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Species Recognition by Song in Two Closely Related Bush Warblers, *Cettia seebohmi* and *C. diphone* (Aves: Sylviidae)

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Abstract. The ability to discriminate conspecifics from heterospecific individuals may contribute to reproductive isolation among species. In birds, song is one of the most important cues in species recognition. We used playback experiments to investigate whether male Japanese Bush Warblers, *Cettia diphone* (Kittlitz, 1831), could distinguish the songs of conspecifics from those of the closely related Luzon Bush Warblers, *C. seebohmi* Ogilvie-Grant, 1894. Japanese Warbler males did not show statistically significant differences in their responses (i.e., the number of songs, rate of L-type song, number of flights, and time spent near the speaker) to song playbacks of the two species. This inability to discriminate between the songs may have been caused by greater costs to males when they misinterpret conspecific rivals for heterospecifics than the converse. In an additional experiment in the Philippines, male Luzon Bush Warblers responded more aggressively to conspecific songs than to heterospecific songs, although the sample size was small. The asymmetry in the results may yield due to the low density of the population in the Philippines, which could lead to less competition among males, although further investigations are needed.
Key words: *Cettia diphone*, *Cettia seebohmi*, playback experiment, song, species recognition.

Introduction

The ability to discriminate conspecifics from heterospecific individuals may contribute to reproductive isolation among species. In a social context, species recognition is important to individuals searching for and choosing a mate, because heterospecific mating produces hybrid offspring that generally have low fitness (Arnold, 1997). Species recognition is also important to individuals defending resources that are necessary for reproduction (e.g., male territory). Because competition for mates, territories, and parentage is most direct between individuals of the same species, heterospecific aggression in this context is a waste of time and energy (Lynch & Baker, 1990).

In birds, species recognition cues include morphology (Ratcliffe & Grant, 1985), plumage (Matyjasiak, 2005), and vocalisations (de Kort *et al.*, 2002; Matyjasiak, 2005). Recognising songs, which are long melodious vocalisations generally uttered by males during the breeding season, is especially crucial, because they function in mate attraction and territory defence against rival males (Searcy & Andersson, 1986; Catchpole & Slater, 1995). Males show aggressive responses to song playbacks (e.g., Vehrencamp, 2001; Hansen & Slagsvold, 2003), and females show copulation solicitation and active nest-building behaviour in response to playbacks (e.g., Searcy, 1984; Okanoya, 2004).

The ability to discriminate between the songs of conspecifics and heterospecifics has been shown in sympatric species. For example, male Nightingales, *Luscinia megarhynchos* Brehm, 1831, increased their song levels (i.e., loudness) more in response to conspecific songs than to

heterospecific songs (Bruum & Todt, 2004). Both the Blue Tit, *Parus caeruleus* Linnaeus, 1758, and the Great Tit, *P. major* Linnaeus, 1758, also showed stronger responses to conspecifics than to other species (Hansen & Slagsvold, 2003). Thus, birdsong is a species-specific signal that plays an important role in reproductive isolation among sympatric species. Among allopatric species, however, there is no selective pressure to discriminate between conspecific and heterospecific songs, because birds of different species do not directly encounter each other during the breeding season. If individuals of a species cannot distinguish their songs from those of a closely related allopatric species, and if the geographic barriers that isolate the species were removed, hybridisation would occur between them. Thus, species recognition among allopatric species is an interesting and important subject in speciation, but it has received little attention.

The Luzon Bush Warbler, *Cettia seebohmi* Ogilvie-Grant, 1894 (previously downgraded to *C. diphone seebohmi* by Delacour, 1942), is closely related to the Japanese Bush Warbler, *C. diphone* (Kittlitz, 1831) (Ogilvie-Grant, 1894; Dickinson *et al.*, 1991; Kennedy *et al.*, 2000). The morphology and plumage of the two species are similar, but the Luzon Bush Warbler has a redder crown, a prominent supercilium, and a greyer underpart (Delacour, 1942; Kennedy *et al.*, 2000). The song of the Luzon Bush Warbler resembles that of other *Cettia* species (Orenstein & Pratt, 1983), and some of its song types are similar to those of the Japanese Bush Warbler (Fig. 1; the results of acoustic analyses will be reported elsewhere). The Luzon Bush Warbler is an endemic resident of northwestern Luzon (Ogilvie-Grant, 1894; Kennedy *et al.*, 2000), while the Japanese Bush Warbler breeds in eastern China, southern Ussuriland, Korea, and Japan (Ornithological Society of Japan, 2000). Although a subspecies of the Japanese Bush Warbler, *C. d. canturians* (Swinhoe, 1860), which breeds in eastern China, spends winter in the Philippines (Kennedy *et al.*, 2000), the two species are not sympatric breeders.

