

Incomplete song divergence between recently diverged taxa: syllable sharing by Orchard and Fuertes' orioles

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ABSTRACT. Orchard Orioles (*Icterus spurius*) and Fuertes' Orioles (*I. fuertesi*) recently diverged from each other, making them an ideal system for investigating trait evolution and mechanisms of reproductive isolation during the early stages of speciation. These taxa differ in adult male plumage coloration and in their breeding and wintering ranges, but quantitative comparisons of their song characteristics have revealed no discernible differences. We assessed evolutionary song divergence in this group by investigating patterns of syllable-type sharing within and between populations. Of 529 distinct syllable types, 142 (26.8%) were shared among individuals, and sharing appeared to decrease with geographic distance. The total number of syllables shared between Orchard and Fuertes' orioles (26; 4.9% of the total) was similar to levels of sharing between populations of Orchard Orioles. Furthermore, hierarchical cluster analyses showed individuals of the two taxa intermixed. Syllables also used as calls were shared more frequently within and between taxa, suggesting that they have evolved more slowly than those used exclusively in songs. Our results show that at least some aspects of song have not yet diverged between these incipient species, either due to cultural exchange or because songs have evolved relatively slowly compared to plumage colors.

RESUMEN. La divergencia incompleta de canciones entre dos taxones que recientemente divergieron: sílabas compartidas por *Icterus spurius* e *I. fuertesi*

Los *Icterus spurius* e *I. fuertesi* divergieron recientemente, lo cual los convierte en un sistema ideal para investigar la evolución de caracteres, y los mecanismos de aislamiento reproductivo durante las primeras etapas de la especiación. Estos taxones difieren en la coloración del plumaje del macho adulto y en sus rangos de reproducción e invernada, pero las comparaciones cuantitativas de las características de sus canciones han demostrado ninguna diferencia discernible. Evaluamos la divergencia evolutiva de las canciones de este grupo mediante la investigación de los patrones del tipo de sílabas compartidas dentro y entre poblaciones. De 529 tipos de sílabas distintas, 142 (26,8%) fueron compartidas entre individuos, y el nivel en la cual comparten estas sílabas parece disminuir con la distancia geográfica. El número total de sílabas compartidas entre *I. spurius* e *I. fuertesi* (26, 4,9% del total) fue similar al nivel en la cual compartieron sílabas las poblaciones de *I. spurius*. Además, el análisis de conglomerados jerárquico demostró que individuos de los dos taxones entremezclaron. Las sílabas también utilizadas como llamadas fueron compartidos con más frecuencia dentro y entre los taxones, lo que sugiere que han evolucionado más lentamente que los que se utilizan exclusivamente en las canciones. Nuestros resultados demuestran que al menos algunos aspectos de la canción aún no han divergido entre estas especies incipientes, ya sea debido al intercambio cultural o porque las canciones han evolucionado de manera relativamente lenta en comparación con los colores del plumaje.

Key words: mating signals, reproductive isolation, song evolution, speciation, syllable types

Bird song functions in mate choice, territorial defense, and species recognition (Collins 2004, Catchpole and Slater 2008). Consequently, songs are thought to play an important role in the process of reproductive isolation during early speciation (Slabbekoorn and Smith 2002, Lachlan and Servedio 2004, Seddon 2005, Price 2008, Seddon et al. 2008). Investigators have used song characteristics to better understand and delineate taxonomic boundaries in a wide variety of avian taxa (Helbig et al. 1996,

Irwin 2000, Balakrishnan and Sorensen 2006, Seddon and Tobias 2007, Brambilla et al. 2008, Fernández-Juricic et al. 2009, Uy et al. 2009). Yet, little is known about the process of vocal divergence during speciation and the possible role that such behavioral traits may play in the formation of species boundaries.

Song characteristics are presumed to evolve rapidly, either as a consequence of sexual selection (Collins 2004, Price and Lanyon 2004) or in response to differences in the social or physical environments where singing takes place (Wiley and Richards 1978, Slabbekoorn 2004). Among species with learned vocalizations, such

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as oscine passerines, songs can change especially rapidly through cultural evolution (Lachlan and Servedio 2004, Derryberry 2007, Luther and Baptista 2009, Price 2010). Song features may also change over time as a consequence of errors during learning, resulting in cultural drift (Lynch 1996, Price 1998). Any of these mechanisms may result in geographic divergence of songs, which can include the formation of regional dialects within species (Podos and Warren 2007).

