

A BIOMETRIC ANALYSIS OF ORIOLE HYBRIDIZATION

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Recent studies of situations involving hybridization in birds have utilized several methods of analysis. These include standard, so-called hybrid index techniques (e.g., Sibley 1950; Short 1963, 1965, 1971) and multivariate analysis (e.g., Rising 1968, 1970; Rohwer 1972). This report describes (1) the potentially very useful technique of "simple allometry," using adjusted means for mensural characters, and (2) analysis of a hybrid index expression for hybridization, developed by the senior author (Misra et al. 1970). With this latter technique it is possible to establish clines across hybrid zones, using as few as two characters if these are reasonably well correlated. Misra (1971) described a method for analyzing hybridization phenomena based on meristic characters. These techniques permit an investigator to explore some genetic aspects of the variation encountered in hybrid situations. Here, for the first time, we apply these methods, developed by the senior author in studies of fishes, to avian hybridization phenomena.

Two subspecies of the Northern Oriole, the "Baltimore" (*Icterus galbula galbula*) and the "Bullock's" (*Icterus galbula bullockii*), hybridize in the central Great Plains. Their interbreeding has been the subject of studies by Sibley and Short (1964), Rising (1970), and Anderson (1971). Data of Sibley and Short for male orioles of the Platte River transect are the subject of the present analysis, as the 11 samples representing this transect are suitably large-sized to afford a clear demonstration of our techniques. The purpose of this report is not so much to add to their study as to demonstrate that our techniques are useful in analyzing situations of avian hybridization, and perhaps even in extending and refining results of the earlier analysis.

HISTORY AND DEVELOPMENT OF METHODS

In their study of hybridization between eastern (Baltimore) and western (Bullock's) populations of

the Northern Oriole, Sibley and Short (1964) assigned scores to treat quantitatively nine color characters comprising three character complexes (their table 3). They also summed the scores to form their "hybrid index," here instead designated "character index" because "hybrid index" has been used formally in a different way (see below). Sibley and Short (1964) used mean scores of nine color characters (shown in their tables 5 and 6) to investigate hybridization between the two parental forms and to establish the zone of hybridization in the Platte River populations. They also analyzed weight, wing length, and tail length, based on mean values (shown in their tables 7 and 8), and demonstrated concordance in effects of hybridization on these mensural and the nine color character gradients.

We support the use of mean values in the study of clines. Further, with two mensural characters that are correlated, the mean value of one character can be "adjusted" statistically for its correlation with the other character. Such adjusted mean values for different populations can be used to investigate clines. There are two advantages to this procedure:

1. The standard error of an adjusted mean generally will be considerably smaller than that of an unadjusted mean (Misra et al. 1970). The standard error of an adjusted mean is reduced by an amount dependent upon its degree of correlation with a second character. Hence, clines which may not be obvious at some or many points along a transect, if based on unadjusted means (as in Sibley and Short 1964), may become obvious when adjusted means are used. The use of unadjusted means probably explains why clines for bill length and tarsal length were not apparent in Sibley and Short's (1964) study.

2. The influence of the first character on the second (due to pleiotropy, linkage, or other genetic effects) is eliminated by use of the adjusted values for the second character. Clines determined from adjusted mean values of the second character then are entirely the characteristic of the second character.

The technique of adjusting the mean of one character to exclude correlative effects of a second character involves the relationship of "simple allometry" between them. The biological importance of the relationship of simple allometry between measurements of two characters, designated X and Y , was studied intensively by Huxley (e.g., 1932, 1950; see also Misra et al. 1970), and simple allometry has been used in treating a great variety of data on animals and plants (see citations in the important reviews by Cock 1966, and by Gould 1966). The development of its use herein is shown in the appendix.

Attention may be drawn to the frequent occurrence of interspecific breeding in natural populations of freshwater fishes reported by ichthyologists. Often, an objective of studying hybridization is to estimate the degree of intermediacy of suspected hybrids and thereby specify the distribution of hybrids in relation to the two parental species. A formula for a "hybrid index," developed mainly by Hubbs and Kuronuma (1942) and Hubbs et al. (1943), has been in use for some 30 years (for more recent examples see Birdsong and Yerger 1967; Tsai and Zeisel 1969; Johnson and Minckley 1969) to estimate the degree of intermediacy of suspected hybrids between the two parental forms. For hybrid individual "*i*" measuring H_i the hybrid index, I_i , is expressed as follows:

$$I_i = \frac{100(H_i - u_1)}{u_2 - u_1}$$

where u_1 and u_2 are the mean values of a trait for the two parental populations.