In this study, we examined whether Japanese Bush Warblers could discriminate between the songs of conspecifics and those of Luzon Bush Warblers. To this end, we used playback experiments in the field to compare the responses of Japanese Bush Warblers to the songs of the two Warbler species. We focused on male aggressive behaviours, because females are very difficult to observe in dense foliage. In addition, we also report the responses of male Luzon Bush Warblers

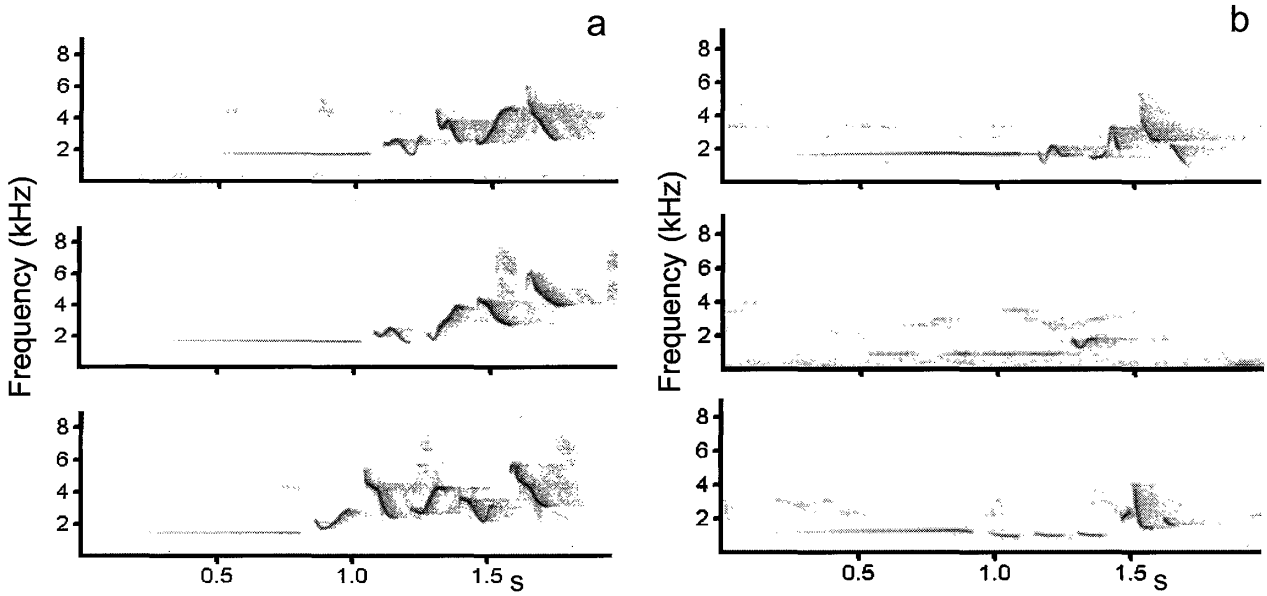

Fig. 1. Sonagrams of the Luzon Bush Warbler, *Cettia seebohmi* (a), and the Japanese Bush Warbler, *C. diphone* (b). Three song types of a male of each species are shown.

to song playbacks of the two species, although the sample size was small due to limited field conditions in the Philippines.

Methods

1. Study area and song recordings

The fieldwork in the Philippines was conducted at one site, an open forest at Ambangeg (16°31′N, 120°50′E; 1355 m alt.) in the Cordillera Mountains of northwestern Luzon. The forest consisted mainly of pine trees, *Pinus* sp., with dense bush and thicket, in which Luzon Bush Warblers were singing. We recorded the songs of Luzon Bush Warblers on 26 and 27 April 2005. Recordings were obtained from 20 males along 9 km of roads, which roughly indicated the density of this species in this area (2.2 males/km).

