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Species rank of Isabela Oriole *Oriolus isabellae* and White-lored Oriole *O. albiloris* reinforced by song playback responses

ANTHONY M. LOWNEY, MERLIJN VAN WEERD & SELVINO R. DE KORT

Introduction

The Isabela Oriole *Oriolus isabellae* and White-lored Oriole *O. albiloris* are endemic to Luzon, northern Philippines (BirdLife International 2014). Historically, the Isabela Oriole was observed in two distinct locations: the northern Sierra Madre mountain range in north-east Luzon and the Bataan Peninsula in western Luzon (Collar *et al.* 1999). However, the species has been reported only three times since 1961, namely (1) 15 km south of Diffun, Quirino province (Gamauf & Tebbich 1995); (2) Mansarong, Baggao, Cagayan province (van der Linde 1995)—neither of which were considered absolutely certain (Collar 1998)—and (3) Ambabok, San Mariano, Isabela province (van Weerd & Hutchinson 2004), all in north-east Luzon. There have been no records from the Bataan Peninsula since 1947 (Collar *et al.* 1999). The current population size of the Isabela Oriole is estimated at fewer than 250 individuals and it is therefore classified as Critically Endangered (BirdLife International 2014). The White-lored Oriole is widely distributed throughout northern Luzon with a population estimated to be greater than 10,000 individuals and it is classified as of Least Concern (BirdLife International 2014). The two species historically occurred sympatrically in the northern Sierra Madre and the Bataan Peninsula (van Weerd & Hutchinson 2004, Collar 2011). Little is known about the ecology of either species except that they are canopy-dwellers and may be associated with secondary forest and forest edge.

Isabela and White-lored Orioles differ in plumage colour, bill length (Collar 1998) and vocalisations (van Weerd & Hutchinson 2004). Isabela Oriole has a stout grey bill, mean length 28 mm, and yellowish lores, while the White-lored Oriole has a slender red bill, mean length 21.8 mm, and broad white lores (Collar 1998). There is little sexual dimorphism in either species (Kennedy *et al.* 2000). The songs of the White-lored Oriole are longer and more tonal or 'flutier' than those of the Isabela Oriole (van Weerd & Hutchinson 2004). Despite these differences the two species show limited DNA sequence divergence, leading to the suggestion that they should 'perhaps' be considered 'a single taxonomic unit' (Jønsson *et al.* 2010). Collar (2011) disagreed with this and argued that the morphological differences, in combination with the historical sympatric occurrence in at least two locations, are sufficient to consider the Isabela and White-lored Oriole as distinct species, which is also in concordance with a recently proposed method for delimiting species (Tobias *et al.* 2010). Clearly, with the Isabela Oriole currently classified as Critically Endangered it is imperative that its taxonomic status is unambiguous. To that end, we conducted playback experiments with both species to test whether they discriminate between each other's songs.

Methods

We used playback experiments to test the hypothesis that the Isabela and White-lored Orioles differentiate between conspecific and allospecific song. Females are expected to discriminate between con- and allospecific stimuli if the consequences of hybridisation are maladaptive (Nelson & Soha 2004). However, females do not respond readily to playback in the field, which prevents testing females (Catchpole & Slater 2008). Therefore, most studies that test species recognition use territorial males as subjects (de Kort *et al.* 2002, Seddon & Tobias 2010). Playback of conspecific songs simulates a territorial intrusion and usually leads to an aggressive response from the territory owner (de Kort & ten Cate 2001, Slabbekoorn & Smith 2002). Playback of territorial songs of allospecifics in general tends to produce lower or no response by a territory owner, especially when two species are sympatric (Falls 1988, de Kort & ten Cate 2001, Catchpole & Slater 2008). Playback of bird songs is therefore a useful method to test for biologically relevant species limits (Alström & Ranft 2003).

If Isabela and White-lored Oriole males discriminate between conspecific and allospecific vocalisations, we infer that females will also respond differentially to the songs of the two species. This would suggest that vocalisations can act as a mating barrier and provide support for the current classification of the two orioles as distinct species.

Study sites

Data were collected in three geographically separated areas: the Sierra Madre, Bataan Peninsula and Northern Cordillera (Figure 1).

