

Conservation implications of song divergence between source and translocated populations of the North Island Kōkako

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Summary

1. Translocation of individuals from healthy source populations to newly colonize or recolonize suitable habitat is a vital tool for the conservation of a species. Demographic, genetic and landscape factors, but also acoustic signals and cultural factors, will all affect translocation success.

2. We investigated variation in song, and response to song, of the endangered North Island kōkako *Callaeas wilsoni* (Bonaparte 1850) in New Zealand in two translocated populations and their source population.

3. We found significant vocal variation between the source population and both translocated populations, the latter of which had reduced repertoire sizes and increased repertoire sharing, as well as structurally different song elements of higher frequency and shorter duration.

4. Despite the song divergence and clear variability in the nature and level of response among populations, we did not find any evidence for discrimination against nonlocal song in our reciprocal playback experiments.

5. *Synthesis and applications.* Vocal divergence and reduced variability in translocated populations suggest founder effects or reduced social interaction rates. The variation could be viewed as cultural erosion and may undermine translocation success. Persistence of response to playback, despite vocal divergence, suggested that social restrictions on gene flow require at least a few decades of separation after translocation. The decision to translocate individuals of threatened species is becoming a more common tool for species conservation world-wide. We argue that it is important to take vocal variation into account during such management decisions as it may affect success of establishment and persistence of translocated populations.

Key-words: behavioural conservation, population isolation, songbird, translocation, vocal divergence

Introduction

Translocation of individuals from threatened to safe habitat patches within a former range can be a last-option conservation strategy to fight extinction in declining species (Griffith *et al.* 1989). In the current context of species decline, translocations are crucially important to protect and restore biodiversity, as shown by the trend of increasing avian transfers from the wild over the past 100 years (Fig. 1). Previous research on translocated populations has addressed management issues (e.g. Scott & Carpenter 1987; Wolf *et al.* 1996), ecological aspects (e.g.

Armstrong *et al.* 1999; Jamieson & Wilson 2003) and also genetic consequences (e.g. Storfer 1999). Translocated populations may face reduced genetic diversity, and greater genetic divergence from source populations, due to founder effects and genetic bottlenecks, which may threaten the survival of populations (Frankham 1996; Segelbacher, Manel & Tomiuk 2008). Although little is known, negative effects may also arise through reduced behavioural diversity and faster rates of cultural evolution (Clemmons & Buchholz 1997; Håkansson & Jensen 2005; Laiolo & Jovani 2007).

New Zealand is one of the world's 25 biodiversity hotspots (Myers *et al.* 2000) and is also one of the places most dramatically affected by habitat destruction and the intro-

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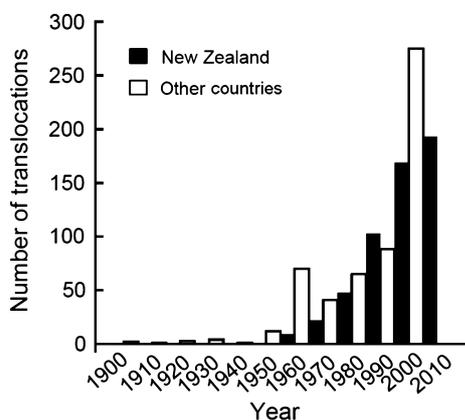


Fig. 1. World-wide (New Zealand and other countries) number of avian translocations every 10 years from 1900 to 2010 (data from Lincoln Park Zoo 2012).

duction of nonnative species (Saunders & Norton 2001; Kelly & Sullivan 2010). Invasive herbivores and predators have had a severe impact on bird habitat and local or global extinction. Intensive pest control in mainland reserves over the last 20 years has allowed numerous translocations of threatened species back to remnants of habitat where they used to occur (Armstrong & McLean 1995; Clout 2001; Saunders & Norton 2001). Translocation has been a successful conservation tool in New Zealand, and it has served as a model implemented world-wide since the 1950s (Taylor, Jamieson & Armstrong 2005; Jamieson, Wallis & Briskie 2006; Fig. 1).