We conducted fieldwork at two study sites in Japan. Both were secondary deciduous forests in Saitama, central Honshu; one at Furusato (36°06′N, 139°18′E; 70 m alt.) and the other at Shogunsawa (36°01′N, 139°20′E; 50 m alt.). Both sites included patches of previously cultivated but presently abandoned lands dominated by dwarf bamboos. We recorded the songs of Japanese Bush Warblers from ten males at Furusato on 16 June 2003, and from five and three males at Shogunsawa on 26 May and 2 June 2003, respectively. The density of this species along the trails in the Shogunsawa area was about 5.4 males/km (Hamao, unpublished data); we could not determine an accurate density of the warblers in the Furusato area, due to scattered cultivated lands.

For all of the recordings, we recorded each singing male for at least 3 minutes using a Sony TCD-D8 DAT recorder with a Sony ECM-G3M directional microphone.

2. Field experiments

We performed playback experiments to compare the responses of male Japanese Bush Warblers to the songs of their own species and to those of Luzon Bush Warblers. The procedure for each playback was as follows: 3 minutes playback (playback 1), 3 minutes silent period, and 3 minutes playback (playback 2). In each playback session, either a Luzon or a Japanese Bush Warbler song was played, random selected by tossing a coin. Four parameters, detailed below, were measured during each playback.

1) Number of songs.

2) Rate of L-type song. The songs of the Japanese Bush Warbler are categorised into H- and L-type songs. These two types were also identified by Momose (1999), Hamao and Ueda (2000), and Park and Park (2000), as alpha and beta types. An L-type song contains more than one note in the introductory whistle portion, and is used as an aggressive signal against close rivals (Momose, 2000; Park & Park, 2000). Therefore, the rate of the L-type song may be a good indicator of an aggressive response.

3) Number of flights farther than 5 m. When a male hears the song playback, it often searches for and approaches the speaker. Therefore, the number of flights during playbacks may indicate an aggressive response (Martin, 1980; Momose, 2000).

4) Time spent within 5 m of the speaker. Because it is difficult in the field to obtain an accurate measure of distance between the subject bird and the speaker, we used the time spent within a certain distance from the speaker as an indicator of aggressive behaviour during playbacks (Matessi *et al.*, 2000; Wiley, 2005).

At the beginning of each experiment, we placed the speaker 10–20 m away from the subject male. If at the start of playback 2, the male was within 10 m of the speaker, we moved the speaker more than 10 m away.

The experiments were conducted for 10 and 8 males at Shogunsawa and Furusato, respectively, in the morning (05:15–11:10 h) on 11 and 12 May 2005. The playbacks were made using a Sony CFM-175TV cassette recorder. In the experiments at Shogunsawa, we used songs recorded at Furusato as stimuli, and vice versa, because male birds show different responses to songs uttered by neighbours and strangers (Momose, 2000; Wiley, 2005). Since there was a distance of

11 km between the two sites, the playback songs from the Japanese Bush Warblers would not have been familiar to the subject males.

A tape for a playback contained all the song types of the recorded male. Songs were recorded on the tape at 15 s-intervals, which is the natural song interval of spontaneously singing Japanese Bush Warblers (Hamao & Ueda, 2000). To avoid pseudoreplication (Kroodsma *et al.*, 2001), we used the song of a different male for each experiment, which made the data from each experiment independent. Therefore, we performed a Wilcoxon's signed-ranks test on the data to compare the responses to the conspecific and heterospecific songs. We used the sequential Bonferroni correction (Rice, 1989) to assess the table-wide type I error rate.

The experiments performed at Ambangeg, the Philippines, took place in the morning (05:30–11:00 h) on 26 and 27 April 2005. Five male Luzon Bush Warblers were used as subjects, and the experimental procedure was similar to that used in Japan. However, we used a Panasonic RQ-L470 cassette recorder for the playbacks, and considered a male response as the time spent within 3 m of the speaker (instead of the 5 m used in Japan) because it is difficult to locate Luzon Bush Warblers due to their habitat (e.g., dense bushes) and infrequent singing. The rate of the L-type song was not measured, because the function of each song type has not been studied in Luzon Bush Warblers. As in Japan, we used the song of a different Japanese Bush Warbler male as the playback stimulus for each experiment (i.e., for the five experiments, we used five males' songs in total); however, we used the song of only one male Luzon Bush Warbler for all experiments because of limited time and sound-editing equipment in the Philippine field. Because of this pseudoreplication and the small sample size, we did not test these results statistically. As in Japan, the subject males would not have been familiar with the song playbacks from their own species, because the songs were recorded 2 km or more away from the experiment site.