The two species occur sympatrically in lowland dipterocarp forest in the San Mariano municipality, Isabela province (17°N 122.167°E), in the northern Sierra Madre. San Mariano is situated on the western edge of the Northern Sierra Madre Natural Park.

The Subic Forest Watershed Reserve (14.750°N 120.333°E) and Bataan Natural Park (14.700°N 120.367°E), both in Bataan province, are located within the Bataan Peninsula. White-lored Oriole is frequently observed in the Bataan Peninsula, but the Isabela Oriole
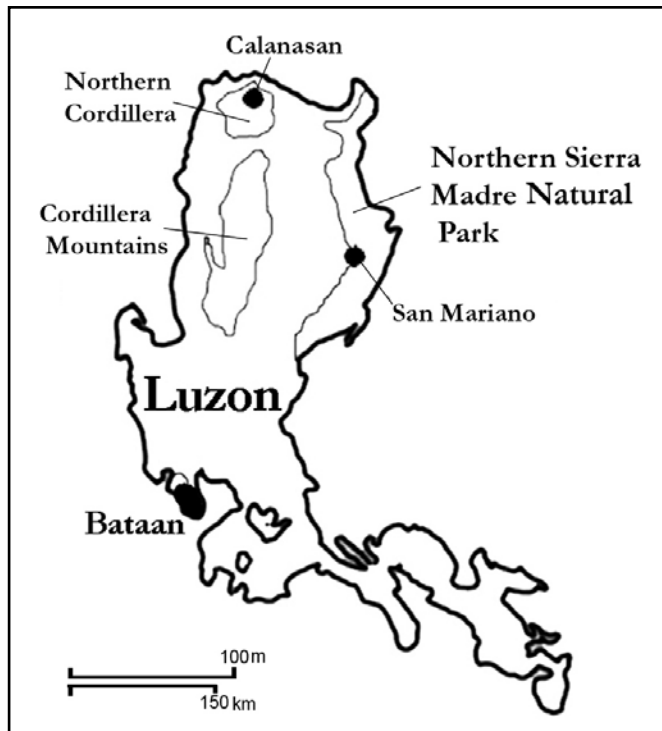
Figure 1. Map of Luzon showing locations of the three study sites.

has not been observed there since 1947 (Collar *et al.* 1999). The Calanasan municipality, Apayao province (18.433°N 121.083°E), is located in northern Luzon and is part of the Cordillera Mountain Range. White-lored Oriole is frequently recorded, but there are no records of the Isabela Oriole; however, Apayao is largely unexplored biologically.

Playback stimuli
Isabela Oriole stimuli were obtained from a single individual because these were the only recordings available for this species at the time (van Weerd & Hutchinson 2004). Two different songs were recorded from this individual and used as separate stimuli. Songs from the White-lored Oriole were recorded from three individuals in the Apayao region by SdK in 2010 at a sampling frequency of 44.1 kHz using a Sennheiser ME67 microphone and a Marantz PMD661. From these recordings eight unique pairs of playback stimuli were made. Songs were selected based on a good signal-to-noise ratio and filtered with a high pass filter set at 500 Hz, with amplitudes standardised across recordings. The songs were played 20 times in a period of 60 s, mimicking the natural song rate of the orioles. Stimuli were played at a volume of 90 db (SPL) measured at 1 m from the speaker using a Precision Gold N05CC sound level meter. The order of playback of the two species was alternated to control for habituation and carry-over effects.

Experimental design and procedure
Playback trials were conducted in each of the three study areas between sunrise and 11h00, and 15h00 to sunset, between December 2011 and March 2012. We walked trails to locate individuals; once an individual was heard, we positioned a wireless speaker (FOXPRO Fury GX7 Digital Game Call) more than 50 m away from the bird. The speaker was operated via a remote control (TX-500 transmitter) from a distance of at least 20 m.

The experimental setup consisted of a pre-playback period (300 s), which allowed for the measurement of baseline activities, and two playback periods (300 s each). The second playback period followed immediately after the first (similar to Slabbekoorn & ten Cate 1997). Each playback period started with 60 s of playback of one of the two species, followed by 240 s of silence. Each trial thus

lasted 900 s. The order in which the two species were presented was alternated between trials.

