

# SINGING BEHAVIOR, MATING ASSOCIATIONS AND REPRODUCTIVE SUCCESS IN A POPULATION OF HYBRIDIZING LAZULI AND INDIGO BUNTINGS<sup>1</sup>

MYRON C. BAKER AND JEANETTE T. BOYLAN<sup>2</sup>

Biology Department, Colorado State University, Fort Collins, CO 80523,  
e-mail: mcbaker@lamar.colostate.edu

**Abstract.** Populations of Lazuli Buntings (*Passerina amoena*) and Indigo Buntings (*P. cyanea*) overlap in their distribution and hybridize in the Great Plains of North America. We conducted a 4-year field study of color-banded Indigo, Lazuli, and hybrid Buntings to address questions about mating behavior, male song and plumage traits, and reproductive success. From previous studies, we knew that males of these two taxa can learn one another's song traits and that song is important in eliciting sexual behavior in females. Here, we explore the possible role of intersexual vocal communication in explaining hybrid matings. We classified males and females as lazuli, indigo, or hybrid on the basis of plumage, and recorded male songs and described their acoustic features. We tested for associations between song traits and plumage phenotypes of the males, and between plumage phenotypes of females and the plumage and song traits of their mates. We found positive assortative mating between male and female plumage types, and between male song phrases and female plumage. Data on reproductive success of the different mating associations suggest lower fitness of pairings involving hybrids, especially those in which the female was hybrid. We conclude that there is selection against hybrids, but that between-species crosses are relatively common because, at least to some degree, females use learned song traits of males in mate choice.

**Key words:** bird song, cultural transmission, hybridization, Indigo Bunting, Lazuli Bunting, *Passerina amoena*, *Passerina cyanea*.

## INTRODUCTION

Populations of the western Lazuli Bunting (*Passerina amoena*) and the eastern Indigo Bunting (*P. cyanea*) overlap and hybridize in the Great Plains of North America (Sibley and Short 1959, Kroodsma 1975, Rising 1983). These species are 1 of the 11 pairs of avian taxa that meet and hybridize to various degrees in the Plains, and one of several east-west pairs that are hypothesized to be examples of secondary contact following allopatric differentiation during the Pleistocene (Remington 1968, Rising 1983). Studies in contact zones between interbreeding taxa can be useful for addressing various issues in speciation theory, such as the role of mate choice and its reproductive consequences (Butlin 1989, Grant and Grant 1989).

As in many other passerine species, male buntings establish territories and attract a female mate by visual and vocal displays (Armstrong 1963, Thompson 1965, 1972). Where these spe-

cies are sympatric, the males are interspecifically territorial (Emlen et al. 1975, pers. observ.) and strongly respond agonistically to each other's songs (Baker 1991). Males of these two buntings are strikingly different in their plumage patterns. With minor variations in first-year birds, mature male Indigo Buntings are cobalt blue overall, whereas male Lazuli Buntings have a turquoise-blue head, throat, and back, a white abdomen, a distinct rusty breast band, and white wing bars (Sibley and Short 1959, Emlen et al. 1975). Thus, the visual cues that males of these species present to females clearly differ.

Males of both species, however, can produce the other's song elements (Emlen et al. 1975). Studies of song learning in Indigo Buntings (Rice and Thompson 1968, Payne 1981) suggest that such cross-species song learning probably occurs during the first year of life up to and including the first breeding season. The acquisition of heterospecific song elements, however, has not been examined in natural populations or beyond the first year of life in captivity (Boylan 1995). One of our initial concerns was the possibility that males in a mixed population might alter their songs to become more attractive to

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<sup>2</sup> Current address: Dallas Zoo, Bird Department, 650 South RL Thornton Freeway, Dallas, TX 75203.

females, depending on social feedback from female responsiveness to male singing. Such strategic flexibility in vocal production was described in Brown-headed Cowbirds (*Molothrus ater*) in a laboratory experiment (West and King 1988). A male bunting of one species may have a song either partly or completely typical of the other species, and both male song and plumage appear to play a role in mating behavior (Baker and Baker 1990). To a given female, a male may present a phenotype that is conspecific in song but heterospecific in plumage. Some of the hybridization that occurs in a mixed population could result from such females making mate choices on the basis of the song phenotype of males despite the differences in plumage (Grant and Grant 1996, 1997).

Our study was designed to address several questions about the speciation process in Indigo and Lazuli Buntings: (1) What is the extent of heterospecific song learning by males in a population containing both species and their hybrids? (2) Do songs of individuals change beyond the first year of life, and, if so, what is the nature of those changes, and are intra- or interspecific mate changes correlated with song changes? (3) What is the relative importance of male plumage phenotype vs. song traits in explaining mating associations and is there positive assortment? (4) Given the mating associations observed, is there evidence of depressed fitness in hybrids? To address these questions, we conducted a 4-year field study in a population in northeast Wyoming where we color-banded the birds, tape recorded songs, and observed mating patterns and their reproductive consequences.