Genotypic and environmentally determined variations among individuals of natural populations often are considerable and, where "metric" traits controlled by numerous genes are employed in an analysis for hybridization, an investigation of the sources that may contribute to the phenotype, P , of the trait measured on an individual may be necessary for a fair appraisal of the hybrid index formula. When discussing interpopulation crosses, generally we have in mind populations that are somewhat reluctant to accept alleles from one another. Possibly the hybrid vigor accompanying relatively large blocks of genes favors their retention in hybrids (Wallace 1968). Falconer (1961) also pointed out the importance of effects of interaction among genes in crosses between such populations. It is thus obvious that twofold effects of the genotype, in addition to the environment effect, E , must be recognized in a study of hybridization, viz., the additive genotype, A , describing the cumulative effects of individual genes and the nonadditive genotype, NA , describing the interaction effects of genes. A model for P -value may then be written as

$$P = A + NA + E$$

which is in line with the model used in quantitative genetics (see e.g., Falconer 1961). The presence of NA may indicate that secondary effects such as specific gene combinations, rather than the genes themselves, play a significant role in determining the status of an intermediate population. In the oriole populations of Sibley and Short (1964), which are

from different geographic areas, deviations from the "norm" determined by the mean values may take place due to the selection of such specific gene combinations induced by the local environments (area effects). It is noted that the formula for the hybrid index is comparable, in the linear scale, with the expression for the "degree of dominance" given in Falconer (1961:113).

Misra et al. (1970) have improved on this method as follows:

1. They derived formulae (see appendix) for the sampling variances $V(I_i)$ of an individual I_i and $V(\bar{I})$ of the mean of I_i -values, for measurements on a mensural trait, so that now hypotheses concerning the degree of intermediacy of individuals and of an entire population of intermediate individuals can be tested.

2. They extended the hybrid index formula to a second trait, based on values adjusted for its correlation with the first trait by using the regression transformation of the equation of simple allometry.

3. They demonstrated that if two characters are at least reasonably correlated, a hybrid index estimate based on the second character will have a small standard error, so that a useful study of the position of the intermediates with regard to two parents can be done. Furthermore, with two fairly well correlated traits, this method may give just about as effective results as a study treating several traits.

Misra (1971) gave a method for estimating and analyzing hybrid indices for meristic traits (see appendix).

From the formula for I , it is obvious that the two parents are represented by values 0 and 100 for I . Normally this is the range of I , although values beyond this range are also conceivable probably due to "overdominance" or "marginal overdominance" (Wallace 1968). Thus, for example, Hubbs (1955) observed that hybrid specimens of fish sometimes could exceed the parental types and attributed it to "heterosis." Interesting interpretations of I -values have been provided by ichthyologists to explain the biological status of an intermediate population (see e.g., Birdsong and Yerger 1967). Thus, value $I = 50$ occurs when the hybrids are exactly intermediate (showing a complete lack of dominance). This would indicate a multiple factor mode of inheritance. Hubbs et al. (1943) reported that a unimodal curve for I -values, with peak near $I = 50$, would confirm the interpretation of the aberrant specimens as hybrids and should the values of two or more unrelated traits

TABLE 1. Frequency distribution of character index for individuals of 11 localities.^a

Locality	Character index												Total frequency	
Blair	12	20	8	3	2	0	0	0	0	0	0	0	0	45
Schuyler	7	22	12	7	3	0	0	0	0	0	0	0	0	51
Silver Creek	3	19	16	11	12	1	0	0	0	0	0	0	0	62
Grand Island	1	13	23	12	5	2	0	0	0	0	0	0	0	56
Elm Creek	0	10	19	18	5	4	0	0	0	0	0	0	0	56
Gothenburg	0	10	11	11	11	5	0	1	0	0	0	0	0	49
Sutherland	0	5	11	9	13	10	5	0	0	0	0	1	0	54
Big Springs	0	0	0	0	4	2	2	2	2	1	1	2	2	18
Crook	0	0	1	1	0	0	0	0	1	1	5	5	2	16
Fort Morgan	0	0	0	0	0	0	0	0	0	1	4	2	5	12
Greeley	0	0	0	0	0	0	0	0	0	1	4	9	12	26

^a Individual character scores are available from the junior author on request.

TABLE 2. Mean scores (\bar{x}) and their standard errors ($SE \bar{x}$) for plumage characters and their complexes.^a