Cultural variation and local song diversity have recently been put forward as potentially critical factors in conservation biology (Gaunt & McCallum 2004; Laiolo 2010) and may affect translocation projects. Geographic variation in birdsong has been well studied in relation to the function and evolution of animal behaviour (reviews in Slabbekoorn & Smith 2002; Podos & Warren 2007). In bird species that learn their vocalizations, young individuals typically copy song elements from parents or other local adults. Copying inaccuracies can accumulate and result in cultural drift and rapid vocal divergence among populations (Ellers & Slabbekoorn 2003). Such vocal differentiation can affect individual success in territory establishment and reproductive activity and thereby alter gene flow between populations (Grant & Grant 1996; Slabbekoorn & Smith 2002). Species-specific heritable song characteristics (e.g. duration, frequency bandwidth and tonality) and learning preferences can reduce variation and enhance species recognition. However, recognition of song variants and preference for local song may result in reproductive divergence (Slabbekoorn & Smith 2002). Vocal divergence of learned songs has also been linked to habitat fragmentation (Laiolo & Tella 2005; Laiolo & Jovani 2007) and may affect population viability (Laiolo *et al.* 2008). Hence, it is important to document song variation and its behavioural consequences in the context of reintroduction and restoration programmes, which are key tools for the Sustainable Biosphere Initiative (SBI) and the Interna-

tional Union for the Conservation of Nature (IUCN) (Sarrazin & Barbault 1996).

The North Island kōkako *Callaeas wilsoni* (Bonaparte 1850) is an endangered songbird endemic to New Zealand (BirdLife International 2009). Kōkako were once widespread on the North Island, but there are currently only 11 surviving and another 11 reintroduced populations in isolated areas with suitable habitat (e.g. Lavers 1978; Innes *et al.* 1999; Basse, Flux & Innes 2003). The initial population decline was due to habitat loss and fragmentation, while more recently several introduced mammalian predators have posed a greater threat. The Department of Conservation has implemented predator control programmes in surviving populations since the 1980s and has also translocated kōkako to areas with both suitable habitat and pest control (Innes *et al.* 2006; Sinclair, Innes & Bradfield 2006; Molles *et al.* 2008). Between 1981 and 2005, 71 individuals were transferred to offshore islands and 117 individuals were transferred to mainland areas between 1996 and 2010 (listed in Molles *et al.* 2008). Recent evidence suggests that kōkako may learn songs after dispersal and prefer to copy local or nearby songs (Valderrama 2012). Previous studies have addressed duetting behaviour (McLeod 1998; Molles, Hudson & Waas 2006) and acoustic variation within and between populations (Hudson 2001; Innes *et al.* 2006; Molles, Hudson & Waas 2006), but there is currently no insight into the behavioural consequences of the variation in these learned vocal traits for translocated populations.

In the current study, we describe vocal variation among three North Island kōkako populations: two populations established by translocation efforts and their source population. We hypothesized vocal divergence would occur in spectral and temporal structure and in a lack of song sharing among populations, and that divergence from the source population would be greater for the first-established translocated population. Furthermore, we hypothesized that vocal divergence among translocated populations and the source population would yield reduced behavioural response levels to song from nonlocal birds. The latter hypothesis was tested using a reciprocal playback experiment with source- and translocated-population songs in the three populations. Our results provide insights into cultural divergence and the potential emergence of behavioural barriers to reproduction, which could be critical for directing future conservation efforts and planning more translocations.