## METHODS

### STUDY AREA AND FIELD METHODS

The study area was located approximately 0.5–5 km south of Beulah, Wyoming, in riparian habitat along Sand Creek, a continuously flowing stream. Bunting territories were located in the dense vegetation near the stream as well as along the more sparsely vegetated gullies and cliffs adjacent to the main stream channel. For the first three years of the study (1988–1990), we began capturing and color-banding buntings in May, early in the period of arrival on the breeding grounds of these migrant species. We documented the establishment of territories of

males and the movements of females from the time of their arrivals. There were usually three workers in the field each day from 06:00 to 15:00. Early in the season, during the arrival of birds, we operated 20 mist nets daily. Thus, we were able to color-band males and females soon after their arrival. Frequent recaptures as well as visual sightings of banded birds allowed us to map the birds' movements, identify singing perches of males, observe male-male interactions, map territories, and observe females being attended and courted by males. As stable pair-bonds were formed, we began to monitor nesting activities of each pair, with the objective of locating all nests and following reproductive success throughout the breeding period. This color-banded population occupied what we will refer to as our intensive study area. We also tape-recorded and examined buntings in areas upstream and downstream from the intensive study area.

### PLUMAGE PATTERN ANALYSES

The plumage patterns of males and females in the intensive study area were examined at capture, and some birds were photographed. Plumage patterns were evaluated according to the criteria used by Emlen et al. (1975), which allow the plumage of males and females to be typed as lazuli, indigo, or hybrid individuals by summing scores assigned to the coloration of plumage of various body regions. For males, scores can range from 0 (typical indigo) to 11 (typical lazuli). Subjective differences among scorers led to some measurement error, arising principally from variation in scoring in head and throat coloration. We therefore judged scores of 0–2 as indigo, 3–8 as hybrid, and 9–11 as lazuli.

We also studied a sample of males outside the intensive study area, mainly for the purpose of recording and analyzing their songs. The plumages of these males were typed as viewed through a spotting scope, and we relied on the major plumage traits to assign them to category: wing bars, breast band, and belly characteristics all exhibit clear alternative character states in the two species, with intermediate states signifying hybrid status. Although some first-year male Indigo Buntings may display whitish feathers among the blue belly feathers (Payne 1992), hybrid males have a heavily mottled blue and white belly together with some degree of a cinnamon breast band. This approach to classifying unbanded birds was found accurate by compar-

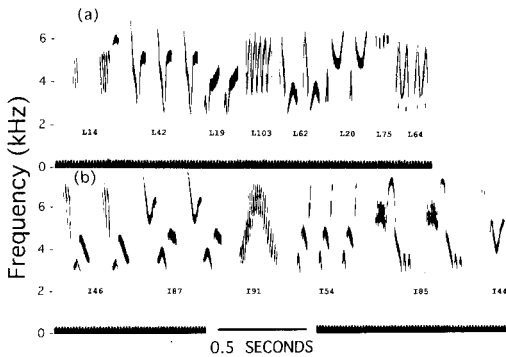


FIGURE 1. A typical song of (a) Lazuli Bunting male and (b) Indigo Bunting male with syllables identified by numbers that correspond to published catalogs. Syllable numbers are prefixed with I (indigo) or L (lazuli).

ing the scores of males from the intensive study area that were scored in hand at the time of capture and banding and also through a scope.

Female plumages of the two species are more similar than are those of males. Female scores (Emlen et al. 1975) range from 0 (typical indigo) to 3 (typical lazuli). We further subdivided these categories to units of 0.5. We judged scores of 0 or 0.5 as indigo, 1, 1.5, or 2 as hybrid, and 2.5 or 3 as lazuli. Our laboratory and field experience with female plumages from allopatric populations of Lazuli and Indigo Buntings helped in evaluating females in our study area in sympatry. In fresh and worn plumage, female indigos have noticeably streaked breasts and no distinct wing bars (see also Pyle et al. 1987), whereas female lazulis lack the breast streaking but have distinct wing bars. As pointed out by Emlen et al. (1975), female indigos also have whitish throat feathers compared to the cinnamon coloration of female lazuli throat feathers.

#### TAPE RECORDING AND ANALYSES

Songs were recorded using a Uher Report Stereo IC recorder and a Uher microphone mounted in a 60 cm parabolic reflector. We recorded a total of 83 males, 36 of which occupied territories in one or more years in our intensive study area, and the remaining 47 of which held territories in neighboring habitat but were not color banded or monitored for reproductive data. These latter 47 were all recorded in the 1988 season. To avoid repeated recording of the same individual in these unbanded males, we moved from one

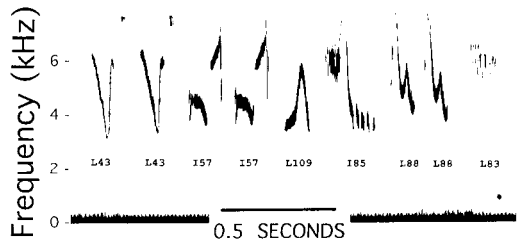


FIGURE 2. A typical mixed song showing Indigo and Lazuli Bunting phrases; some phrases contain repeated syllables.

singing male to the next, continuing onto new territories without retracing the route.

All songs were displayed and analyzed on a Kay Elemetrics DSP Sonograph (model 5500) using settings of flat shaping, 300 Hz transform, Hamming window, and no averaging. Syllables present in each song were compared to catalogs of the syllable repertoires of each species that had been recorded in allopatric populations (Thompson 1970, 1976, Baker and Boylan 1995). The morphology of syllables in this sympatric population matched well with the published catalogs. We found no new syllables and no hybrid syllables.