Results

1. Behavioural changes by song playbacks

The following is a description of the behavioural response of one subject male of the Japanese Bush Warbler to playbacks and also during 3-min silent periods before and after each experiment. Although the male sang at a distance of 11 m from the speaker during the silent period preceding the experiment, it approached the speaker during playback 1, which was the song of the Luzon Bush Warbler (Figs 2, 3a). Because the male remained within 2 m of the speaker during the silent period following playback 1, we moved the speaker 19 m away just before playback 2. At the start of playback 2, which was the song of its

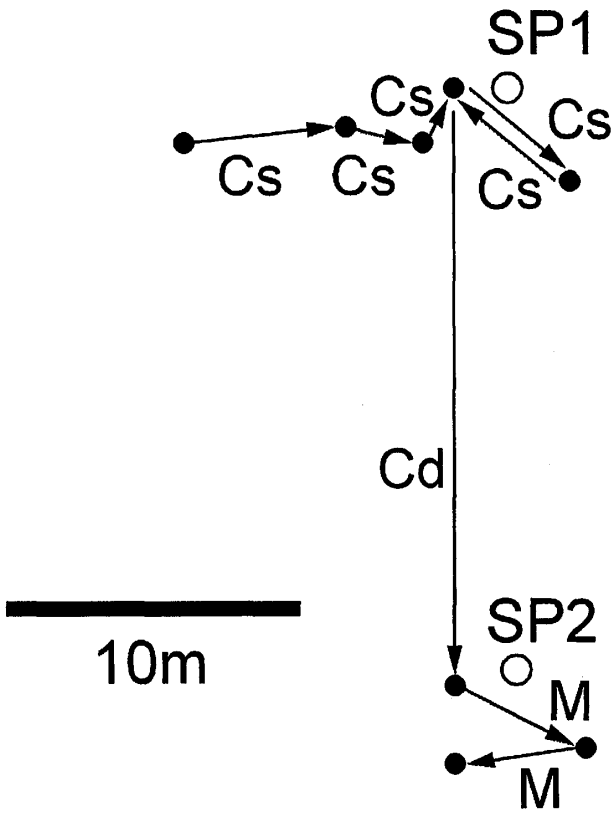

Fig. 2. Locations (dots) and movements (arrows) of a male Japanese Bush Warbler, *Cettia diphone*, in a playback experiment. SP1 and SP2 show the locations of the speaker during playbacks 1 and 2, respectively. Cs, Cd, and M indicate the movements during playback 1 (song of the Luzon Bush Warbler, *C. seebohmi*), playback 2 (song of the Japanese Bush Warbler), and the silent period, respectively.