Orchard (*Icterus spurius*) and Fuertes' (*I. fuertesi*) orioles provide an ideal system for investigating trait evolution during early speciation. These closely related songbirds diverged <200,000 years ago and, based on molecular analyses, exhibit little or no interbreeding (Baker et al. 2003). In a comparison of mitochondrial DNA sequence data, Baker et al. (2003) showed that some Orchard and Fuertes' orioles share mtDNA haplotypes, indicating that these taxa are not reciprocally monophyletic, likely due to incomplete lineage sorting since divergence. However, they do exhibit significant differences in haplotype frequencies, indicating little or no gene flow (Baker et al. 2003). These species have similar breeding behaviors, broadly overlapping morphological measurements, and similar female and subadult male colorations (Graber and Graber 1954, Scharf and Kren 1996, Jaramillo and Burke 1999). However, they differ in adult male plumage coloration, with no overlap in measures of color variation (Hofmann et al. 2007, Kiere et al. 2007). Adult male Orchard Orioles are chestnut and black, whereas adult male Fuertes' Orioles have lighter ochre plumage rather than chestnut coloration (Graber and Graber 1954, Howell and Webb 1995). In both species, subadult (second year, or SY) males have different plumage than adult (after second year, or ASY) males (Jaramillo and Burke 1999, Hofmann et al. 2007).

Previous authors have described the songs of Fuertes' Orioles as "less rich and loud" (Chapman 1911) or "softer and less brilliant" (Graber and Graber 1954) than the songs of Orchard Orioles, and this was one of the initial reasons for describing them as separate species (Chapman 1911). However, a recent quantitative comparison of song characteristics of these taxa, using 16 song measurements also used by Price et al. (2007) to compare the songs of other species in the genus *Icterus*, revealed no

statistically significant differences between them (Sturge, unpubl. data). The songs of Orchard and Fuertes orioles do not differ significantly in duration, the duration and rate of delivery of syllables, number of syllable types per song, and a variety of frequency measures (Sturge, unpubl. data).

Songs may differ in ways not captured by such quantitative measurements, however, including the fine-scale morphology of syllables. Although syllables are generally transmitted culturally (Catchpole and Slater 2008), syllable types can persist for many years in populations and can reveal past interactions and patterns of relationship (Jenkins 1978, Payne and Payne 1993, Ficken and Popp 1995, Lynch 1996, Price 1998). Moreover, syllable sharing between populations may provide an important tool for investigating historical patterns of divergence or intermixing (Podos and Warren 2007). However, to our knowledge, no one to date has examined patterns of syllable-type sharing between recently diverged species.

We investigated potential song divergence between Orchard and Fuertes' orioles by comparing patterns of song syllable-type sharing within and between populations, using the same songs compared previously (Sturge, unpubl. data). Syllable-type sharing within, but not between, these taxa would indicate vocal divergence, whereas significant sharing across taxonomic lines would indicate either continued cultural exchange since divergence or a relatively slow rate of vocal evolution compared to changes in plumage.

METHODS

Study taxa. Orchard Orioles and Fuertes' Orioles have alternately been described as distinct species (*Icterus spurius* and *I. fuertesi*; Chapman 1911, Clements 2007) or as subspecies (*I. s. spurius* and *I. s. fuertesi*; Blake 1953, Graber and Graber 1954, Howell and Webb 1995, AOU 1998). Here we treat them as recently diverged species, following recent recommendations based on genetic and phenotypic evidence (Baker et al. 2003, Clements 2007, Hofmann et al. 2007, Kiere et al. 2007).