Materials and methods

STUDY SITES

We conducted our study in three isolated mainland reserves (Fig. 2). Te Urewera National Park, the source population, sustains the largest surviving population of North Island kōkako. In total, Te Urewera has been estimated to harbour 366 birds in five intensively managed core areas, 21.5% of all extant kōkako (Moorcroft *et al.* 2010). We conducted our experiment in Otamatuna (38°20'3"S, 177°83'8"E), the largest core area (25.3 km²),

where an estimated 224 individual kōkako reside. Between 2001 and 2007 kōkako were transferred from Otamatuna to two areas. In 2001, five pairs were transferred to Boundary Stream Mainland Island (T1), held in a captive breeding programme for 3 years and then released along with their offspring in 2004. In 2007, another 10 birds from the same source population were released. Boundary Stream Mainland Island covers an area of 8 km² in the Maungaharuru range in the south-east of the North Island (39°6'24"S, 176°49'5"E). Only 3.62 km² in T1 are covered with old-growth podocarp (*Podocarpaceae*) broadleaf forest, and 10 pairs and nine single kōkako surviving at this site may have faced greater spatial restriction and competition over resources than suggested by the overall reserve size. At the time of this study, T1 comprised seven founders from the first introduction plus five of the founders' offspring and three females from the second translocation. Ngapukeriki Reserve (T2) is located in the eastern Raukumara ranges (37°50'33"S, 177°42'10"E) and comprises an area of 4.5 km². In 2005, 19 birds from Otamatuna were translocated to the reserve; the estimated population in 2010 was 18 kōkako. T1 is separated from the source population by around 90 km, and T2 is about 65 km away from the source.

Yearly monitoring surveys have been carried out by the Department of Conservation in both translocated populations. We thoroughly surveyed three areas in Otamatuna in 2009, where we collected recordings and conducted our playback experiment. We determined population density as the number of neighbours within minimum convex polygons. Euclidean distances to the nearest neighbour in each population were also calculated. The mapped locations of the pairs correspond to approximately the centres of their territories. We used Quantum GIS (Quantum GIS Development Team 2010) for spatial analyses. The density in the source population was considerably greater (0.62 individuals per hectare) compared with T1 and T2 (0.14 individuals per hectare for both T1 and T2). Furthermore, the nearest neighbour distances were significantly greater in the translocated populations than in the source population ($\chi^2 = 27.31$, d.f. = 2, $P < 0.01$). The mean distance (\pm SE) between neighbouring pairs

was 279.59 m (± 26.77) in T1, 324.86 m (± 48.72) in T2 and 125.43 m (± 12.70) in the source population.

SONG DIFFERENTIATION

We collected recordings of natural song (i.e. not simulated by playback) in all three populations between 2009 and 2011. Focal pairs were recorded between 0500 and 1000 h using Sennheiser directional microphones (ME66, ME67 and MKH416; Sennheiser, Berlin, Germany) and Marantz portable PC card recorders (PMD660; D&M Professional, Kanagawa, Japan). All recordings were collected in a Wave file format at a 44.1 kHz sampling rate and 16 bits per sample. We visualized (using SYRINX-PC; J. Burt, Seattle, WA, USA) and classified all songs collected into phrase types (a stereotyped short sequence of one or more syllables) to document phrase type repertoires for 11 pairs from the source population and eight pairs from each translocated population. We sampled the complete repertoire of phrase types for all target pairs based on stabilized curves of cumulative number of phrase types vs. number of phrase types recorded. We calculated the repertoire of phrases for each pair recorded and an overall repertoire for each population. We characterized the spectral and temporal structure of the songs from each population by measuring one example of each phrase type recorded from every pair. We measured peak frequency, fundamental frequency, peak frequency of the loudest harmonic, minimum frequency and maximum frequency, as well as duration and number of syllables. All measurements were made using AVISOFT SASLAB PRO, version 5.1.14 (Avisoft Bioacoustics, Berlin, Germany). Spectrograms were produced with a fast Fourier transformation of 1024 points, 25% frame size, 224 Hz bandwidth (43 Hz resolution) and 93.75% overlap on a Hamming Window. We used high- and low-pass filters to reduce noise at frequencies out of the kōkako song range (<0.25 and >15 kHz) and also spot-filtered other species' song before making measurements.