Three predetermined response parameters were recorded during each trial: the shortest distance between the bird and the speaker (distance); number of vocalisations (songs) including any vocalisation type; and response latency. Response latency was defined as the time(s) between onset of the playback and the first song produced by the subject. If no response occurred during a playback period, latency was set at 301 seconds. Distance was estimated in five bands: 1–10 m (10), 11–20 (20), 21–30 (30), 31–50 (50) and > 50 (51).

Statistical analyses
To test if the experimental procedure altered the behaviour of the subjects we compared the behavioural parameters for the pre-playback with the first post-playback period using a Wilcoxon signed-rank test. We then compared the two playback periods to test whether the birds behaved differently in response to the con- and allospecific vocalisations using a Wilcoxon signed-rank test. All statistical analyses were performed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA).

Results
Four Isabela Orioles were observed during this study, all in San Mariano in the Sierra Madre, and two of these individuals were successfully exposed to playback treatments. Seventeen White-lored Oriole were exposed to playback treatments: eight in Bataan, six in San Mariano, and three in Apayao. The small sample size of two for Isabela Oriole precluded statistical analyses of the results for this species.

Response to playback stimulus compared with baseline behaviour
Compared to pre-payback periods, White-lored Oriole showed an increase in song rate (Wilcoxon signed rank test; $Z = -2.366$, $n=8$, $p= 0.018$) and approached the speaker closer ($Z = -2.214$, $n = 8$, $p = 0.027$) following playback of conspecific songs, but not following playback of the Isabela Oriole stimuli (song: $Z = -0.844$, $n = 9$, $p = 0.398$, and distance: $Z = 0$, $n = 9$, $p = 1$ respectively).

Interspecific playback
Both species showed a stronger response to conspecific playback stimuli in number of vocalisations (see Figure 2; for the White-lored Oriole: Wilcoxon signed rank test; $Z = -2.178$, $n = 17$, $p = 0.029$). Subjects approached the speaker closer in response to conspecific playback stimuli (Figure 2; for the White-lored Oriole: $Z = -2.048$, $n = 17$, $p = 0.041$). The results for response latency were ambiguous, with the Isabela Oriole responding slightly later to conspecific stimuli (Figure 2a) and the White-lored Oriole responding later to allospecific stimuli (Figure 2d; for the White-lored Oriole: $Z = -1.349$, $n = 17$, $p = 0.121$); neither result was significantly different.

Recordings
Sound recordings of vocalisations were obtained from three of the four Isabela Orioles, all in San Mariano in the northern Sierra Madre. These three Isabela Orioles produced two song types, both consisting of a single whistle that either rises slightly in frequency and lasts around 0.5 s or descends in frequency and lasts 0.4 s. Both are repeated irregularly every 1–2 s. White-lored Oriole recordings were obtained from 25 individuals at all three sites and these recordings showed considerable variation within and between sites (Figure 3). These songs often contained two or three notes and tended to be longer and flutier than those of the Isabela Oriole. The most frequently recorded song in Bataan was a descending two-note whistle lasting around 0.6 s, repeated regularly every 8–12 s. In the Sierra Madre the commonest song consisted of three short notes lasting up to 1 s repeated regularly every 2.5–3 s. In the Cordillera
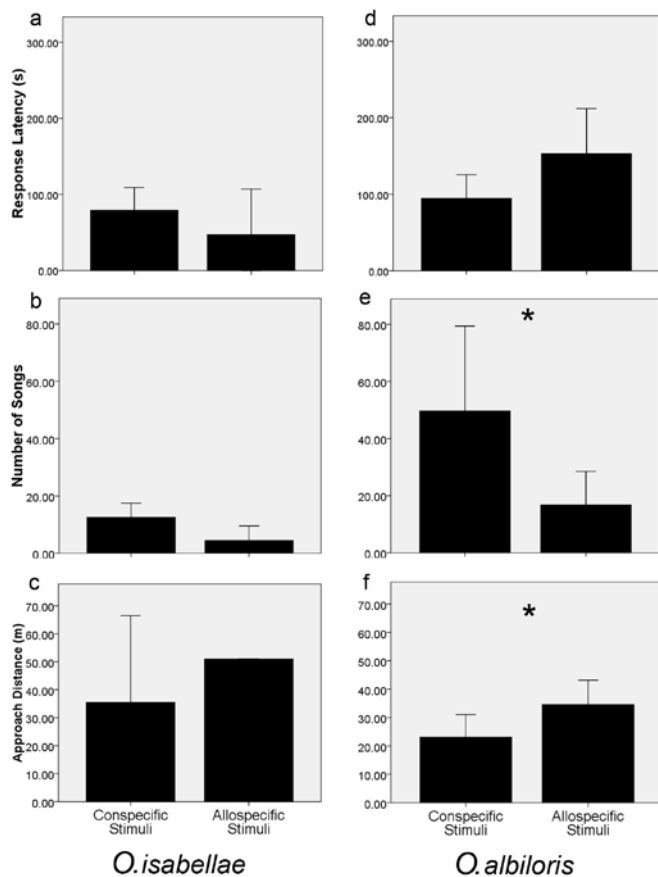
Figure 2. Response measures (mean $\pm$ 2 se) to conspecific and allospecific stimuli for Isabela Oriole (a–c) and White-lored Oriole (d–f). For Isabela Oriole $n = 2$ and White-lored Oriole $n = 17$. *Significant differences ($p<0.05$)