In addition to plumage differences, Orchard and Fuertes' orioles have different breeding and wintering ranges (Fig. 1) and also differ in migratory behavior. Orchard Orioles breed



Fig. 1. Recording locations for Orchard Orioles (filled circles) and Fuertes' Orioles (open circles) across their ranges in eastern North America. The breeding range of Orchard Orioles is indicated in light gray and the breeding range of Fuertes' Orioles in dark gray (adapted from Baker et al. 2003). Inset: Locations where Orchard Orioles were recorded in Maryland and Delaware.

across most of the eastern United States and the central plains of Mexico, and winter from southern Mexico to northern South America (Scharf and Kren 1996, Jaramillo and Burke 1999). Some populations are itinerant breeders, migrating to western Mexico to raise a second brood before wintering (Rohwer et al. 2009). Fuertes' Orioles have a more restricted breeding range in eastern Mexico, including most of coastal Veracruz and southern Tamaulipas, and apparently migrate southwest to Mexico's Pacific coast (Jaramillo and Burke 1999). Vagrant Fuertes' Orioles have occasionally been found as far north as Texas (Dickerman 1964). Although the breeding grounds of these two taxa are disjunct, Orchard Orioles are known to migrate through the range of the Fuertes' Oriole and can sometimes be heard singing in those areas during migration (Graber and Graber 1954). Indeed, Chapman (1911) collected a male Orchard Oriole in early April

when he collected the type series for Fuertes' Oriole. Thus, although there is no evidence of interbreeding (Baker et al. 2003), Orchard and Fuertes' orioles presumably come into contact during migration or due to occasional vagrants.

Song recording. We recorded the vocalizations of 17 Orchard Orioles in Maryland and Delaware, USA, and 13 Fuertes' Orioles in Veracruz, Mexico, during the breeding seasons (April–July) of 2008 and 2009 (Table 1). All recordings were made at a sampling rate of 48 kHz using a digital recorder (Model PMD670, Marantz, Sagami-hara, Japan) with an omnidirectional microphone (Model ME62 with K6 power supply, Sennheiser Electronic Corporation, Wennebostel, Germany) in a parabolic dish (Telinga Microphones, Tobo, Sweden). We also obtained recordings from an additional 10 Orchard Orioles recorded between 1954 and 1998 from the Macaulay Library of Natural Sounds at Cornell University (Table 1).

Table 1. Recordings of the songs of male Fuertes' Orioles (*Icterus fuertesi*) and Orchard Orioles (*I. spurius*) used in our study.