Typical songs of a lazuli male and an indigo male are illustrated in Figure 1 together with identification numbers of syllable types corresponding to those types in the catalogs of Baker and Boylan (1995). Buntings often repeat syllables of a given type in doublets or, less often, triplets. We followed the convention of referring to a syllable type, whether occurring singly in the song or as a repeated unit, as a phrase (Shiovitz 1975). In classifying the song of a male, we counted only phrases. Thus, for example, the mixed song in Figure 2 consists of six phrases (six different syllable types: L43, I57, L109, I85, L88, L83) made up of nine individual syllables (three phrases contain doublets), and we scored this male as singing two indigo phrases and four lazuli phrases.

To examine mate preferences of females for males with songs of different phrase compositions, we created a hybrid song category. A song consisting of two or more lazuli phrases and two or more indigo phrases was classified a hybrid song (e.g., Fig. 2). A lazuli song contained either all lazuli phrases or no more than one indigo phrase. Half the males classified as singing lazuli songs included only lazuli phrases in their

songs, and in the remaining half each male included only one indigo phrase amidst the lazuli phrases. An indigo song contained either all indigo phrases or no more than one lazuli phrase. Eighty-five percent of the males classified as singing indigo songs included only indigo phrases in their songs, and in the remaining 15% each male sang a song that contained one lazuli phrase amidst the indigo phrases. We felt that this classification scheme was reasonable based upon previous studies of the White-crowned Sparrow (*Zonotrichia leucophrys*), which indicated that replacement of a single phrase in the song of a home dialect with a phrase from an alien dialect had no detectable effects on the number of copulation solicitation displays elicited from females (Baker et al. 1987). No similar data are available for the buntings.

We also examined the temporal pattern of phrase delivery. Quantification of the time intervals between repeated syllables within a phrase in lazuli songs (Emlen et al. 1975, Baker 1991) has shown them to be short and similar in duration to intervals between dissimilar syllables (different phrases). In contrast, intervals between phrases in Indigo Bunting songs are significantly longer than between syllable repetitions within phrases. Furthermore, between-phrase intervals are longer in indigos than in lazulis, whereas intervals between repeated syllables are the same.

We used the measurement of inter-phrase interval (Baker 1991) to assign the temporal pattern feature of the songs as indigo, lazuli or hybrid. The interval between phrases in indigo songs averages 0.090 sec (99% CI = 0.008,  $n = 173$ ) and in lazuli songs 0.060 sec (99% CI = 0.005,  $n = 94$ ). Subtracting the 99% confidence interval from the indigo mean gives 0.082 sec, and adding the 99% confidence interval to the lazuli mean gives 0.065 sec. Therefore, we considered an inter-phrase interval equal to or greater than 0.082 sec as indigo, equal to or less than 0.065 sec as lazuli, and 0.066–0.081 sec as hybrid.

Because we were interested in the flexibility of male songs as social signals addressed to potential mates, we tape recorded males between and within breeding seasons. In the intensive study area, 15 males were recorded in two or more summers for a total of 24 between-season comparisons: 9 were recorded for two years, 3 for three years and 3 for four years. By the

plumage scores of these 15 males, 10 were lazuli, 2 were indigo, and 3 were hybrid. Fourteen of these also were recorded twice within a season. We emphasized recording each territorial male resident in the intensive study area during two time periods: early season (late May–early June, median date 27 May) and mid season (mid June–mid July, median date 5 July). We recorded an average of 14 songs for each male in each time period. In the fourth year of the study, 1991, we recorded those territorial males banded in a previous year, but no other research was conducted. Most of the songs of a male recorded in a session were identical and complete. Sometimes a song would be truncated with one or more syllables dropped from the ending of the song and these were ignored for comparisons between seasons or years.

#### MEASUREMENT OF REPRODUCTIVE SUCCESS

We constantly monitored mating behavior, and when a pair was formed we began intensive observations of nesting activity and reproductive success. We found most nests during the egg stage. The dense vegetation, difficult viewing circumstances, and secretive behavior of the females often led to several days of effort to find a single nest. To reduce predation, abandonment, and parasitism by Brown-headed Cowbirds, we held our nest visits to a minimum, but this meant that we were often able to determine only if a nesting attempt produced eggs, nestlings, and fledglings. After we found a nest in the egg stage, our subsequent observations, from a distance, of parents' feeding trips to nestlings allowed us to monitor nest progress and, subsequently, if fledglings resulted. After fledging, we were able to determine the presence of fledglings by observing parent feeding trips, but because of the bird's movements we could not reliably and consistently determine the number of fledglings.

We did not attempt to take the age of the parents into account when evaluating reproductive success. Previous research on Indigo Buntings (Payne 1989) with large samples from two different populations showed that breeding success of females did not vary with age nor did male breeding success vary with age in one of the two populations. The only significant age effect in males was that in one population the first-year males were less successful than third-year males, but not different in success from second, fourth,

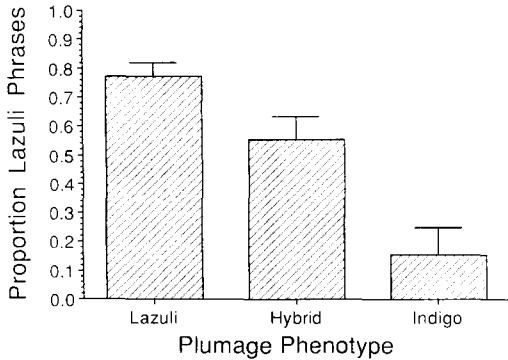


FIGURE 3. Proportion (+ SE) of Lazuli Bunting phrases sung by Lazuli ( $n = 46$ ), hybrid ( $n = 24$ ), and Indigo Bunting ( $n = 13$ ) males. Remaining proportion for each category of male comprise Indigo Bunting phrases.

fifth, or sixth year males. In addition, our sample size was too small to attempt further subdivision by parental age. For convenience in describing patterns of association in contingency tables, we assume mating associations are a result of female preferences. Williams' correction is applied to  $G$ -tests (Sokal and Rohlf 1981). Values presented are means  $\pm$  SE.