Character	Population																					
	Blair (45)	Schnyler (51)	Silver Creek (62)	Grand Island (56)	Elm Creek (56)	Gothenburg (49)	Sutherland (54)	Big Springs (18)	Crook (16)	Fort Morgan (12)	Greeley (26)											
	\bar{x}	$SE \bar{x}$	\bar{x}	$SE \bar{x}$	\bar{x}	$SE \bar{x}$	\bar{x}	$SE \bar{x}$	\bar{x}	$SE \bar{x}$	\bar{x}	$SE \bar{x}$										
Superciliary line	0.13	0.051	0.10	0.042	0.13	0.043	0.07	0.035	0.25	0.068	0.25	0.084	0.61	0.116	2.11	0.384	3.19	0.321	3.75	0.124	3.96	0.037
Forehead color	0.00	0.008	0.00	0.008	0.03	0.033	0.04	0.036	0.11	0.060	0.24	0.110	0.40	0.122	1.67	0.324	2.75	0.348	3.67	0.215	3.46	0.174
Sides of neck	0.18	0.085	0.12	0.066	0.39	0.110	0.36	0.102	0.79	0.140	0.82	0.140	1.04	0.146	2.33	0.324	3.12	0.352	3.67	0.215	3.92	0.075
Ear covert color	0.04	0.032	0.00	0.077	0.05	0.028	0.02	0.019	0.07	0.035	0.12	0.069	0.19	0.076	1.72	0.350	3.38	0.329	3.92	0.079	3.96	0.037
Throat color	0.29	0.068	0.29	0.064	0.34	0.068	0.52	0.076	0.68	0.076	0.94	0.106	1.19	0.111	2.61	0.306	3.56	0.292	3.75	0.136	3.85	0.070
Head-pattern complex	0.18	0.057	0.08	0.038	0.21	0.052	0.18	0.052	0.39	0.075	0.45	0.092	0.70	0.107	2.11	0.323	3.12	0.317	3.67	0.136	3.88	0.062
Wing bar	0.56	0.081	0.86	0.096	1.18	0.081	1.00	0.072	1.02	0.074	1.14	0.100	1.28	0.097	2.89	0.247	3.44	0.249	3.92	0.172	3.77	0.099
Wing coverts	0.31	0.076	0.61	0.088	0.98	0.096	1.05	0.108	1.34	0.089	1.33	0.117	1.81	0.098	2.83	0.226	3.56	0.197	3.92	0.079	3.88	0.062
Wing-pattern complex	0.64	0.084	0.96	0.088	1.35	0.076	1.25	0.073	0.38	0.065	1.53	0.071	1.76	0.069	2.83	0.196	3.31	0.211	3.83	0.107	3.69	0.105
Tail base	0.29	0.081	0.33	0.066	0.58	0.093	0.77	0.101	0.61	0.100	0.61	0.111	0.93	0.141	2.39	0.211	3.06	0.299	3.42	0.219	3.65	0.121
Tail tip	0.13	0.051	0.31	0.071	0.36	0.065	0.46	0.076	0.55	0.091	0.69	0.105	1.15	0.115	2.44	0.275	3.31	0.245	3.67	0.136	3.85	0.070
Tail-pattern complex	0.36	0.084	0.51	0.070	0.66	0.085	0.80	0.082	0.77	0.079	0.90	0.079	1.19	0.120	2.44	0.179	3.00	0.250	3.42	0.185	3.65	0.093
Character index	1.18	0.158	1.55	0.153	2.21	0.160	2.23	0.145	2.54	0.149	2.88	0.202	3.65	0.242	7.39	0.658	9.44	0.700	10.92	0.471	11.23	0.159

^a Sample sizes are shown in parentheses with the names of localities.

TABLE 3. Distribution of intermediate localities according to their differences from Blair and Greeley samples, based on mean scores (\bar{x}).

Character	Localities which differ significantly in their \bar{x} values from \bar{x} of the samples from	
	Blair	Greeley
Superciliary	Sutherland and West	Crook and East
Forehead	Gothenburg and West	Big Springs and East
Sides of neck	Elm Creek and West	Crook and East
Ear coverts	Big Springs and West	Big Springs and East
Throat	Grand Island and West	Big Springs and East
Head-pattern complex	Elm Creek and West	Crook and East
Wing bar	Schuyler and West	Big Springs and East
Wing coverts	Schuyler and West	Big Springs and East
Wing-pattern complex	Schuyler and West	Big Springs and East
Tail base	Silver Creek and West	Big Springs and East
Tail tip	Schuyler and West	Crook and East
Tail-pattern complex	Silver Creek and West	Crook and East
Character index	Silver Creek and West	Crook and East

in a given set of hybrids yield such unimodal curves intervening between those for the parental species, the assumption of hybridity would appear to be established. By using adjusted values of a trait, Y , for its correlation with another trait to estimate hybrid indices as explained earlier, one meets the criterion of exhibiting the degree of intermediacy as if Y were unrelated to X . If individual hybrid index values cluster around the value $I = 50$, this may indicate that most hybrid specimens belong to the F_1 generation if the parental phenotypes are both present in the sample along with the hybrids. An intermediate value of I associated with an increase in variability and a larger range than those for the parental populations would indicate the occurrence of backcrossing and intercrossing of hybrids. Values of I other than 50 would indicate that the hybrids are nonintermediate. Thus $I = 25$ indicates that the hybrids are three times closer to one parent than to the other. This or any other assigned value of I can, of course, be tested for significance by the method of Misra et al. (1970) and Misra (1971).

RESULTS

REANALYSIS OF COLOR CHARACTERS

The situation of hybridization and introgression in orioles of the Platte Valley transect based on mean values has been discussed in detail by Sibley and Short (1964). The techniques of Misra et al. (1970) and Misra (1971) can be extended easily to the case of several intermediate populations. The hybrid index method of analyzing for the degree of intermediacy is easy to apply and straightforward to understand. These provide an interesting alternative to some of the multivariate procedures that are used in analysis of introgression, particularly when one realizes that the assumptions of these procedures are not always met in biological data (see e.g., Namkoong 1966 who also pointed out that additive gene action is often assumed in the multivariate procedures). The hybrid index method

is appropriate for the analysis of oriole data in which populations at the two ends of the east-west transect may be regarded as "fixed" for the following reasons:

1. The biological problem, as presented by Sibley and Short (1964), is to establish the zone of hybridization, if it exists, between two parental forms, in this case *galbula* and *bullockii*, on the basis of data for intermediate populations.