Species	Age ^a	Recording source and date
<i>Icterus fuertesi</i>	SY	R. J. Sturge, Tlacotalpan, Veracruz, Mexico, 31 May 2009
<i>Icterus fuertesi</i>	SY	R. J. Sturge, Tamiahua, Veracruz, Mexico, 5 June 2009
<i>Icterus fuertesi</i>	SY	R. J. Sturge, Tlacotalpan, Veracruz, Mexico, 1 June 2009
<i>Icterus fuertesi</i>	SY	R. J. Sturge, Las Barrancas, Veracruz, Mexico, 9 June 2008
<i>Icterus fuertesi</i>	SY	R. J. Sturge, Tecolutla, Veracruz, Mexico, 4 June 2008
<i>Icterus fuertesi</i>	ASY	R. J. Sturge, Tlacotalpan, Veracruz, Mexico, 31 May 2009
<i>Icterus fuertesi</i>	ASY	R. J. Sturge, Las Barrancas, Veracruz, Mexico, 30 May 2008
<i>Icterus fuertesi</i>	ASY	R. J. Sturge, Tamiahua, Veracruz, Mexico, 6 June 2009
<i>Icterus fuertesi</i>	ASY	R. J. Sturge, Tamiahua, Veracruz, Mexico, 6 June 2009
<i>Icterus fuertesi</i>	ASY	R. J. Sturge, Tuxpan, Veracruz, Mexico, 4 June 2009
<i>Icterus fuertesi</i>	ASY	R. J. Sturge, Tlacotalpan, Veracruz, Mexico, 2 June 2009
<i>Icterus fuertesi</i>	ASY	R. J. Sturge, Tlacotalpan, Veracruz, Mexico, 1 June 2009
<i>Icterus fuertesi</i>	ASY	R. J. Sturge, Las Barrancas, Veracruz, Mexico, 9 June 2009
<i>Icterus spurius</i>	SY	N. D. Hagemeyer, Bombay Hook Nat. Wild. Ref., DE, USA, 27 June 2009
<i>Icterus spurius</i>	SY	R. J. Sturge, Blackwater Wildlife Refuge, MD, USA, 21 June 2008
<i>Icterus spurius</i>	SY	R. J. Sturge, Blackwater Wildlife Refuge, MD, USA, 20 June 2008
<i>Icterus spurius</i>	SY	R. J. Sturge, Blackwater Wildlife Refuge, MD, USA, 20 June 2008
<i>Icterus spurius</i>	SY	R. J. Sturge, Centennial Park, Columbia, MD, USA, 9 May 2009
<i>Icterus spurius</i>	SY	R. J. Sturge, Ellis Bay Wildlife Management Area, MD, USA, 1 July 2008
<i>Icterus spurius</i>	SY	R. J. Sturge, Ellis Bay Wildlife Management Area, MD, USA, 1 July 2008
<i>Icterus spurius</i>	SY	R. J. Sturge, Univ. Maryland Baltimore County, MD, USA, 4 May 2008
<i>Icterus spurius</i>	ASY	R. J. Sturge, Halethorpe Farm Ponds, MD, USA, 29 June 2008
<i>Icterus spurius</i>	ASY	N. D. Hagemeyer, Bombay Hook Nat. Wild. Ref., DE, USA, 27 June 2009
<i>Icterus spurius</i>	ASY	R. J. Sturge, Blackwater Wildlife Refuge, MD, USA, 20 June 2008
<i>Icterus spurius</i>	ASY	R. J. Sturge, Blackwater Wildlife Refuge, MD, USA, 20 June 2008
<i>Icterus spurius</i>	ASY	R. J. Sturge, Blackwater Wildlife Refuge, MD, USA, 20 June 2008
<i>Icterus spurius</i>	ASY	R. J. Sturge, Blackwater Wildlife Refuge, MD, USA, 20 June 2008
<i>Icterus spurius</i>	ASY	N. D. Hagemeyer, Kinder Farm Park, Columbia, MD, USA, 8 June 2009
<i>Icterus spurius</i>	ASY	N. D. Hagemeyer, Jug Bay Wetlands Sanctuary, MD, USA, 2 June 2009
<i>Icterus spurius</i>	ASY	R. J. Sturge, Ellis Bay Wild. Mgmt. Area, MD, USA, 1 July 2008
<i>Icterus spurius</i>	ASY	R. J. Sturge, Ellis Bay Wild. Mgmt. Area, MD, USA, 1 July 2008
<i>Icterus spurius</i>	SY	Arthur A. Allen, Adams County, OH, USA, 18 May 1954 ^b
<i>Icterus spurius</i>	SY	Robert C. Stein, NE, USA, 20 June 1961 ^b
<i>Icterus spurius</i>	SY	Curtis Marantz, Cayuga County, NY, USA, 17 May 1998 ^b
<i>Icterus spurius</i>	ASY	Mark Robbins, Nodoway County, MO, USA, 14 May 1991 ^b
<i>Icterus spurius</i>	ASY	Geoffrey Keller, Apalachicola National Forest, FL, USA, 30 April 1992 ^b
<i>Icterus spurius</i>	ASY	Geoffrey Keller, Charleston County, SC, USA, 2 June 1994 ^b
<i>Icterus spurius</i>	ASY	Arthur A. Allen, Elsa, IL, USA, 22 May 1954 ^b
<i>Icterus spurius</i>	ASY	William W. H. Gunn, Point Pelee, Ont., Canada, 11 May 1954 ^b
<i>Icterus spurius</i>	Unk	Geoffrey A. Keller, Lost Maples Rec. Area, TX, USA, 4 May 1986 ^b
<i>Icterus spurius</i>	Unk	Theodore A. Parker III, Baton Rouge, LA, USA, 26 May 1988 ^b

^aSY = second year, ASY = after second year, and Unk = unknown age.

^bRecordings obtained from the Macaulay Library of Natural Sounds, Cornell University.

Although song features can change over such time periods (Derryberry 2007), examination of these recordings suggested that syllable contents of songs did not differ between years any more than between locations. Our sampling covered the breeding range of Orchard Orioles across the United States and southern Canada (no recordings were available from northern Mexico) and across the entire breeding range of Fuertes' Orioles (Fig. 1).