RESULTS

COMPARISONS OF MALE PLUMAGE AND PHRASE STRUCTURE OF SONG

Lazuli males ( $n = 46$ ) on average sang songs composed of  $4.5 \pm 0.4$  lazuli phrases and  $1.4 \pm 0.3$  indigo phrases. Indigo males ( $n = 13$ ) on average sang songs composed of  $4.3 \pm 0.6$  indigo phrases and  $1.1 \pm 0.7$  lazuli phrases. Males in hybrid plumage ( $n = 24$ ) on average sang songs composed of  $3.2 \pm 0.6$  lazuli phrases and  $2.4 \pm 0.4$  indigo phrases. Analysis of variance comparing the proportion of lazuli phrases constituting the songs of the three kinds of males (Fig. 3) showed significant heterogeneity (ANOVA on arcsine transformed proportions:  $F_{2,80} = 15.4, P = 0.001$ ). All three pairwise comparisons were significantly different (Fisher's LSD multiple comparisons test, all  $P_s < 0.05$ ; Fig. 3).

Applying our definitions of lazuli, hybrid, and indigo songs, classified by their phrase compositions, we found positive association between male plumage and type of song. Males in lazuli plumage were more likely to sing lazuli songs; males in indigo plumage were more likely to sing indigo songs (Table 1;  $G_4 = 20.6, P <$

TABLE 1. Number of males of each plumage category that sang with phrases or temporal patterns characteristic of Lazuli, hybrid, or Indigo Bunting songs.

Male plumage	Song types					
	Lazuli		Hybrid		Indigo	
	Phrase	Temporal	Phrase	Temporal	Phrase	Temporal
Lazuli	32	22	6	18	8	6
Hybrid	13	9	4	10	7	5
Indigo	2	2	0	3	11	8

0.001). Overall, 85% of indigo males sang indigo-phrased songs, and 70% of lazuli males sang lazuli-phrased songs. Hybrids were as likely to sing lazuli songs as they were to sing indigo songs and were not more likely to sing hybrid songs.

MALE PLUMAGE AND TEMPORAL STRUCTURE OF SONG

Male plumage and temporal pattern of songs also were not independent (Table 1;  $G_4 = 11.9, P < 0.02$ ). We found positive association between lazuli males and lazuli song and between indigo males and indigo song. Overall, 62% of indigo males sang with an indigo temporal pattern, and 48% of lazuli males sang with a lazuli temporal pattern. Values for hybrid males in each temporal pattern type were close to those expected by random association.

CHANGES IN PHRASE CONTENT OF SONGS WITHIN A BREEDING SEASON

Changes in phrase content occurred within a breeding season. Considering all 37 comparisons between the song structure of the early and mid-season, there were 52 phrases added to the beginning or ending of songs, 20 phrases inserted or substituted within songs, and 17 phrases lost from the sampled song pool. Thus, 89 song changes occurred from the early to the mid-season, or an average of 2.4 changes in phrase composition for each comparison.

CHANGES IN PHRASE CONTENT BETWEEN BREEDING SEASONS

Song changes also occurred between breeding seasons. Some between-year comparisons revealed that up to 100% of the phrases could be exchanged (e.g., Fig. 4). Fifteen males altered their songs from one year to the next by adding, deleting, or substituting one or more phrases. Because some males were recorded in more than

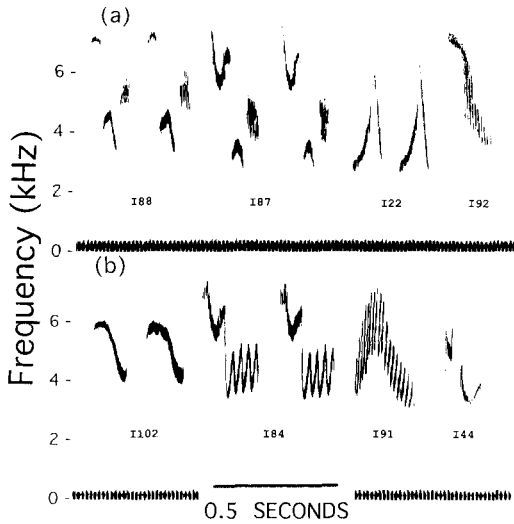


FIGURE 4. Song of same male recorded in (a) 1988 and (b) 1989 showing that a completely new set of Indigo Bunting phrases in 1989 replaced those sung in 1988.

two years, we had 24 between-year comparisons available. We addressed the question of how many changes occurred in the song of a male in year Y compared to year Y-1. There were 23 phrases added to songs in Y relative to Y-1, 14 new phrases substituted, and 7 phrases lost. This gives a total of 44 song changes from Y-1 to Y in the 24 possible comparisons, or 1.8 song changes (range 1-8) for each year-to-year comparison in the sample. Thirty-nine songs were sung by the 15 birds recorded in two or more years. The average length of a song was about 5.9 phrases. Therefore, in an average male's song, approximately 30% of the phrases changed between successive years. For any given individual, there was no particular pattern of using phrases from one species or the other in these song changes. No matter what species-typical phrases were in the song in year Y-1, either indigo or lazuli or both kinds of phrases might be incorporated into the song in year Y.

