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S01-2 Breaking down reproductive isolation between closely related species

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Abstract One of the functions of bird song is its use in species identification, which contributes to the maintenance of reproductive isolation between closely related species (Welty, 1975). For species that can modify their vocalizations, the isolating function of song difference sometimes breaks down. Formerly allopatric Chinese bulbuls (*Pycnonotus sinensis*) and Styan's bulbuls (*P. taivanus*) live under similar ecological conditions on Taiwan and have songs that resemble one another. In the 1950s they came into secondary contact and the two species hybridized. This study analyzes the vocalizations of these taxa recorded from both allopatric and sympatric populations, and demonstrates vocal modification in sympatric populations.

Key words Reproductive isolation, Hybrid zone, Vocal types, Bulbuls, *Pycnonotus sinensis*, *Pycnonotus taivanus*

1 Introduction

Bird songs are signals commonly used in intra- and inter-sexual communication. Because songs can be heard at a distance, they are effective and efficient cues for species identification. Many studies have demonstrated that birds rely on vocal signals in territorial defense and in mate selection. For example, *Streptopelia* doves react more strongly to playback of congeneric than heterogeneric doves, and more to allopatric than sympatric members of species pairs (de Kort and ten Cate, 2001). Even among subspecies, song barriers have been found, as in the chiffchaff (*Phylloscopus collybita*) (Salomon, 1989). Differences in vocalization between species are powerful isolating mechanisms (Martens, 1996), and many studies have focused on the role of bird song in promoting speciation (citations in Slabbekoorn and Smith, 2002). In contrast, this paper discusses the role of bird song in facilitating the breakdown of species isolation.

If differences in vocalization between species function as isolating mechanisms, such differences should be especially effective between closely related sympatric species where potential for hybridization is greater. Songs are often found to be more similar in distantly than closely related species; closely related species with similar songs, moreover, may differ in how they use them (Smith, 1996; Jones, 1997). Thus differences in songs evidently contribute significantly to premating separation of closely related species.

Many bird species learn their songs (Marler and Peters, 1977; Kroodsma, 1982; Slater, 1983, 1986; Jarvis et al., 2000). Although many species preferentially learn the songs of their own species (Marler and Peters, 1977; Baker, 1983; Clayton, 1988; Beecher, 1996), or of their own population or their male parents (Clayton, 1988; Baptista and Schuchmann, 1990; Grant and Grant, 1997), mistakes happen and songs of another species are sometimes learned (Baptista, 1972; Baptista and Morton, 1981). This may facilitate hybridization.

Nearly 10% of bird species hybridize in nature (Grant and Grant, 1992). The vocalizations of some hybridizing pairs have been studied, including the turtle doves *Streptopelia vinacea* and *S. capicola* (de Kort et al., 2002), buntings *Passerina amoena* and *P. cyanea* (Payne, 1996; Baker and Boylan, 1999), flycatchers *Ficedula hypoleuca* and *F. albicollis* (Gelter, 1987), and Darwin's finches *Geospiza fortis*, *G. fuliginosa* and *G. scandens* (Grant and Grant, 1992). The songs of some well differentiated but hybridizing subspecies have also been documented, as in the common chiffchaff, *Phylloscopus collybita* (Salomon, 1989; Martens, 1996) and great tit, *Parus major* (Martens, 1996). In such cases, failure to discriminate between conspecific and heterospecific signals has led to hybridization (e.g. Baker and Boylan, 1999; de Kort et al., 2002).

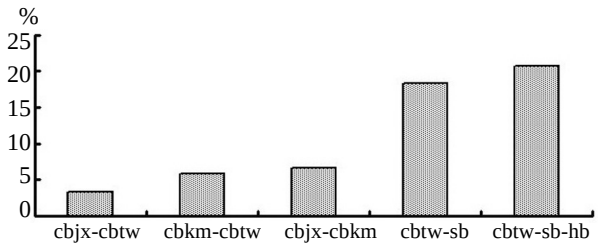

Fig. 1 Proportion (%) of syllable types shared
The closer the populations, the greater the syllable sharing. key to acronyms: cb = Chinese bulbul; sb = Styan's bulbul; hb = hybrid Chinese × Styan's bulbuls; tw = Taiwan; jx = Jiangxi, China; km = Kinmen Island.

In this paper we examine the songs of allopatric, parapatric and sympatric populations of Chinese (*Pycnonotus sinensis*) and Styan's (*P. taivanus*) bulbuls that hybridize in a zone of sympatry on Taiwan. We do so to test the hypotheses that (1) when two closely related taxa come into secondary contact, learnt songs will increase the chance that songs will contain characteristics of both parental forms; and that (2) this will, in turn, weaken the isolating function of species-specific song, so creating the initial circumstances for facilitating hybridization.