2. Most of the specimens of the populations at the two ends of the east-west transect have been identified as parental forms, at least on the grounds that, for color characters, they have been assigned "limiting scores" of 0 for *galbula* and 2 or 4 for *bullockii* (Sibley and Short 1964:134) to identify them with "pure" parental forms.

Table 1 gives the frequency distributions of scores (x) of character indices for individuals of 11 samples, and the mean score values (\bar{x}) of 13 characters (i.e., plumage characters and their complexes) are shown in table 2. These were calculated from the original data sent to Misra by Short. There are minor variations in some of these mean values from those published in tables 5 and 6 of Sibley and Short (1964). However, it will be noted that these discrepancies are small. Table 2 also gives standard error estimates (SE \bar{x} of \bar{x} values) for nine color characters and their three complexes, which were not shown by Sibley and Short (1964), and for the character indices. With the knowledge of these standard error values, investigation of hybridization can be done accurately and the distribution of localities of intermediate populations, based on the differences of their mean scores from those of the Blair and Greeley populations, can be ascertained (table 3).

Results of table 3 are based on tests done at the 5% probability level of significance (which is also the level used throughout this report).

From these tables it is observed that each character indicates a high degree of introgression between the two forms and the existence of a hybrid zone through which genetic contact between eastern *galbula* and western *bullockii* is maintained. Zones of hybridization vary with the characters. All zones, however, are within the range of Crook and Schuyler. Although the population at Fort Morgan is similar to the Greeley population with regard to all characters, *bullockii* genes seem to have spread as far east as Crook for some characters (viz., the forehead, ear coverts, throat, wing bars and coverts, wing-pattern complex, tail base, and the tail tip). *Galbula* genes may have spread as far west as Sutherland for the ear coverts. Populations at Big Springs and Sutherland are clearly distinct from phenotypically pure *bullockii* or *galbula* populations with regard to all characters, with the exception of the ear coverts, for which the population at Big Springs alone is distinct from the two extreme populations. The thus defined hybrid zone (see Short 1969 for definition of hybrid zones based on sympatry or lack of sympatry of parental forms) occurs between Big Springs and Sutherland for all characters, and over an even wider area for some of them.

At this point analysis of hybrid indices for 13 characters was undertaken to examine the pattern of hybridization, in particular. Table 4 gives values of mean hybrid indices of nine intermediate samples for 13 characters. These hybrid-index values and their standard errors were estimated by the method given by Misra (1971). Table 5 gives the distribution of the intermediate localities according to the degree of intermediacy, measured by I , achieved for these characters. The distribution is based on the estimates of 95% confidence intervals for I . Any degree of hybridization can be examined by considering the corresponding value of I . However, for the purpose of preparing table 5 and following the framework of Sibley and Short's (1964) study, values of I chosen were: (a) 0 and 100 which indicate pure *galbula* and *bullockii* characteristics, respectively; (b) ranges 0-50 and 50-100 of I indicating introgression and the thus defined hybrid zone; and (c) 50 which is an exact intermediate value indicating the center of a hybrid zone. Continuity of the gradient is now shown more clearly, with the center of the hybrid zone shown definitively to be in the vicinity of Big Springs for the character index,

the head-pattern group, the tail base, and the tail tip. For the tail-pattern complex and the wing-pattern group, the indicated hybrid zone is centered just east of Big Springs (toward Sutherland) and it is considerably narrower than the area estimated on the basis of Sibley and Short's mean score values. The existence of localities with values of I in the ranges $0 < I < 50$ and $50 < I < 100$ on two sides of the hybrid zone and interposed between the two parental forms clearly shows that considerable introgression is occurring through the hybrid zone and into both parental populations. The general similarity among various characters in regard to the distribution of localities is more obvious on the basis of these hybrid-index values. Variation among characters in the degree of intermediacy shown at various localities can also be examined from the figures in table 4.

It is observed that there have been differences among characters in the extent to which *galbula* genes may have spread westward from Blair, and *bullockii* genes eastward from Greeley. The spread of *galbula* genes westward is wider for the head-pattern group (as far west as Gothenburg for the superciliary), less so for the tail base, tail-pattern complexes, and the character index, and hardly any for the tail tip and the wing-pattern group. The spread eastward of *bullockii* genes is relatively less, extending only as far east as Crook for the forehead, ear coverts, throat color, tail base, and the wing-pattern group, and as far east as Fort Morgan for all other characters.