#### CAUSES OF SONG CHANGES IN MALES

We examined two social influences as possible causes of change in the phrase composition of the songs of males: change caused by female influence (West and King 1988) and change caused by male influence (Payne 1983). For the question of female influence, we examined the set of males that altered their songs from one

year to the next to determine whether the song change was correlated with a new female mate, provided the plumage type of the female differed in the two years. We had only six useful cases with which to examine this issue: in three cases, there was a matching change in song phrases to female plumage and in three cases the change in song phrases did not result in a closer match with the plumage of the new female.

For the question of male influence, we determined whether the change in a male's song had any relationship to the songs sung by neighboring males. Shifts in territories and recruitment of new males each year might lead to alterations in song resulting from males matching syllables to the songs of new neighbors. Although matching of songs between males has been documented in an Indigo Bunting population in Michigan (Payne et al. 1981), no similar study has been made in an indigo-lazuli hybridizing population. In our study area, matching also occurred and did so without regard to whether males were indigo, lazuli, or hybrid plumage phenotypes. We obtained suitable data on 66 pairs of neighbors. We applied the 4-0 and 4-1 matching criteria used by Payne et al. (1981). With this approach, two birds in question had in common four matching phrases in the same order with either no (4-0) or only one (4-1) insertion of a different phrase in the sequence. Thus, bird A may have phrases 3-10-16-44-57-43 and bird B have 3-10-16-44-52 for a 4-0 match, whereas bird C may have 66-42-99-13-77 compared to bird D with 66-42-19-99-13-88 for a 4-1 match.

We recorded 15 cases of males changing songs from one season to the next and the associated songs of neighbors of these males in both years. Thus, we asked if a song change was made from year Y-1 to year Y did it then match the song of a new neighbor in Y? In 4 of the 15 cases such matching did occur; in the remaining 11 cases no matching with a new neighbor occurred in Y. In all four of the matching cases, the male that changed songs and matched a new neighbor was an adult that had produced a stable song and bred in the previous year. Regardless of changes in neighbors, by the 4-0 criterion there were 22 pairs of neighboring males with matching songs over the three seasons. An additional four pairs of males matched by the 4-1 criterion.

## MATING ASSOCIATIONS BY MALE AND FEMALE PLUMAGE TYPES

We observed a total of 62 pairings for which we had color-banded the birds, mapped territories, and followed reproductive success. In 1988, there were 14 matings comprising 14 different males and 14 different females. In 1989, with 4 cases of bigamy and 1 case of a female switching mates and breeding twice, there were 19 matings comprising 15 different males and 18 different females. Seven of these males and one of the females, constituting 10 matings, also had bred in 1988, but only one of these pairings in the 1989 sample was of a male and female who also had paired in 1988. The remaining six males had new mates in 1989. In 1990, with 6 cases of bigamy, 1 of trigamy, and 2 cases of females switching mates and breeding twice, there were 29 matings involving 21 different males and 27 different females. Five males constituting six matings also had bred in both 1988 and 1989 with different females. One male with two matings had bred in 1989 with a different female. One male with three matings with different females had bred in 1988 but was not present in the study area in 1989. Six of the females had bred in the previous year. For the three seasons of data, there were 36 individual males and 52 individual females for 62 matings.

Thus, we observed a great deal of turnover in mating associations. Most matings were socially monogamous within a season, but there was only one case in which the same two birds paired in successive years. We do not know if individual females have set preferences for particular male phenotypes, which may be carried on for several years. However, we had 52 individual females in our total of 62 pairings and we have considered their first matings in a separate analysis. Repeat matings over 2 or more years were too few for statistical treatment. The frequencies of the three plumage types for both males and females in the mating population did not change significantly over the three breeding seasons (male:  $G_4 = 2.9$ ,  $P > 0.5$ ; female:  $G_4 = 4.1$ ,  $P > 0.5$ ). For these reasons, we treated the 62 matings as independent events and pooled the data for the three seasons.

Analysis of the pairings (Table 2) revealed significant deviation from independence of association of male and female plumage types ( $G_4 = 13.6$ ,  $P < 0.01$ ). Lazuli females preferred lazuli males, indigo females preferred indigo males,

TABLE 2. Number of females of each plumage category mated with males that had plumage (P), song phrases (SP), or song temporal patterns (ST) characteristic of Lazuli, hybrid, or Indigo Buntings.

Female plumage	Male traits								
	Lazuli			Hybrid			Indigo		
	P	SP	ST	P	SP	ST	P	SP	ST
Lazuli	24	25	18	13	9	21	5	8	3
Hybrid	5	7	4	3	0	3	1	1	1
Indigo	1	5	5	3	0	2	7	6	4

and hybrid females mated at random. We also examined mating associations from two additional perspectives. First, we considered only the first matings of the 36 individual males in the sample. In this set of pairings, as in the full set of 62 pairings, we found that lazuli females preferred lazuli males and indigo females preferred indigo males ( $G_4 = 17.0$ ,  $P < 0.01$ ). Second, we considered the first matings of the 52 individual females. In this set of pairings, we again found that lazuli females preferred lazuli males and indigo females preferred indigo males ( $G_4 = 13.1$ ,  $P < 0.02$ ). For subsequent analyses, we used the full data set of all 62 matings. However, because one of the males was only captured and plumage typed, but not tape recorded, we had 61 matings for analyses of the association of male song traits with female plumage.