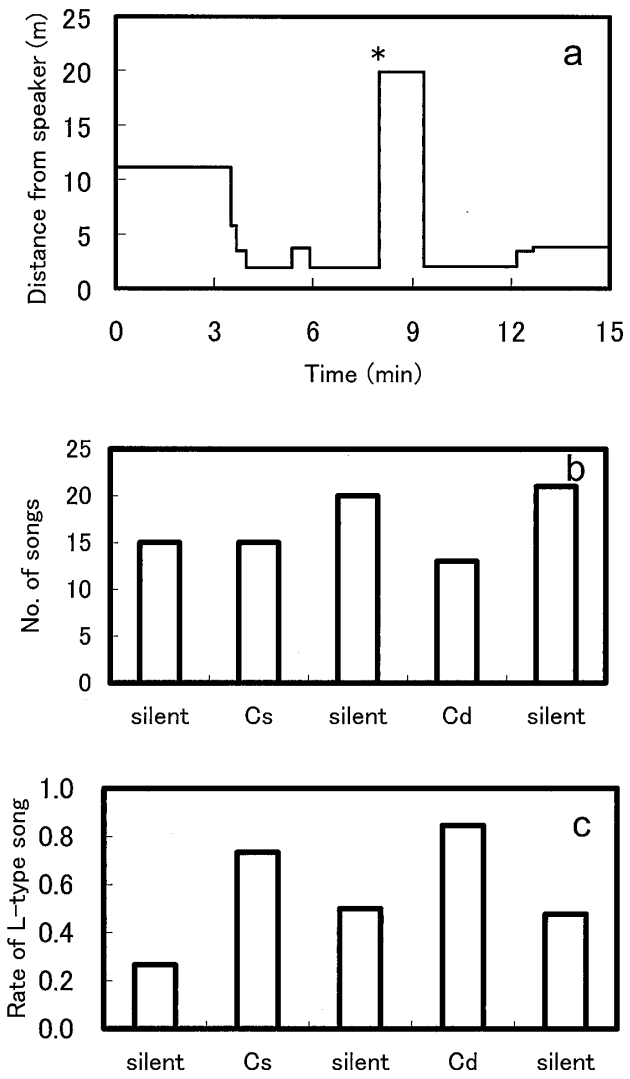

Fig. 3. The distance from the speaker (a), number of songs (b), and rate of the L-type song (c) of a male Japanese Bush Warbler, *Cettia diphone*, during a playback experiment. An asterisk indicates the time when the speaker was moved.

own species, the male flew to a tree about 2 m away from the speaker (Figs 2, 3a). During and after playback 2, it spent time near the speaker (Figs 2, 3a).

No clear trend was observed by changing the number of songs uttered by the subject male during each 3-min period, but singing decreased during song playbacks of its own species (Fig. 3b). The rate of the L-type song increased during song playbacks of both species (Fig. 3c). Thus, we conclude that the playbacks changed the behaviour of the male.

2. Response of Japanese Bush Warblers to playbacks

We compared the responses of male Japanese Bush Warblers to the song playbacks of both species. The number of flights was greater during the playback of Japanese Warbler songs than during Luzon Warbler songs (Fig. 4c), but the number of songs, the rate of the L-type song, and the time spent near the speaker did not differ between the song playbacks of the two species (Fig. 4a, b, d). None of the four parameters showed a statistically significant difference between the songs of the two species (Wilcoxon's signed-ranks test, all $P>0.1$).

3. Response of Luzon Bush Warblers to playbacks

Male Luzon Bush Warblers actively sang during the playback of Japanese Warbler songs, whereas they seldom sang during the playback of conspecific songs (Fig. 5a). The subject males flew more frequently during the playback of conspecific songs than during those of Japanese Warblers (Fig. 5b). The time spent near the speaker did not differ between the playbacks (Fig. 5c). Thus, male Luzon Bush Warblers were prone to reduce singing and to actively search for the speaker when they heard the song of a conspecific, but not the song of a heterospecific.

Discussion

We did not find statistically significant differences between the responses of male Japanese Bush Warblers to the songs of their own species and those of Luzon Bush Warblers (Fig. 4). This does not directly indicate that their responses to conspecific and heterospecific songs are similar, because it cannot be ruled out that we failed to detect the differences (type II error in statistical tests). However, we argue that male Japanese Bush Warblers did not, in fact, show clear differences in their responses to conspecific and heterospecific songs. This is because song playbacks successfully simulated territorial intrusions by rival males, as evidenced by male Japanese Bush