We noted the age (subadult/SY or adult/ASY) of each recorded singer. Overall, we recorded eight subadult and nine adult Orchard Orioles and five subadult and eight adult Fuertes Orioles. The Macaulay Library recordings of Orchard Orioles included three subadults, five adults, and two individuals of unknown age (Table 1). Although female Orchard Orioles occasionally sing (Scharf and Kren 1996), only male songs were included in our study. We did not mark birds for individual identification. However, we recorded songs of more than one singer in an area only when they were observed >200 m apart (greater than the width of two typical territories) or if they were both clearly visible for the duration of the recordings. For Orchard Orioles with no close territorial neighbors, some recordings were made at the same location over multiple days and attributed to the same individual. We avoided using recordings from the same location in different years.

Syllable-type scoring. We generated spectrograms of oriole songs using Raven Pro 1.3 (Cornell Lab of Ornithology, Ithaca, NY; frequency resolution = 135 Hz; time resolution = 10.7 ms). Following previous authors, we defined continuous sound traces on spectrograms as notes and defined syllables as notes or groups of notes that always had the same frequency, duration, and morphology (e.g., Marler 2004, Catchpole and Slater 2008). Notes separated by > 0.01 s were classified as different syllables, and syllables < 0.5 s apart were considered parts of the same song (also see Price et al. 2007, 2008). As with some other oriole species (e.g., Price et al. 2008), both Orchard and Fuertes' Orioles have some syllables used both during territorial singing bouts and while calling in other contexts (see below). We defined songs as vocalizations that included four or more syllables, based on observations of typical singing behavior in the field. Single syllables preceded and followed by > 0.5 s intervals, as well as rare

cases where two or three syllables were produced < 0.5 s apart, were defined as calls and were not included in our study.

We classified syllables into distinct types based on comparisons of acoustic measurements in spectrograms (also see Price and Lanyon 2004). Syllables were classified as the same type only if they were consistently similar in duration, frequency range, starting and finishing frequencies, frequencies at inflection points, and duration of any internal notes. A subset of syllables was independently scored by NDH and RJS to ensure agreement and consistency in our syllable classifications. Most syllable types (80.3%) consisted of a single whistled note or a group of such notes (Fig. 2A–C). Other syllables had harmonics or included rapid frequency modulation (Fig. 2D–E). These latter syllables were also often encountered as calls in our study, and were therefore referred to as “call-type” syllables. The former, whistled syllable types were only used in songs and were therefore referred to as “song-type” syllables. In all, we initially identified 570 distinct syllable types.

Syllable sharing. Our analyses focused on comparing the syllable compositions of songs within and between taxa. In general, our criteria for classifying syllables as the same type were strict, and thus our measurements of syllable-type sharing between individuals were relatively conservative. For initial analyses, we used every recorded song from each individual. Preliminary k-means cluster analyses of these songs in R (R Development Core Team 2011) showed that the songs of each individual almost always clustered together exclusively. Three Orchard Orioles from Blackwater National Wildlife Refuge in Maryland were an exception because their syllables intermixed with each other. K-means clustering correctly assigned songs to individuals 87.1% of the time, and differences between the songs of individuals were generally far smaller than differences between individuals. Thus, for further analysis, we used hierarchical cluster analysis using four randomly selected songs from each oriole (or fewer when four clear recordings were not available; mean = 3.5, SE = 0.1), which sorted individuals based on the presence or absence of 529 distinct syllable types. Hierarchical cluster analyses using a similarity matrix were conducted using all syllables, only call-type syllables ($N = 104$), and only song-type syllables ($N = 425$). We also compared the