## MATING ASSOCIATIONS: MALE SONG TRAITS AND FEMALE PLUMAGE

We examined associations between male song and female plumage in two ways. First, we compared the plumage type of the females to the song type of their mates as defined by the component phrases of the songs. The types of song phrases sung by the male mate and the plumage of the female in the 61 pairings did not combine randomly (Table 2;  $G_4 = 10.9$ ,  $P < 0.05$ ). Lazuli females preferred males singing hybrid-phrase songs and discriminated against indigo-phrase songs. Hybrid females preferred males singing lazuli-phrase songs, and indigo females were mated to males singing indigo-phrase songs more often than expected in random mating. Second, we examined the relationship between female plumage and male song by considering the temporal pattern of the song as defining its type as lazuli, hybrid, or indigo. Analysis of the 61 matings showed that these traits combined randomly (Table 2;  $G_4 = 6.4$ ,  $P > 0.1$ ).

## STRENGTH OF ASSOCIATIONS: FEMALE PLUMAGE AND MALE TRAITS

The comparison of the plumage, song phrase, and song temporal pattern of the males with the plumage of their mates indicated that male plumage and male phrase traits formed significant associations with female plumage. To determine the relative statistical importance of these two male traits in explaining mating associations, we used a 3-way log-linear model (Fienberg 1977; PROC CATMOD, SAS Institute 1997). The model that provided a good fit to the data of the 3-way table (female plumage  $\times$  male plumage  $\times$  male phrase type) had no significant 3-factor interaction ( $G_3 = 1.92$ ,  $P > 0.58$ ), and this term was dropped from the model. With the female plumage  $\times$  male plumage term already in the model, adding the female plumage  $\times$  male phrase term did not explain a significant additional amount of variation ( $G_2 = 3.09$ ,  $P > 0.1$ ). Conversely, with the female plumage  $\times$  male phrase term already in the model, adding the female plumage  $\times$  male plumage term explained a significant additional amount of variation ( $G_3 = 8.47$ ,  $P < 0.05$ ).

We also ran a 3-way log-linear model (PROC CATMOD, SAS Institute 1997) on a reduced data set of 30 matings by excluding cases involving hybrid categories of female plumage, male plumage, and song phrase. The effect of adding either male plumage or male song phrase individually to the null model was significant (plumage:  $G_1 = 10.3$ ,  $P < 0.005$ ; phrase:  $G_1 = 6.0$ ,  $P < 0.03$ ). With male plumage already in the model, however, adding the male phrase term did not significantly reduce the variation ( $G_1 = 0.9$ ,  $P > 0.4$ ), whereas with the phrase term already in the model, adding the plumage term significantly reduced the variation ( $G_1 = 5.1$ ,  $P < 0.03$ ). Thus, both these analyses support the conclusion that mating associations are better predicted by male plumage traits than by phrase characteristics of the male's song.

## REPRODUCTIVE SUCCESS IN RELATION TO PLUMAGE TYPES OF MATES

We used three measures of reproductive success for each mated pair: the presence of eggs in the nest, the presence of at least one nestling, and the production of at least one fledgling. We examined first the reproductive success of pure matings (lazuli  $\times$  lazuli, indigo  $\times$  indigo) compared to mixed matings (lazuli  $\times$  indigo) at three

TABLE 3. Reproductive success (percent success in parentheses) of mating associations among Lazuli and Indigo Buntings and their hybrids.

Mating association	No. pairs	Number of pairs producing		
		Eggs	Nestlings	Fledglings
Lazuli $\times$ Lazuli and Indigo $\times$ Indigo	31	31 (100)	25 (81)	17 (55)
Lazuli $\times$ Indigo	6	5 (83)	4 (67)	4 (67)
$G^a$			0.46	0.27
Neither parent hybrid	37	36 (97)	29 (78)	21 (57)
Male parent hybrid	16	14 (88)	10 (63)	4 (25)
Female parent hybrid	6	5 (83)	1 (17)	1 (17)
$G^b$		2.00	8.36*	6.55*

<sup>a</sup>  $G$ -test comparing proportion of pairs in the two mating categories that produced nestlings and fledglings.

<sup>b</sup>  $G$ -test comparing proportions of pairs in the three mating categories that produced eggs, nestlings, and fledglings.

\*  $P < 0.05$ .

different phases of the nesting cycle (Table 3). Both categories of mating association had similar reproductive success whether compared at the egg phase, the nestling phase, or the fledgling phase.

These 37 matings were therefore combined as a mating association in which neither parent was hybrid and compared to two other mating associations: male only was a hybrid, and female only was a hybrid. All three of these mating associations had similar high success when compared in the egg production phase of the nesting cycle (Table 3). However, when examined at either the nestling phase or the fledgling phase, pairs in which one member was hybrid were less successful than pairs in which neither parent was hybrid. The least successful group was that in which only the female was hybrid (Table 3).