We conducted 351 pairwise comparisons using Jaccard's similarity coefficient to calculate phrase repertoire sharing among individuals within and among all three populations. The Jaccard

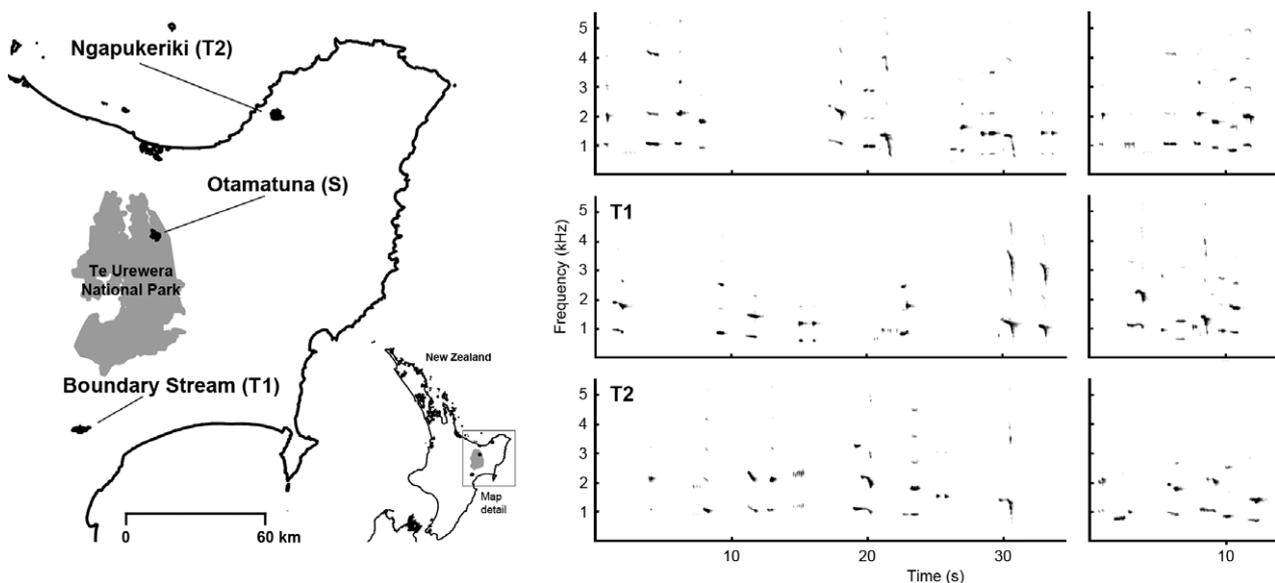


Fig. 2. Map of the three study areas and corresponding example sonograms of long (left panels) and short (right panels) themes from the source (S) and the two translocated populations (T1 and T2).

coefficient was calculated as $s_{ij}/[s_{ij} + s_i + s_j - c_{ij}]$, where s_{ij} is the number of phrases common to pairs i and j ; s_i is the number of phrases in the repertoire of i but absent from the repertoire of j (s_j is the reciprocal); we controlled for different sample sizes (e.g. length of recording per pair) by incorporating the difference in phrase repertoire size between each of the two pairs compared as the term c , an adjustment that has been shown to improve the robustness of song similarity estimates (Tracy & Baker 1999; MacDougall-Shackleton *et al.* 2009). Jaccard's coefficients can range from a minimum of 0 when there is no similarity, up to 1 when all song elements are shared between two pairs.

PLAYBACK EXPERIMENT

Stimulus preparation

We selected and processed songs with the highest available signal-to-noise ratio. We used a band-pass filter procedure in Adobe AUDITION version 3.0 to remove noise below 0.3 kHz and above 15 kHz. We also faded the volume of other bird sounds that did not overlap *kōkako* song frequencies to background noise level to avoid playing other species sounds with the stimuli. Finally, we normalized all the songs to optimally exploit the available dynamic range. Male and female *kōkako* sing duets comprising stereotyped sequences, or 'themes', of up to eight phrases produced with immediate variety (every subsequent phrase type is a different one from the available repertoire); these themes are typically shared by all members of a song neighbourhood (Molles, Hudson & Waas 2006). We selected one long theme and one short theme from each pair in each population for use as playback stimuli (Fig. 2). For each stimulus, the long theme was followed by a natural pause length of silence and the subsequent short theme; this pattern was then repeated once yielding a total of 4 themes per stimulus. We used themes from different pairs for each trial to reduce potential problems of pseudo-replication (Slabbekoorn & Bouton 2008). In addition, subjects and stimulus pairs were selected from at least two territories apart to reduce the impact of familiarity with immediate neighbours.