From these results as from those of Sibley and Short (1964), it is clear that the eastern *galbula* and western *bullockii* populations are in genetic contact, maintained between the two forms through intermediate populations which show an increasing degree of hybridization from east (*galbula*) to west (*bullockii*). The increase is gradual but small from Blair to Sutherland, followed west of the latter locality by sudden jumps in the hybrid-index values, which are noticeable for all characters and indicate the occurrence of a major shift in hybridization. The point of exact intermediacy is in the immediate vicinity of Big Springs (for all characters except the wing-pattern group and the tail-pattern complex), or in the area between Big Springs and Sutherland (for the wing-pattern group and the tail-pattern complex). The occurrence of primary hybridization resulting in the production of F_1 individuals must be rare due to the following reasons: (1) individuals of the intermediate populations in the neighborhood of Big Springs show an increase in variability of

TABLE 4. Mean hybrid-index values (*I*) and their standard errors (*SE I*) for plumage characters and their complexes, of nine intermediate localities.

Character	Locality																	
	Schuyler		Silver Creek		Grand Island		Elm Creek		Gothenburg		Sutherland		Big Springs		Crook		Fort Morgan	
	<i>I</i>	<i>SE I</i>	<i>I</i>	<i>SE I</i>	<i>I</i>	<i>SE I</i>	<i>I</i>	<i>SE I</i>	<i>I</i>	<i>SE I</i>	<i>I</i>	<i>SE I</i>	<i>I</i>	<i>SE I</i>	<i>I</i>	<i>SE I</i>	<i>I</i>	<i>SE I</i>
Superciliary line	-0.4	1.73	-0.1	1.74	-1.6	1.63	3.0	2.23	2.9	2.58	12.5	3.31	51.7	10.16	79.8	8.59	94.5	3.84
Forehead color	0.0	0.00	0.9	1.11	2.1	1.56	3.1	1.85	7.1	3.25	11.8	3.49	48.1	9.69	79.4	10.83	105.9	8.29
Sides of neck	-1.6	2.93	5.6	3.76	4.8	3.59	16.2	4.43	17.0	4.45	22.9	4.59	57.6	9.13	78.7	9.98	93.4	6.81
Ear covert	-1.1	0.83	0.1	1.08	-0.7	1.21	0.7	1.21	2.0	1.93	3.6	2.07	42.8	8.98	85.0	8.51	98.9	2.49
Throat color	0.1	2.63	1.4	2.71	6.4	2.87	11.0	2.89	18.3	3.57	25.2	3.73	65.3	8.98	92.0	8.81	97.3	4.99
Head-pattern complex	-2.7	1.87	0.9	2.09	0.0	2.08	5.8	2.54	7.3	2.92	14.2	3.29	52.1	8.93	79.5	8.88	94.1	4.51
Wing bar	9.6	3.92	19.3	3.62	13.8	3.40	14.4	3.44	18.3	4.06	22.5	4.02	72.6	8.57	89.7	8.88	104.6	6.25
Wing coverts	8.3	3.27	19.0	3.45	20.3	3.75	28.8	3.36	28.4	3.98	42.1	3.66	73.7	6.94	91.0	6.41	100.9	4.13
Wing-pattern complex	10.4	4.01	23.3	3.85	19.9	3.74	24.0	3.63	29.1	3.83	36.6	3.90	71.8	7.68	87.5	8.39	104.6	6.40
Tail base	1.3	3.12	8.7	3.68	14.2	3.89	9.4	3.85	9.6	4.11	18.9	4.89	62.3	7.25	82.4	9.86	92.8	7.99
Tail tip	4.9	2.36	6.0	2.24	8.9	2.47	11.3	2.82	15.1	3.17	27.3	4.46	62.2	7.66	85.6	7.04	95.2	4.49
Tail-pattern complex	4.7	3.33	9.3	3.65	13.6	3.59	12.5	3.55	16.4	3.94	25.1	4.55	63.3	6.47	80.2	8.55	92.8	7.08
Character index	3.69	2.19	10.3	2.24	10.5	2.17	13.5	2.17	16.9	2.58	24.6	2.96	61.8	6.86	82.2	7.36	96.9	3.91

TABLE 5. Distribution of intermediate localities along the east-west transect according to their degrees of intermediacy based on *I*-values.

Character	Values of <i>I</i>		
	0	0 < <i>I</i> < 50	50 < <i>I</i> < 100
Superciliary	Schuyler to Gothenburg	Sutherland	Crook
Forehead	Schuyler to Elm Creek	Gothenburg and Sutherland	Crook and Fort Morgan
Sides of Neck	Schuyler to Grand Island	Elm Creek to Sutherland	Fort Morgan
Ear coverts	Schuyler to Sutherland	Big Springs	Crook and Fort Morgan
Throat color	Schuyler and Silver Creek	Grand Island to Sutherland	Crook and Fort Morgan
Head-pattern complex	Schuyler to Grand Island	Elm Creek to Sutherland	Fort Morgan
Wing bar	Schuyler	Schuyler to Sutherland	Crook and Fort Morgan
Wing coverts	Schuyler	Schuyler to Sutherland	Crook and Fort Morgan
Wing-pattern complex	Schuyler	Schuyler to Sutherland	Crook and Fort Morgan
Tail base	Schuyler	Schuyler to Sutherland	Crook and Fort Morgan
Tail tip	Schuyler	Silver Creek to Sutherland	Crook and Fort Morgan
Tail-pattern complex	Schuyler	Schuyler to Sutherland	Fort Morgan
Character index	Schuyler	Silver Creek to Sutherland	Fort Morgan

TABLE 6. Estimates of means and their standard errors (SE) for the body weight and adjusted wing and tail lengths.