Although we did not observe extra-pair copulation, in spite of intensive behavioral observations, it is possible that extra-pair fertilization occurred in our study population. Extra-pair fertilization has been documented in eastern populations of Indigo Buntings (e.g., Westneat 1990). Lacking genetic data on parentage in our birds, we have assumed that the observed patterns of reproductive success are attributable to the mated pairs, as defined by their observed social behavior. If extra-pair fertilizations occurred in our reproductive data, we assume they were random among pairs and would not explain the differences in success of the mating types.



## DISCUSSION

### MIXED SONG PHRASES

In this mixed population of Indigo, Lazuli, and hybrid Buntings, we found that a male of any of these three plumage morphs may sing a song composed of phrases of either or both species. The ability of any bunting morph to sing phrases of both species also was documented by Emlen et al. (1975) for a sample of males ( $n = 20$ ) in a population near Chadron, Nebraska. Our laboratory studies of Lazuli Buntings, hand-raised in the presence of male Indigo Buntings acting as visual and song learning models, showed that syllables were learned from the heterospecific males (Boylan 1995). As we did not know the experiences of the males in our field study prior to their first tape recording, we could not determine whether the early rearing auditory environment was influential in the way our laboratory studies suggest.

### CHANGES IN PHRASE COMPOSITION

Because our study was of color-banded birds for a 4-year period, we also were able to document that breeding age males could change their songs within a reproductive season and from one season to the next. We found only weak evidence that changes in some male songs from one year to the next were correlated with the kind of female mate in the next year, or that the changes were correlated with the songs of new male neighbors in a subsequent year. The amount of song matching between males in the population as a whole, however, was substantial, and this suggests that male-male interactions are probably the most important explanation of altered songs. Because we recorded a number of individuals more than once within each season, we also were able to examine matching in this context. Six of 8 first-year males came to match a territorial neighbor within their first breeding season. Detailed studies of mechanisms of song development in Indigo Buntings indicate a sensitive phase for learning that extends into the first breeding season (Payne et al. 1988). A major influence on the acquisition of definitive adult song structure occurs through social learning from older conspecific males during territory establishment in the young bird's first spring. Apparently, a similar process occurs in Lazuli Buntings (Greene et al. 1996). Our results on

first-year males tend to parallel these previous studies.

In general, however, our results also suggest more prolonged plasticity in vocal learning, beyond the first breeding season, than appears to occur in earlier reports. Our data indicate song changes in adult males well beyond their first breeding season. These changes most often were of a few phrases rather than a complete replacement of elements, although the latter did occur. It seems probable that alterations in songs of adult males are socially induced, but we were unable to obtain the details of social interactions on a fine-grained time scale that would reveal this influence. Nor did we find evidence of a plastic song phase during which vocal change might occur, as has been shown in Indigo Buntings in the laboratory (Margoliash et al. 1991).

Taken together with our observations on matching by first-year males, the few data we obtained on the alteration of songs by particular males faced with new neighboring males or a new female mate lead us to the conclusion that males do not strategically alter the phrase content of their songs in response to mating opportunities. For an allopatric population of Indigo Buntings, Payne (1983) also reached the conclusion that song changes in first year males were not addressed to females. A previous laboratory experiment failed to alter songs by exposing the males to heterospecific females (Baker 1994). Even though production of heterospecific song phrases may influence mate choices by females, it seems most likely that the incorporation of heterospecific phrases into a male's song results from male-male interactions and not from female shaping.

### SONG PHRASES AND TEMPORAL PATTERNS

The relationships between male plumage and song characteristics differ somewhat depending on whether song phrases or temporal patterns are compared to plumage. Males with lazuli plumage sang songs composed of about 77% lazuli phrases, but only about 48% of these lazuli plumage males sang with a lazuli temporal pattern. Indigo plumage males were slightly different with 85% of their song composed of indigo phrases and 62% delivered with an indigo temporal pattern. Males in hybrid plumage sang songs composed of 56% lazuli phrases: about 42% of these males sang with the lazuli temporal pattern and another 42% sang with a hy-

brid temporal pattern. Thus, males of lazuli and indigo plumage phenotypes were more likely to sing phrases corresponding to their own plumage type than they were to sing with their own species temporal pattern. Hybrid males had a slight tendency to sing more lazuli phrases in either a lazuli or hybrid temporal pattern.

Overall, there appear to be no absolute constraints on what kinds of phrases a bunting of a given type of plumage can sing nor in what temporal pattern it can sing them. The more evenly-mixed phrase composition of songs of hybrid males could be viewed as an indication that there is some heritable constraint on phrase and temporal pattern learning, but it also could simply reflect the fact that such males may have experienced an early auditory environment containing both indigo and lazuli songs. Some indigo or lazuli males in our field population may have originated in populations where there were few or no songs of the alternative species and subsequently immigrated into our study population, bringing those species-typical song traits established elsewhere. Others may have originated in a mixed population.

#### MATING ASSOCIATIONS: PLUMAGE AND SONG

Mating associations judged by the plumage phenotypes of males and females showed a pattern of positive assortative mating. Although the assortment was statistically significant, there were several matings between species, mostly between indigo males and lazuli females, as well as backcrosses. Interspecific crosses constituted about 10% of the matings over the three years of the study. If we assume the morph ratios in our sample of 83 singing males are representative of the population, hybrids constitute 29% of the sample. Thus, 10% of the matings would not appear sufficient to produce the 29% of the population that exhibits hybrid plumage, assuming steady-state demography. This possible differential may suggest that some hybrid plumages were backcross progeny. Because we lack information on the numbers of genes involved in the plumage differences, it is not possible to estimate the proportion of backcross progeny that exhibit hybrid phenotypes.