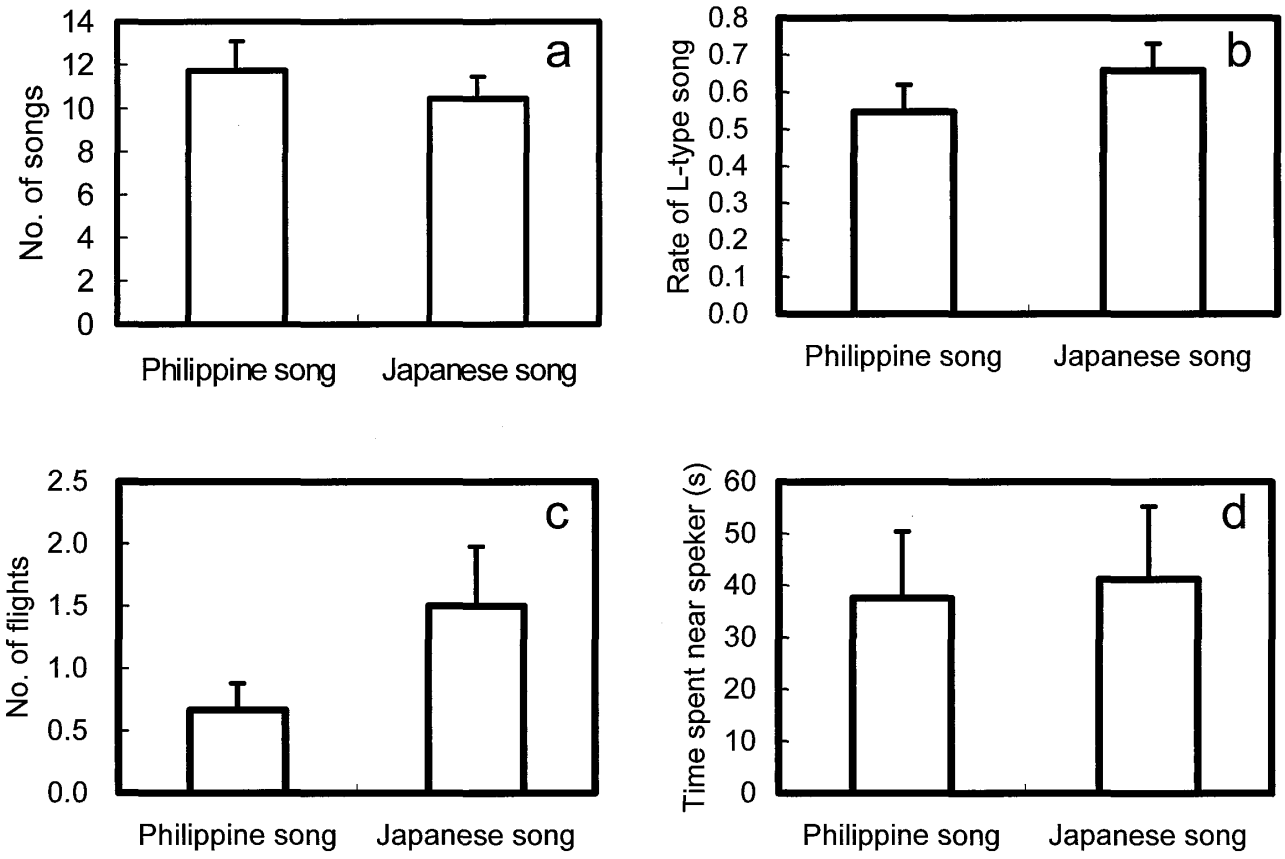

Fig. 4. The responses of male Japanese Bush Warblers, *Cettia diphone*, to song playbacks of its own species and the Luzon Bush Warbler, *C. seebohmi*. Means±S.E. are shown.

Warblers approaching the speaker and increasing their L-type songs (Figs 2, 3), which are considered aggressive signals against close rivals (Momose, 2000; Park & Park, 2000). We also carefully designed the experiment to avoid pseudoreplication: all subject males were exposed to the songs of different males, thereby excluding any potential bias toward any one male's song. Furthermore, we adopted a statistically sensitive design that included within-individual comparisons between the two playbacks. Therefore, it appears that male Japanese Bush Warblers do not discriminate between the songs of conspecifics and those of Luzon Bush Warblers.

Among sympatric species, male discrimination between conspecific and heterospecific songs has been reported (Hansen & Slagsvold, 2003; Bruum & Todt, 2004). Since Luzon and Japanese Bush Warblers are allopatric breeders, it is impossible that the males of the two species would compete for mates or territories via misrecognition. Thus, the lack of selective pressure on species recognition would lead to an inability to discriminate between conspecific and heterospecific songs. However, if acoustic differences were to develop over a long period of time, males would be able to discriminate between conspecific and heterospecific songs, irrespective of locality. The songs of Luzon Bush Warblers and Japanese Bush Warblers differ, although some song types are similar (Fig. 1). Therefore, whether the difference in song structure affected the results is unclear.

A possible factor influencing our results is that species recognition is better developed in females than in males. If a female accepts a heterospecific male, she will suffer the great costs of hybrid mating (outbreeding depression: deleterious effects on fitness that sometimes occur as a result of outcrossing). If a female rejects a conspecific male by misrecognition, it is not costly, because females generally have many opportunities to