2 Methods

2.1 The taxa

Styan's bulbul is endemic to lower altitudes in eastern and southern Taiwan (Cheng, 2002). The Chinese bulbul is found in southeastern China, Hainan, northern Indochina, and on northern and western Taiwan at low to mid altitudes. Mountains in central Taiwan confine both species to the periphery of the island. There both species are common in city parks, farms, orchards, and most types of secondary vegetation. Over the last 50 years, the range of Chinese bulbul has been expanding on Taiwan into the range of Styan's bulbul, and the two taxa hybridize where they overlap.

2.2 Sound recording

A Nagra IS tape recorder coupled with a Sennheiser MKH 816 shotgun microphone were used to record the songs of Chinese and Styan's bulbuls in northern, central, southern and eastern Taiwan where the bulbuls are parapatric and sympatric. We also recorded the songs of Chinese bulbuls on Kinmen Island (24°27'N, 118°24'E) and in Jiangxi, China (28°41'N, 115°53'E) where they are allopatric with respect to Styan's bulbuls. In areas where the two bulbuls are sympatric on Taiwan, we sound recorded the songs of hybrids as well. Each song exemplar was obtained from a different individual.

2.3 Sound analyses

We produced sound spectrograms with Canary Version 1.2 software. Spectrograms of almost all bulbul songs reveal syllables that have two parts, allowing the syllables to be described by five traits: the duration of each part (2 traits), the initial frequency, the frequency of the transition point, and the terminating frequency. Frequency values were taken at the point where energy was highest, as determined by the power spectrum. We grouped all syllables into syllable types by first performing a Principle Component Analysis, and then using the values of the five trait axes in a cluster analysis to group and separate the syllable types. The following analysis of song variation is based on these types.

3 Results

We analyzed 1 731 syllables extracted from 244 bulbul songs and grouped these into 167 syllable types (Table 1). Most syllable types were rare and often unique to one population or taxon. The proportion of unique types in the three populations of Chinese bulbuls and Styan's bulbul did not differ statistically (chi-square, $P>0.05$). Twenty-eight syllable types were recorded in more than one population or taxon (Table 1). Sharing occurred generally between neighboring regions; and the proportion of syllable types shared between two populations increased with the proximity of the populations (Fig. 1). Parapatric and sympatric Chinese and Styan's bulbuls shared significantly more syllable types than allopatric populations of Chinese bulbuls (chi-square, $P<0.013$).

The majority of shared and unique syllables were rare. Chinese bulbuls had 42 unique phrases, Styan's 22, and hybrids 46. Only five shared types were very common, and these formed more than 80% of the songs of both taxa on Taiwan (types A–E; Table 2). Four of them were also found on Kinmen, and two on Jiangxi, but all were relatively rare there. On Taiwan, the frequency of the five common types differed significantly between the two taxa (chi-square, $P<0.0001$). Thus type C was more common in Chinese bulbuls and type E in Styan's (Fig. 2); hybrid (sympatric) populations had syllable frequencies similar to Styan's.

The five common syllable types also occurred at significantly different frequencies in the four regions of Taiwan (chi-square, $P<0.001$). Type A and B syllables were relatively rare everywhere, while type C was very common in northern and central Taiwan, and type E very common in

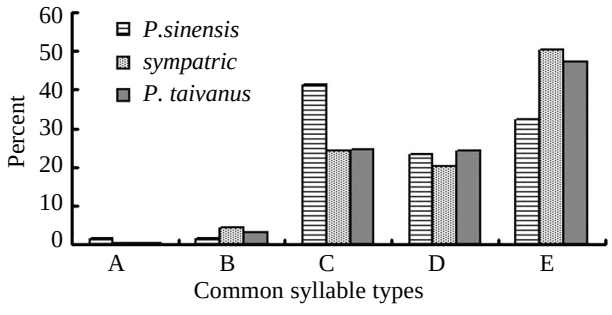

Fig. 2 Distribution of syllable types among the songs of two taxa of bulbuls and their hybrids
Sympatric populations include hybrids and parental types.

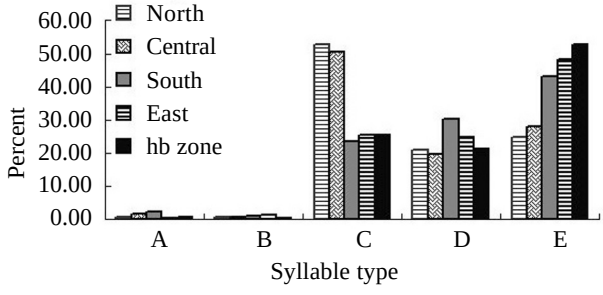

Fig. 3 Distribution of the five commonly shared syllable types (A–E) in different sectors of Taiwan
Syllable distribution in hybrid bulbuls shows a high degree of similarity to that in eastern Taiwan, which was sung only by Styan's bulbuls.