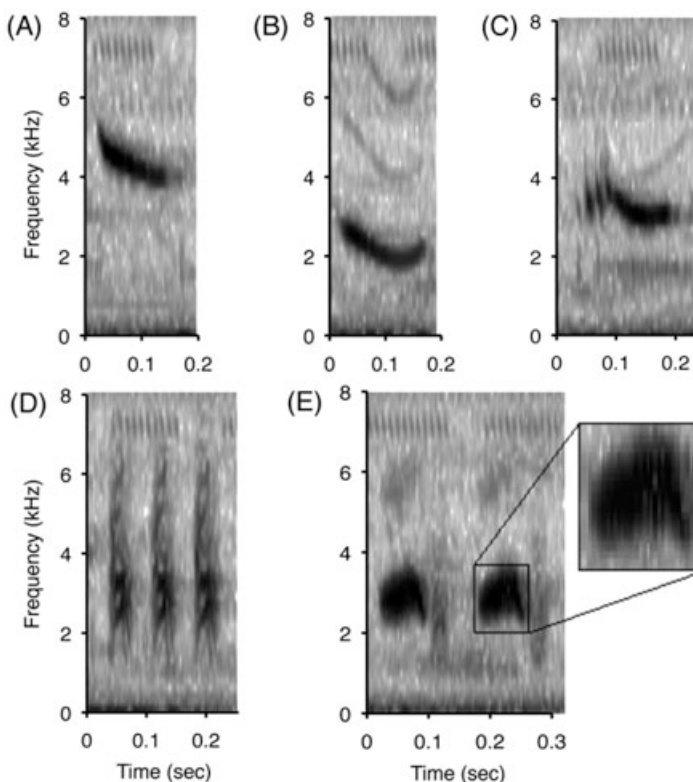


Fig. 2. Sound spectrograms showing examples of syllable types: (A and B) syllables comprising single whistled notes, (C) a syllable including multiple whistled notes, (D) syllables with visible harmonics, and (E) buzzed syllables with rapid frequency modulation (magnified in inset). Top syllables (A–C) are song-type syllables that occurred only in songs, whereas the bottom syllables (D and E) are call-type syllables that sometimes also occurred as calls. These examples are from a single Orchard Oriole song, but syllables with these characteristics occurred in both taxa.

number of syllable types per song and per individual between species and between age classes within each species using two-tailed *t*-tests. Hierarchical cluster analyses and *t*-tests were performed using SPSS (version 18.0, PASW Statistics, Chicago, IL), and differences were considered significant for *P* values < 0.05. Values are presented as means \pm SE.

RESULTS

Unrooted hierarchical cluster analyses using all syllable types generated a dendrogram with Orchard and Fuertes' Orioles intermixed (Fig. 3). Birds from different geographic regions and recorded in different years were also intermixed. However, when the hierarchical cluster analysis was completed using only call-type

syllables, correct cluster assignment increased to 79.3% for Orchard Orioles and 84.6% for Fuertes' Orioles, and correct assignment decreased when only song-type syllables were used.

Of the 529 syllable types included in the study, 142 (26.8%) occurred in the repertoires of more than one individual and 26 (4.9%) were shared between Orchard and Fuertes' Orioles. Levels of syllable-type sharing between oriole taxa were not unlike the patterns of sharing across other parts of their ranges, which was highest in local populations and decreased with geographic distance. For example, 33.1% (49/148) of Fuertes' Oriole syllables were shared within that species, similar to the 34.1% (84/246) of syllable types shared within a similarly sized geographic range of Orchard Orioles in Maryland and Delaware (MD/DE,

