I. b. bullockii-C. mexicanus major* (T.L., P.P., E.P.); *D. oryzivorus-Q. quiscula versicolor* (S.H., Acro., M.P.); *D. oryzivorus-I. parisorum* (S.H., Acro., M.P.); *A. p. mearnsi-A. p. phoeniceus* (S.H., Acro., P.P.); *A. p. nevadensis-A. p. phoeniceus* (S.H., Acro., M.P.).

The forms with two means of no significance include: *Q. quiscula versicolor-I. parisorum* (S.H., Acro.); *S. magna argutula-I. b. bullockii* (S.H., Acro.); *S. magna argutula-I. spurius* (Acro., M.P.); *S. magna argutula-S. neglecta confluenta* (Acro., M.P.); *S. magna argutula-A. p. mearnsi* (S.H., Acro.); *I. spurius-I. parisorum* (T.L., S.H.); *I. spurius-A. p. phoeniceus* (Acro., M.P.); *I. spurius-A. p. nevadensis* (Acro., M.P.); *I. b. bullockii-A. p. nevadensis* (Acro., M.P.); *D. oryzivorus-A. p. nevadensis* (T.L., M.P.); *D. oryzivorus-A. p. floridanus* (M.P., P.P.); *A. p. nevadensis-A. p. mearnsi* (S.H., Acro.); *A. p. phoeniceus-A. p. floridanus* (Acro., T.L.); *S. magna argutula-A. p. nevadensis* (S.H., M.P.).

The forms with only one mean of no significance include: *D. oryzivorus-C. mexicanus major* (Acro.); *D. oryzivorus-A. p. phoeniceus* (M.P.); *D. oryzivorus-I. b. bullockii* (M.P.); *D. oryzivorus-S. neglecta confluenta* (E.P.); *D. oryzivorus-I. spurius* (E.P.); *D. oryzivorus-A. p. mearnsi* (M.P.); *S. neglecta confluenta-Q. quiscula versicolor* (Acro.); *S. neglecta confluenta-Q. quiscula versicolor* (Acro.); *S. neglecta confluenta-C. mexicanus* (M.P.); *S. neglecta confluenta-A. p. floridanus* (Acro.); *S. neglecta confluenta-A. p. mearnsi* (Acro.); *I. spurius-I. b. bullockii* (Acro.); *I. spurius-Q. quiscula versicolor* (M.P.); *I. spurius-A. p. floridanus* (Acro.); *I. spurius-A. p. mearnsi* (Acro.); *S. magna argutula-Q. quiscula versicolor* (M.P.); *S. magna argutula-C. mexicanus major* (M.P.); *Q. quiscula versicolor-I. b. bullockii* (M.P.); *Q. quiscula versicolor-A. p. floridanus* (Acro.); *Q. quiscula versicolor-A. p. nevadensis* (M.P.); *I. parisorum-I. b. bullockii* (M.P.); *I. parisorum-C. mexicanus major* (Acro.); *I. parisorum-A. p. nevadensis* (E.P.); *I. parisorum-A. p. mearnsi* (M.P.); *I. parisorum-A. p. floridanus* (M.P.); *C. mexicanus major-I. spurius* (M.P.); *C. mexicanus major-A. p. phoeniceus* (E.P.); *C. mexicanus major-A. p. mearnsi* (E.P.).

The following species and subspecies could be completely separated on quantitative variations: *S. neglecta confluenta-I. b. bullockii*; *S. neglecta confluenta-A. p. phoeniceus*; *S. neglecta confluenta-A. p. nevadensis*; *Q. quiscula versicolor-C. mexicanus major*; *Q. quiscula versicolor-A. p. mearnsi*; *A. p. nevadensis-C. mexicanus major.*

It appears that the statistical analysis of measurement means of sperm parts within members of this avian family has much potential as a taxonomic tool in ornithology.

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