Locality	Mean \pm SE for the		
	Body wt.	Wing length	Tail length
Blair	35.63 \pm 0.263	96.39 \pm 0.307	75.41 \pm 0.368
Schuyler	37.13 \pm 0.235	95.84 \pm 0.277	75.04 \pm 0.350
Silver Creek	37.64 \pm 0.343	97.01 \pm 0.281	76.44 \pm 0.302
Grand Island	37.60 \pm 0.313	96.84 \pm 0.253	76.46 \pm 0.123
Elm Creek	35.63 \pm 0.292	96.84 \pm 0.242	76.60 \pm 0.280
Gothenburg	36.67 \pm 0.297	96.93 \pm 0.260	76.97 \pm 0.364
Sutherland	37.13 \pm 0.284	96.60 \pm 0.266	77.52 \pm 0.279
Big Springs	38.35 \pm 0.482	99.54 \pm 0.348	78.55 \pm 0.563
Crook	37.99 \pm 0.591	100.66 \pm 0.677	80.56 \pm 0.747
Fort Morgan	38.45 \pm 0.514	101.26 \pm 0.549	81.20 \pm 0.427
Greeley	36.16 \pm 0.350	102.02 \pm 0.363	81.48 \pm 0.344

frequencies and larger ranges of scores, for various characters, over those of the two parental forms (table 1); and (2) in the analysis of hybridization for mensural characters, presented later in this report, I -values for individual specimens were estimated—the distributions of these estimated I -values were trimodal rather than unimodal for intermediate populations, including Big Springs. These observations indicate the occurrence of backcrossing and intercrossing of hybrids.

We also note that (1) populations to the east of Big Springs show values of I which are mostly less than 50 for various characters, and taper off to zero, slowly, as we proceed eastward, whereas the switch from 50 to 100 westward is fairly rapid; and (2) frequency distributions of scores show greater variability and wider range for populations to the east of Big Springs than for those to the west. This is also obvious for the I -values since relative sizes of the standard errors of mean I -values (table 4) are considerably larger for populations to the east of Big Springs than for those to the west. This is true even though samples for populations in the west are much smaller than those in the east. These observations indicate that (1) the *galbula* population east of the hybrid zone shows more introgression from *bullockii* than vice versa; and (2) variability of recombinant populations is still considerably high and selection has not yet had time to reduce it enough so as to fashion a cline between two geographically complementary populations of a single species, as suggested by Sibley and Short (1964).

REANALYSIS OF MENSURAL CHARACTERS

We now consider the analysis of the data of Sibley and Short (1964) on the weights and the wing and tail lengths of male orioles, first

that based on mean values. Table 6 gives mean weights and their standard errors, as well as means and their standard errors for wing and tail lengths adjusted for their correlations with weight using the relationship of simple allometry, as explained above. For adjustment purposes, measurement of weight was used for X in the equation $Y = BX^a$, of simple allometry. This is because body weight has often been used by numerous workers to measure the general body size, and with Sibley and Short's (1964) data also it seemed appropriate to do so. It is also noted that the wing or tail length is a one-dimensional trait, whereas body weight is a measure of the body as a whole. Simple allometry of the wing and tail lengths with the cube-root of the body weight rather than the body weight itself was therefore considered for the estimation purpose, a procedure similar to the one followed by Cock (1963). Statistically weighted means of individual adjusted values and their standard errors were computed as explained in Misra et al. (1970) and in the appendix. From the expression for an adjusted Y -value, given in the appendix, it is obvious that an adjusted value will be the same as the corresponding unadjusted value, within the limits of sampling error, if a is not significant, i.e., if x and y are uncorrelated. It was observed on the basis of t -tests that these correlations were significant only for Schuyler, Elm Creek, and Big Springs for the wing and tail lengths, and also for Greeley in the case of the tail length. Again, very small discrepancies occur in the mean weights shown in table 6 and those of table 8 of Sibley and Short (1964). Locality means for all characters increase more or less steadily along the east-west transect. Several localities differ in their mean weights, as confirmed by t -tests. However, as pointed out by

TABLE 7. Estimates of hybrid-index values and their standard errors (SE) for mean lengths of the wing and tail for nine intermediate localities.

Locality	Hybrid-index estimate \pm SE for the mean	
	Wing length	Tail length
Schuyler	-9.7 ± 7.93	-6.1 ± 8.65
Silver Creek	11.0 ± 7.11	17.0 ± 7.14
Grand Island	8.1 ± 6.88	17.3 ± 7.57
Elm Creek	8.0 ± 6.75	19.6 ± 6.80
Gothenburg	9.6 ± 6.90	25.6 ± 7.64
Sutherland	21.5 ± 6.62	34.7 ± 6.37
Big Springs	55.9 ± 7.57	51.6 ± 10.16
Crook	75.9 ± 13.05	84.8 ± 13.25
Fort Morgan	86.5 ± 11.26	95.3 ± 8.88

Sibley and Short (1964), the pattern is unclear due to the small difference between the extreme populations in their mean weight. Differences among localities with regard to the mean wing and tail lengths are less obvious (the pattern, however, became clear when hybrid indices were analyzed, see below). The hybrid zone seems to extend between Silver Creek and Sutherland with regard to wing length, and between Silver Creek and Big Springs for tail length.