the most frequently recorded song consisted of a single whistle that increases in frequency and lasts up to 1s, repeated regularly every three seconds (Figure 3).

Discussion

Both the Isabela and White-lored Oriole responded more strongly to conspecific than to allospecific songs. Although the results for the Isabela Oriole are limited due to the small sample size, the combined results suggest that there is a species barrier based on vocalisations. Despite the structural variation in the White-lored Oriole songs between sites, all individuals tested responded more strongly to the conspecific songs that originated from one location (Apayao in the Cordillera) than to the Isabela Oriole songs.

The differences in biometrics (bill size), plumage (bill colour, lore colour) (Collar 1998) and acoustics (this paper) strongly suggest species delimitation (Tobias *et al.* 2010) between the sympatric Isabela and White-lored Oriole despite the limited genetic divergence between them (Jønsson *et al.* 2010).

Only four Isabela Orioles were observed during this study, all within Northern Sierra Madre Natural Park. No Isabela Orioles were

found in Bataan, a site with historical records of the species, or in the northern Cordillera. Northern Sierra Madre Natural Park is one of the largest protected areas within the Philippines (Mallari *et al.* 2001) but environmental law enforcement is weak, with hunting, logging and agricultural encroachment currently threatening habitats and species (van der Ploeg *et al.* 2011). Moreover, government plans for infrastructural development and mining may increase pressure on the biodiversity within the park. The Isabela Oriole is one of 20 globally threatened bird species found within the park; others include the Philippine Eagle *Pithecophaga jefferyi* and Green Racquet-tail *Prioniturus luconensis* (van der Ploeg & van Weerd 2010). For several endemic pigeon, parrot and hornbill species, the Northern Sierra Madre Natural Park is one of the last reserves on Luzon large enough to sustain viable populations (Española *et al.* 2013). Furthermore, the park is home to a large number of other globally threatened species including 28 tree species (van Weerd & Udo de Haes 2010), the Critically Endangered Philippine Crocodile *Crocodylus mindorensis* (van Weerd 2010) and a newly described species of frugivorous monitor lizard *Varanus bitatawa* (Welton *et al.* 2010). Effective conservation of Northern Sierra Madre Natural Park is crucial for the survival of these species, including the Isabela Oriole.