Compared to our results, Emlen et al. (1975) found fewer hybrids in their samples and concluded that there was no evidence of introgression, a conclusion shared by Kroodsma (1975)

from his study in North Dakota and Montana. For our general study area, as sampled by singing males, the proportion of birds in the three plumage morph categories ( $n = 83$ : lazuli 46, hybrid 24, indigo 13) did not differ significantly ( $G_2 = 3.76$ ,  $P = 0.16$ ) from those found by Kroodsma (1975) in his North Dakota/Montana sample ( $n = 153$ : lazuli 78, hybrid 35, indigo 40). The incidence of hybrid phenotypes was 23% in the North Dakota/Montana sample and 29% in our sample from northeast Wyoming; however, these values are larger than the 6–7% hybrids found by Emlen et al. (1975), who used a pooled set of morphometric and plumage measurements for their descriptive morph ratios. It is possible that the morph ratios vary a good deal from one population to another.

In examining mating probabilities, the association of the female plumage with that of the male mate provided the strongest statistical result, whereas the kinds of phrases in the male's song did not add any significant additional explanation of the residual variation in the mating associations. This trend may imply, as was suggested by Emlen et al. (1975), that females use male plumage as the more reliable trait for mate choice, perhaps ignoring song characteristics. Baker and Baker (1990), in a laboratory assay of female sexual responsiveness to male traits, however, found that females used male plumage and song about equally in assessment. In that study, omitting hybrid phenotypes and using only indigo and lazuli males, females, and songs, subject females were tested to answer two questions. First, if the male plumage is conspecific and the song is heterospecific, what is the response relative to all-conspecific or all-heterospecific stimuli? Second, if the male plumage is heterospecific and the song is conspecific, what is the response relative to all-conspecific or all-heterospecific stimuli? The findings suggested that both of these treatments resulted in similar effects on female response, a response that was intermediate to that elicited by all-conspecific and all-heterospecific stimuli.

Considering the laboratory and field results together, and assuming external validity of the laboratory experiments, we find that both the male song phrases and male plumage are significantly associated with the plumage of the female. For the field data, male plumage and kinds of phrases sung are correlated features, but the somewhat stronger association of male plumage

with that of the female resulted, upon log-linear modeling, in the song phrase feature of the male phenotype not adding significant additional prediction of the mating associations.

We conclude that both male plumage and the phrase content of male song are good predictors of mating associations and both may play some role in mate choice decisions of females. Hence, some of the hybridization in bunting populations of the Great Plains may be caused by a female choosing a heterospecific or hybrid male because he sings a song typical of her own species. Boylan (1995) also has shown that a preference by females for heterospecific males can be induced by the auditory environment experienced in early life. Field studies of hybridization in Galapagos finches (*Geospiza*) have reached similar conclusions (Grant and Grant 1997).

Other studies of hybridization between congeneric species pairs in the Great Plains range from those in which assortative mating by plumage phenotypes was clearly demonstrated (orioles, *Icterus galbula*, *I. bullockii*, Edinger 1985) to those that found no evidence of assortative mating (Northern Flicker, *Colaptes auratus*, Moore 1987). In the oriole case, despite Baltimore (*I. galbula*) and Bullocks (*I. bullockii*) morphs exhibiting interspecific territoriality, there is almost no sharing of song components between the two in sympatry (Edinger 1985). This may seem surprising given that some neighboring Baltimore Oriole males match songs (Beletsky 1982, Edinger 1985). Compared to the buntings, it appears that the orioles are more constrained to learn conspecific song elements than are the buntings. In the orioles, there is a stronger association between male plumage and conspecific song features than there is in the buntings.

#### MATING ASSOCIATIONS: REPRODUCTIVE SUCCESS

We found that matings between conspecifics and matings between heterospecifics did not differ by our measures of nestling and fledgling production. A significant loss in reproductive success was found for matings involving hybrids, however, and those matings in which the female was hybrid were especially unsuccessful. Thus, the depressed fertility in female hybrid buntings may represent a case of Haldane's Rule (Haldane 1922) of low hybrid fitness in the heterogametic sex. Similar results have been observed

in natural populations of hybridizing Collared and Pied Flycatchers (*Ficedula albicollis*, *F. hypoleuca*, respectively; Tegelström and Gelter 1990), and low fitness in female hybrids has been documented in Carrion and Hooded Crows (*Corvus corone corone* and *C. c. cornix*, respectively; Saino and Villa 1992).

#### CONCLUSIONS

Culturally acquired male song features that play a role in female mate choice present an interesting evolutionary problem when secondary contact occurs between formerly isolated populations. In the bunting case examined here, the major divergence in plumage traits and the lowered fitness of hybrids suggest gene pool divergence of the two species, echoing the conclusion of Emlen et al. (1975). However, it appears that the mechanisms of female choice of mate did not, or perhaps cannot, evolve to the extent that females ignore male singing behavior and base preferences entirely upon conspecific plumage traits. Thus, there appears to be no absolute brake on the occurrence of hybridization between Lazuli and Indigo Buntings.

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