In view of the fact that a -values for the wing-weight and tail-weight combinations were unlike for some localities, we did not compare locality-means on the basis of an analysis of covariance. Instead, comparisons were based on standard errors estimated separately for locality-means (table 6). We conducted no study of hybridization based on direct comparison of a -values (following Misra 1973), as these values are small for several localities.

Table 7 gives mean hybrid-index values (I) of nine intermediate samples for the adjusted wing and tail lengths. It is observed that the gradients for wing length and tail length are similar and are in agreement with those indicated for the color characters earlier. The following observations also were made:

1. The null hypothesis that $I = 50$ is acceptable for the Big Springs population and no other population.

2. The value I for Big Springs is significantly different from the two parental values of 0 and 100.

3. Intermediate values of I exist on either side of $I = 50$.

Estimates of I for the adjusted wing and tail lengths of individual specimens of all intermediate localities were also obtained by the method of Misra et al. (1970). All I -values

for a locality were then divided into three classes, viz., those which were: (a) $<$ Mean $I - 2$ SE; (b) Between Mean $I - 2$ SE and Mean $I + 2$ SE; and (c) $>$ Mean $I + 2$ SE, to see whether or not the distribution of I values for a locality was unimodal.

It was observed that the distribution for none of the intermediate localities was unimodal, as there was a considerably high proportion of specimens in either of the classes (a) and (c).

Observations on introgression activities of populations with respect to the two mensural characters are thus similar to those made for the color characters earlier, viz. (1) the zone of hybridization is centered at Big Springs, and (2) considerable introgression occurs through the hybrid zone and into the two parental forms. Primary hybridization resulting in the production of F_1 individuals is a rare event, as observed by Sibley and Short (1964). The genetic contact between eastern *galbula* and western *bullockii* populations is maintained through intermediate populations which, judged from their hybrid index values, form a continuum from east to west. The spread westward of *galbula* genes is greater than the spread eastward of *bullockii* genes.

SUMMARY

A formula for "hybrid index," developed mainly by Hubbs and Kuronuma (1942) and Hubbs et al. (1943) has been in use by ichthyologists for some 30 years to study the status of an intermediate population in relation to its two parents. It was noted by Misra et al. (1970) that the expression for the hybrid index is comparable, in a linear scale, with that for the "degree of dominance" (Falconer 1961:113). The hybrid index expression is thus not only mathematically simple to apply, especially in comparison to a multivariate analysis which is sometimes used to analyze data on parents and their hybrids, but also provides potential to investigate the genetic aspects of variations in populations in a direct and straightforward manner. The senior author (in Misra et al. 1970) used the potentially very useful technique of "simple allometry" to estimate and analyze hybrid indices, for mensural traits, of hybrids considered individually and as a group. With this technique it is possible to establish clines across hybrid zones, using as few as two characters if these are reasonably well correlated. Misra (1971) gave a method of analyzing hybrid indices for meristic traits. Here for the first time we apply these methods to avian hybridization phenomena. Applications of these methods are

illustrated for the data of Sibley and Short (1964) on male orioles of the Platte River transect. This transect is represented by 11 samples of suitably large size.

Eleven areas were compared on the basis of the mean scores for nine plumage color characters reported by Sibley and Short (1964), the head pattern, wing pattern, tail pattern, and the character index (designated "hybrid index" in Sibley and Short 1964). This was followed by an analysis of hybrid indices for these 13 characters. Continuity of the gradient is shown clearly, with the center of the hybrid zone shown definitively to be in the vicinity of Big Springs for the character index, the head-pattern group, tail base, and the tail tip. For the tail-pattern complex and the wing-pattern group, the indicated hybrid zone is not centered exactly at Big Springs but in its immediate neighborhood, on the side toward Sutherland, and it is considerably narrower than the area estimated on the basis of Sibley and Short's mean score values. Wing and tail lengths were statistically adjusted for their correlations with the body weight and then analyzed for hybridization. It is observed that the gradients for wing and tail lengths are similar and in agreement with those indicated for the color characters. It is also clear that the eastern *galbula* and the western *bullockii* populations are in genetic contact, which is maintained through intermediate populations. These populations show a gradually increasing degree of hybridization from east to west. The increase is small from Blair to Sutherland, followed by sudden jumps in the hybrid-index values, which are noticeable for all characters, and indicate the occurrence of a major shift in hybridization. The analysis also indicated that (1) primary hybridization resulting in the production of F_1 individuals must be very uncommon; (2) backcrossing and intercrossing of hybrids occurs; and (3) the *galbula* population east of the hybrid zone shows more introgression from *bullockii* than vice versa.