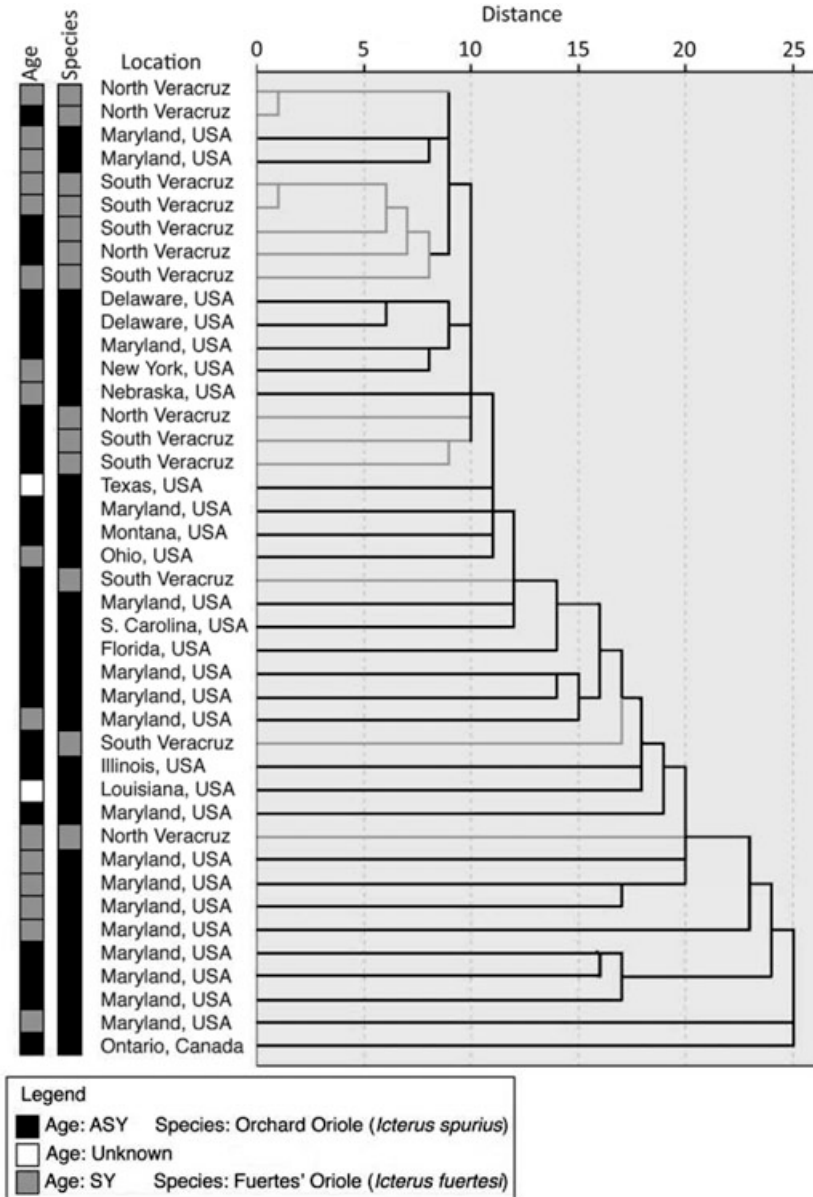


Fig. 3. Hierarchical cluster analysis dendrogram based on the presence or absence of 529 syllable types among SY (second-year) and ASY (after second year) Orchard Orioles ($N = 29$) and Fiertes' Orioles ($N = 13$). Distance between individuals on the tree reflects fusion values based on the presence or absence of syllable types in each bird's songs. Individuals from the two oriole taxa were intermixed.

Fig. 1). Only 3.2% (13/407) of syllable types were shared between MD/DE birds and other Orchard Orioles and, similarly, only 3.5% (11/311) of syllable types were shared between those same non-MD/DE Orchard Orioles and Fiertes' Orioles. The two most thoroughly sam-

pled populations, Fiertes' Orioles and MD/DE Orchard Orioles, shared 4.0% (15/379) of their syllable types. Thus, overall, we found no apparent discontinuities in syllable-type sharing across the combined breeding ranges of these species.

Although call-type syllables made up only a small proportion (19.7%) of the total syllables identified, they were almost twice as likely to be shared among birds; 43.3% (45/104) of call-type syllables were shared whereas only 22.8% (97/425) of song-type syllables were shared. Most (69.2%) of the 26 syllables shared between species were also call-type syllables.

Mean numbers of syllable types per song did not differ between Fuertes' Orioles (11.7 ± 0.4) and Orchard Orioles (11.8 ± 0.3 ; $t_{144} = 0.2$, $P = 0.84$). Similarly, the number of syllable types recorded from each individual did not differ between Fuertes' Orioles (16.2 ± 1.4) and MD/DE Orchard Orioles (19.9 ± 1.5 ; $t_{30} = 1.8$, $P = 0.084$), despite the fact that we recorded some Orchard Orioles ($N = 4$) over multiple days. We also found no effect of male age on the number of syllable types in songs for either Fuertes' Orioles (SY = 11.4 ± 0.7 syllables/song; ASY = 11.8 ± 0.4 syllables/song; $t_{41} = 0.5$, $P = 0.64$) or Orchard Orioles (SY = 11.0 ± 0.5 syllables/song; ASY = 11.7 ± 0.6 syllables/song; $t_{66} = 0.9$, $P = 0.39$). None of the cluster analyses provided evidence of groupings based on age class (Fig. 3).

DISCUSSION

Our results suggest that syllables in the songs of Orchard and Fuertes' orioles have not diverged from each other since the initial separation of these species. Patterns of syllable-type sharing between species were not appreciably different from those within species, and cluster analyses based on syllable sharing showed that individuals of the two taxa intermixed. We also found no differences between species in the number of syllable types per song or produced by individual birds over time, indicating that these orioles organize their song syllables in similar ways during singing bouts. Our results add to previous quantitative comparisons of the songs of Orchard and Fuertes' orioles, which showed no significant differences in a variety of frequency and temporal measurements (Price et al. 2007, Sturge, unpubl. data).