Table 1 Syllable types and sample sizes for different populations

Population*	Song no	Note no	Total syllable types	Unique types	Shared types	%
cbkm	18	92	31	23	8	25.81
cbjx	12	53	34	29	5	14.71
cbtw	82	604	60	41	19	31.67
sb	53	382	37	19	18	48.65
hb	79	600	46	27	19	41.30
sum	244	1731	169	139	28	16.57

*cbkm=Chinese bulbul from Kinmen Island, cbjx=Chinese bulbul from Jiangxi, cbtw=Chinese bulbul from Taiwan, sb=Styan's bulbul, hb=hybrids.

the south and east. Bulbul songs in Taiwan can be divided into two regional groups according to syllable frequency: the northern and central group, and the south and east group. Because only Styan's bulbul occurs on eastern Taiwan, Chinese bulbul syllables in the south are thus more similar to those of Styan's than to conspecifics elsewhere. The distribution of syllable types in hybrids was similar to that of Styan's bulbul (Fig. 3).

Some syllables often occurred in sequence to form phrases, either repeated or in combination with others. For two-syllable phrases, those containing type C syllables occurred more often in Chinese bulbul songs (52.57%). Type E syllables were most common in Styan's bulbul and hybrid phrases (47.67% and 54.79% respectively). All three populations used phrases containing type D syllables around 30% of the time. Syntax in two-syllable phrases was very similar between hybrids and Chinese bulbuls (Mantel test, CB:HB, g=7.304, P<0.0001), and between Chinese and Styan's bulbuls (Mantel test, CB:SB, g=2.283, P=0.0025); but syntax similarity between hybrid and Styan's bulbuls was not significant. For allopatric populations, the great differences in syllable types and their frequency made comparisons of syntax impossible. For three-syllable phrases, Chinese and Styan's bulbuls and their hybrids had 18 phrases in common. Chinese bulbuls and hybrids shared another 21 phrases, whereas Chinese and Styan's bulbuls shared only three more, and Styan's bulbul and hybrids only two more.

These comparisons reveal that the hybrids produce common syllables in much the same frequencies as Styan's bulbuls, but in a syntax much more like that in Chinese bulbuls.

4 Discussion

This study found that the songs of Chinese and Styan's bulbuls differ markedly between allopatric populations, but share many characteristics in parapatric populations and merge in sympatric populations. If convergence of syllable and song structure is accepted as evidence for song learning (Kroodsma, 1982; Gaunt et al., 1994), then Styan's and Chinese bulbuls on Taiwan demonstrate mutual learning and song modification. Evidence for learning comes from an avicultural report of a caged Chinese bulbul, reared among hwameis (*Garrulax canorus*), singing hwamei-type songs, as well as our captive-bred hybrid bulbuls singing unusual songs due, apparently, to their keeper talking habitually to them during their rearing.

Between related sympatric species, signals and their recognition are assumed to diverge and enhance species isolation (Miller, 1982; Saetre et al., 1997; de Kort and ten Cate, 2001). For allopatric species, however, there is no selective pressure to maintain that isolation through interspecific signal divergence. The two taxa of bulbuls were allopatric on Taiwan before secondary contact occurred. No signal isolating mechanism had evolved due either to allopatry or recency of speciation or both. Birds may learn songs from neighbors because of the need to match in countersong (McGregor and Krebs, 1984). The function of countersinging is primarily territorial. Because the two species of bulbuls are ecological equivalents, interspecific territoriality and countersinging became necessities with their sympatry. The fact that bulbuls learn their songs facilitates their acquiring the community language; and this has accelerated the merging of songs in the hybrid zone. Since vocalized differentiation between closely related species is the "first line of defense" in maintaining species boundaries in zones of secondary contact, species that modify their songs through learning should be more prone to

Table 2 Distribution of syllables among types

Population	Unique types	Major type	Other shared types	% in major type
Kinmen	52	35	5	38.04
Jiangxi	42	3	8	5.66
Taiwan CB	45	512	47	84.77
SB	23	311	48	81.41
hybrid zone	41	536	23	89.33

hybridization. This is reflected in the interaction between Chinese and Styan's bulbuls on Taiwan.

Hybridization is known between the thrush nightingale (*Erithacus megarhynchos*) and nightingale (*E. luscinia*) in a narrow zone from Denmark to the Balkans (Hagemeijer and Blair, 1997). Becker (1995) suspected that hybridization was triggered by the high percentage of non-breeding males in the overlap zone. Because members of these two species are mixed singers that regularly add the songs of the other species to its own, we propose that hybridization is initially facilitated by the breaking down of their vocal isolation.

The songs of Taiwan hybrid bulbuls are similar to those of Styan's bulbul in frequency but the Chinese bulbul in syntax. The population sizes of the two parental taxa are comparable in the area where songs were recorded. However, mixed pairs tend to comprise Chinese bulbul males and Styan's bulbul females rather than the converse. How this affects vocal asymmetry in the offspring has yet to be determined.

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