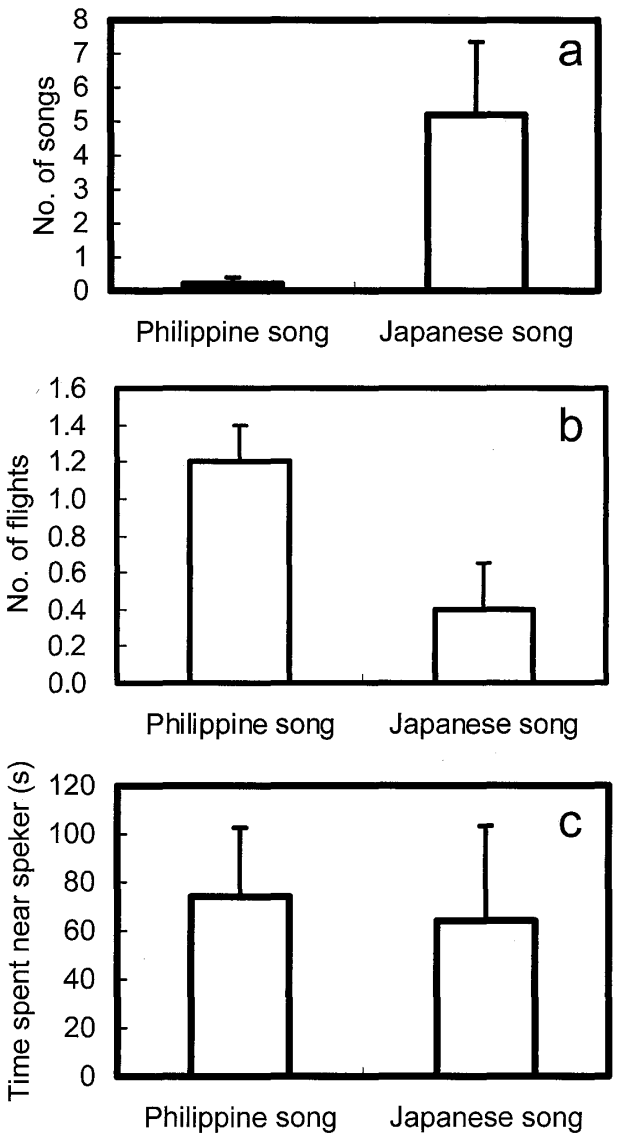

Fig. 5. The responses of male Luzon Bush Warblers, *Cettia seebohmi*, to song playbacks of conspecifics and the Japanese Bush Warbler, *C. diphone*. Means±S.E. are shown.

mate. Therefore, the females may have developed an acute ability to differentiate between conspecifics and heterospecifics. In contrast, males suffer most if they regard conspecific rivals as heterospecifics, because such a mistake could result in the loss of their territory and paternity. Males that court heterospecific females suffer only the cost of approaching and displaying more or less in vain. Therefore, it is less important for males, in this context, to develop a strong ability to discriminate between conspecifics and heterospecifics. Thus, if males are generally less discriminating than females (Searcy & Brenowitz, 1988), male Japanese Bush Warblers may not have been able to discriminate between the songs of their own species and those of Luzon Bush Warblers. Our findings provide supporting evidence that male discrimination ability between allopatric species is not developed.

In contrast to male Japanese Bush Warblers, male Luzon Bush Warblers seemed to respond more aggressively to conspecific songs than to heterospecific songs. If this is true, there is an asymmetric trend in species recognition between the two species of warblers. This difference may be caused by the high breeding density of Japanese Bush Warblers (see Methods); males may discriminate less due to a higher risk of losing their resources to competitors. However, a well-designed experiment with a large sample size is required to evaluate further the discrimination ability of the Luzon Bush Warbler.

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近縁なウグイス2種，*Cettia seebohmi* と *C. diphone* の音声による種の認知

濱尾章二・Maria J. S. Veluz・西海　功

同種の個体と他種の個体を識別する能力は，動物種間の生殖隔離を成立させる要因の一つである．一般に，鳥類では形態や羽色とともに，さえずりがこのような種の認知の重要な手がかりになると考えられている．そこで本研究では，日本のウグイス *Cettia diphone* の雄が自種のさえずりとフィリピンのウグイス *C. seebohmi* のさえずりを区別できるかどうかを，野外における音声再生実験によって検証した．それぞれの種のさえずりに対する雄の排他的行動をさえずり回数，威嚇の信号といわれるL型さえずりの割合，飛翔回数，スピーカーから5m以内にいる時間の4つの変数によって比較したところ，雄の反応に統計的に有意な差は認められなかった．雄がさえずりを区別しない理由として，同種を異種と誤認するコストが異種を同種と誤認するコストに比べて大きいことが考えられる．これに対して，実験回数は少ないものの，フィリピンのウグイスの雄は日本のウグイスのさえずりよりも自種のさえずりに対して強く反応する傾向が見られた．フィリピンでは生息密度が低いために雄間の競争が弱いことが影響しているのかも知れない．