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APPENDIX

The equation of simple allometry is given by the power function,

$$Y = BX^a$$

where a is a constant called the "coefficient of allometry" (Cock 1966) and is genetically controlled. It may be estimated as the slope of the line of regression of $\ln Y$ (or y) on $\ln X$ (or x). The other constant, B , of the power function is usually of no biological importance and is read, merely for the convenience of calculation, as the value of Y when $X = 1$. Misra et al. (1970) have shown that Y_{aj} or "adjusted" value of Y , which is the measurement of Y for specimen "j" may be given as follows:

$$Y_{aj} = Y_j \cdot \exp(-a[x_j - \bar{x}]),$$

where \bar{x} is the mean of x_j -values in a sample of n individuals. Standard error of \hat{Y}_{aj} or the estimate of Y_{aj}

$$= \hat{Y}_{aj} \left\{ \frac{1-r^2}{n-2} \left[\sum_{j=1}^n y_j^2 - \frac{(\sum_{j=1}^n y_j)^2}{n} \right] \left[1 + \frac{1}{n} + \frac{2(x_j - \bar{x})^2}{\sum_{j=1}^n x_j^2 - (\sum_{j=1}^n x_j)^2/n} \right] \right\}^{\frac{1}{2}}$$

where r is the coefficient of correlation between x_j and y_j values of the sample. Estimate of the mean of Y_j values could also be used as an estimate of the mean of Y_{aj} values. Misra et al. (1970), however, used the weighted mean of Y_{aj} values, using reciprocal of the variance estimate as the weight factor.

Incidentally, it may be noted that the parameter a , or the coefficient of allometry which, as mentioned earlier, is genetically controlled and is of much biological importance, provides a separate basis for comparing populations (Misra 1973).

Adjusted Y -values for the hybrids and adjusted means for the parents may be used in the hybrid-index expression to estimate the hybrid indices, \hat{I}_j . For estimates made from n_i ($i = 1$ for parent 1, $= 2$ for parent 2, and $= 3$ for hybrid populations) individuals, its sampling variance, $V(\hat{I}_j)$, is given as follows:

$$V(\hat{I}_j) = \frac{10^4}{(\hat{\mu}_2 - \hat{\mu}_1)^4} \left[\frac{(H_j - \hat{\mu}_2)^2 \hat{\sigma}_1^2}{n_1} + \frac{(H_j - \hat{\mu}_1)^2 \hat{\sigma}_2^2}{n_2} + (\hat{\mu}_2 - \hat{\mu}_1)^2 \hat{\sigma}_3^2 \right],$$

where $\hat{\sigma}_i$ are estimates of variance for samples from the three populations. Standard error of \hat{I}_j is, of course, the square root value of $V(\hat{I}_j)$. Hubbs et al. (1943) used the simple mean of \hat{I}_j -values to estimate the mean hybrid index \bar{I} . Misra et al. (1970) used the weighted mean instead, using the reciprocal of $V(\hat{I}_j)$ as the weight factor, and expressed $V(\bar{I})$ as =

$$\left\{ \sum_{j=1}^{n_3} [V(\hat{I}_j)]^{-1} \right\}^{-1}$$

The method of Misra (1971) for estimating and analyzing hybrid indices for meristic traits may be briefly described as follows: Consider, again, samples of n_i ($i = 1$ and 2 for the two parental forms and $= 3$ for their hybrids) individuals from the two parent populations and their hybrids. An individual may take one of the s possible values, say. All individuals may be considered as distributed in a $3 \times s$ table with p_{ik} ($i = 1, 2, 3; k = 1, 2, \dots, s$) as the proportions of individuals of the i th population which are in the k th class. Proportion p_{ik} may be considered as the mean in the i th population of a variable which takes only the values 0 and 1. Mean u_i for population "i" is estimated as,

$$\hat{u}_i = \sum_{k=1}^s \hat{p}_{ik} X_k,$$

and its sampling variance, $V(\hat{u}_i)$, as

$$V(\hat{u}_i) = \frac{1}{n_i} \left[\sum_{k=1}^s X_k^2 \hat{p}_{ik} (1 - \hat{p}_{ik}) - 2 \sum_{\substack{k, m=1 \\ k \neq m}}^s X_k X_m \hat{p}_{ik} \hat{p}_{im} \right].$$

Hybrid-index estimate, showing the position of the mean for hybrids, may be expressed as,

$$\hat{I} = \frac{100(\hat{u}_3 - \hat{u}_1)}{\hat{u}_2 - \hat{u}_1},$$

and the estimate $V(\hat{I})$ of its sampling variance as

$$V(\hat{I}) = \frac{10^4(\hat{u}_3 - \hat{u}_1)^2}{(\hat{u}_2 - \hat{u}_1)^2} \left[\frac{V(\hat{u}_3) + V(\hat{u}_1)}{(\hat{u}_3 - \hat{u}_1)^2} + \frac{V(\hat{u}_2) + V(\hat{u}_1)}{(\hat{u}_2 - \hat{u}_1)^2} - \frac{2V(\hat{u}_1)}{(\hat{u}_3 - \hat{u}_1)(\hat{u}_2 - \hat{u}_1)} \right].$$

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