Clawson (1980) found that the songs of subadult (SY) and adult (ASY) male Orchard Orioles differed significantly in their temporal and frequency characteristics, and that females could discriminate between subadult and adult males based on their songs. However, we found

no significant differences between age classes in the number of syllable types per song for either Orchard Orioles or Fuertes' Orioles, suggesting that syllable types might be learned early in life and remain unchanged thereafter.

Given that Orchard and Fuertes' orioles may regularly come into contact during migration and due to occasional vagrants (Chapman 1911, Dickerman 1964), syllable-type sharing may be a result of cultural transmission between species. With the exception of a few songbird groups (e.g., mimids and sturnids), most songbirds do not learn their song syllables from other taxa (Catchpole and Slater 2008). However, cases of interspecific syllable learning have been reported in wild populations of some species (e.g., Eberhardt and Baptista 1977, Adkisson and Conner 1978, Payne et al. 1984), and such learning may be especially likely among closely related species that have similar songs and are known to hybridize (Baker and Boylan 1999, Severinghaus et al. 2006, Price 2010). Although there is no evidence of hybridization between Orchard and Fuertes' Orioles (Baker et al. 2003), several factors may nevertheless foster cultural transmission between them, including similarities in their song characteristics (Sturge, unpubl. data), broadly overlapping morphological features (Graber and Graber 1954), and regular contact outside of the breeding season (Chapman 1911).

An alternative possibility is that these shared vocalizations represent the retention of ancestral syllable types that have persisted since the divergence of Orchard and Fuertes' Orioles, much like the retention of DNA haplotypes due to incomplete lineage sorting (Lynch 1996, Baker et al. 2003). These retained vocal patterns may in turn reflect retention of ancestral learning or production biases (Podos and Warren 2007). The syllables most frequently shared between birds in our study, call-type syllables, were also the vocalizations that most clearly assorted according to species and geographic region in hierarchical cluster analyses. Call-type syllables were nearly twice as likely to be shared as song-type syllables, both within and between species, suggesting that these components of song are relatively stable across broad geographic areas and have been less subject to evolutionary change during the history of these birds. Indeed, given the estimated time since divergence (Baker et al. 2003), our data suggest that call-type

syllables may have remained unchanged for perhaps thousands of years.

Syllable sharing between male Orchard and Fuertes' Orioles is especially interesting given that they exhibit clear differences in adult male coloration (Jaramillo and Burke 1999, Hofmann et al. 2007, Kiere et al. 2007). Kiere et al. (2007) found no overlap between taxa in a quantitative spectral analysis of color variation, and Hofmann et al. (2007) found significant differences in pigment concentrations, presumably reflecting fixed genetic differences. Thus, song syllables appear to have diverged more slowly than male plumage colors. Both songs and plumage colors presumably function as mating signals and each therefore has a potentially important role in premating reproductive isolation (Price 2008). At least one previous study has suggested that plumage colors play a stronger role in species recognition than song among incipient species (i.e., *Monarcha* flycatchers, Uy et al. 2009), and our results are consistent with this idea. However, detailed field experiments with Orchard and Fuertes' Orioles, including both song playbacks and mount presentations within and between species (Uy et al. 2009), are needed to further investigate this possibility.

Our study provides intriguing evidence that some characteristics of song may diverge more slowly than often assumed (e.g., Slabbekoorn and Smith 2002, Lachlan and Servedio 2004, Seddon and Tobias 2007, Derryberry 2007, but see Laiolo 2012). Our results also highlight the importance of assessing multiple traits and exploring rates of trait divergence in defining species boundaries. Songs are frequently used to delineate taxonomic boundaries in birds (Price 2008), but the songs of Orchard and Fuertes' Orioles show few consistent differences in their syllable contents despite clear evidence that these species are genetically distinct (Baker et al. 2003). Moreover, just as different plumage regions and pigment types appear to diverge at different rates (Hofmann et al. 2007), some types of syllables may evolve more gradually than others.

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