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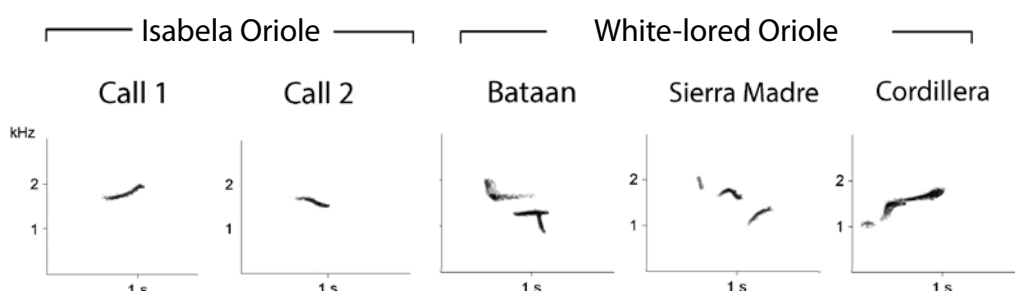
Figure 3. Two Isabela Oriole song types from San Mariano, Luzon, and the most frequently recorded White-lored Oriole songs from each site.

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New Black-necked Crane *Grus nigricollis* subpopulation recorded in southern Tibet, China

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Black-necked Crane *Grus nigricollis* is classified as Vulnerable (BirdLife International 2015) with an estimated total population of 10,070–10,970. It breeds in wetlands on the Qinghai–Tibetan plateau, China, with a small population in adjacent Ladakh, India (BirdLife International 2001, 2015). Six wintering areas have been identified at lower altitudes on the Qinghai–Tibet and Yunnan–Guizhou plateaus in China, and it also winters in Bhutan and Arunachal Pradesh, India. On the Qinghai–Tibetan plateau, it is considered that there are three main distinct populations—west, central and east (Wu *et al.* 1993). The west population is the largest, estimated to be 7,400 individuals, and it occupies the most southerly breeding grounds (Bishop & Tsamchu 2007), mainly in Nagqu prefecture, Ngari prefecture, Angren county, Xigazê prefecture and Damxung, Lhasa, Tibet Autonomous Region (Tsamchu *et al.* 2008). However, breeding has also occasionally been reported south of latitude 30°N: one pair was photographed on 'Tinki plain' on 15 June 1922 by Longstaff and Norton (Hingston 1927); a pair was seen at 15,000 feet (4,570 m) at Tingri on 7 July 1924 (Hingston 1927); in the 1991 breeding season, one nest (29.467°N 85.900°E) and two broods of four chicks (29.867°N 83.733°E, 29.983°N 83.533°E) were reported by an International Crane Foundation expedition (Dwyer *et al.* 1992)—the most southerly breeding record for the species. Li & Bishop (1999) included an undated report of breeding in Tingri county.

In summer 2014, we carried out a 13-day survey along the road network in the Pumqu River basin, an area very little surveyed previously for cranes. During the course of our survey, we recorded 111 adult Black-necked Cranes and 22 chicks, confirming the existence of a new subpopulation in this area.

Fieldwork was carried out from 16 to 28 July 2014 in the Pumqu River basin, which is situated in south-west Tibet just north of the border with Nepal and Sikkim between 27.817°N 85.633°E and 29.083°N 88.950°E. The source of the river is the Yebokangjiale glacier on the north face of Mt Shishapangma, Nyalam county, Tibet Autonomous Region, China; it flows eastwards through Tingri county and turns south towards Nepal to the east of Mt Everest. The basin is about 320 km long from west to east and up to 120 km in breadth from north to south, with a total catchment area of about 25,000 km². For 280 km, from the glacier outflow to Tingri town, the upper and middle reaches of the river lie in broad valleys with gentle gradients at 4,500 m or more in altitude. There are many winding reaches and meanders with well-established wetlands, which offer the potential to be good habitat for the Black-necked Crane. However, in lower reaches, the gradient increases as the river flows off the plateau through narrow, deep V-shaped valleys and the steep slopes and fast-flowing torrents are not crane-friendly habitats.

We used the direct counting method to search for the Black-necked Crane. At vantage points chosen for their extensive views, we used a telescope to search the appropriate habitats on either side of the road. The positions of cranes were determined by the simultaneous use of compass and wireless GPS Logger (Holux M-241), to record coordinates and direction relative to the observation points. Then we used frame-of-reference and cross-locating methods to position cranes on the map. In addition, we interviewed residents to obtain information on where cranes had been seen to help improve the efficiency and accuracy of the survey. They were also questioned about historical distribution, time of