Experimental design

We tested a total of 23 pairs between the 16th of August and the 16th of November 2010. Six pairs were tested in T1: one female in this group was released in 2007 (tested 3 years post-translocation), while all other birds tested were founders or their offspring (9 years post-translocation). Eight pairs were tested in T2 (5 years since translocation; focal birds included founders and their offspring). We presented each focal pair with two playback treatments: (i) songs from the source population and (ii) songs from their own translocated population. A reciprocal experiment was performed by testing nine pairs in the source population, where we presented four pairs with T1 songs (9 years since translocation) and five pairs with T2 songs (5 years since translocation). Comparisons between vocal responses and proximity measures yielded no differences based on the origin of translocated-population stimuli. Therefore, we pooled data from T1 and T2 stimuli for the analysis of responses in the source population. Similarly, responses of founder pairs and offspring in translocated populations were combined for analysis as their responses did not differ. We balanced the order of the treatments in all populations by switching the treatment order for each new pair tested.

Pairs from contiguous territories were tested at least 2 days apart to avoid an impact of previous playback-induced activity levels of neighbours on current response strength to playback in their own territory. We recorded natural singing behaviour before presenting subjects with stimuli to allow comparisons of vocal behaviour before and after playback.

Playback procedure and acoustic variables

We conducted our trials between 0600 and 0900 h. Each trial included a 10-min period before any playback was broadcast, during which we recorded natural vocalizations. Immediately after the preplayback period, we set up a single wireless speaker and playback system (Foxpro FX5, www.gofoxpro.com), positioned 3 m above the ground and facing up (to avoid potential bias due to directionality associated with neighbour positions relative to the territory), and 5–10 m from the song post used by the focal birds during the preplayback period. We then started playback using the speaker remote control. Speaker volume was standardized at 70 dB(A) for the peak amplitude at 1 m from front of the speaker. Each trial began with one of the two stimuli (local or nonlocal; duration 130 s), followed by a 10-min silence. This postplayback period was followed immediately by the second stimulus (duration 130 s) and a second 10-min silent period. Consequently, pairs were recorded and monitored before, during and after playback for almost 35 min. Two observers, blind to the order of stimulus categories, recorded approach and vocal responses using, respectively, an M-Audio Micro Track 24/96 digital recorder with a portable clip microphone and a Marantz portable PC card recorder (PMD660) with a Sennheiser directional microphone (ME67). Prior to trials, observers were trained to estimate distances to one object and between two objects. Approach variables included (i) proportion of time spent ≤ 15 m from the playback speaker and (ii) proportion of time pair members spent ≥ 10 m apart when they were 0–15 m from the speaker. We used SYRINX-PC software (J. Burt) to visualize our sound recordings and calculated the following vocal response variables during postplayback periods: (i) singing rate (i.e. number of phrases per second), (ii) phrase type rate (i.e. number of different phrase types per second), (iii) Shannon-Wiener diversity index for phrase types produced, (iv) innovation versatility (i.e. number of switches to a new phrase type relative to the total number of switches, taking into account the available number of different phrase types relative to the total number of phrases produced) and (v) number of new phrase types relative to the phrase types sung during the preplayback period.

STATISTICAL ANALYSIS

Song similarity within and between populations was evaluated using a Kruskal–Wallis test. We performed a principal component analysis (PCA) to obtain composite variables that describe spectral characteristics of the songs from the three populations. Prior to analysis, correlations were examined to avoid including redundant measures in composite variables. Analysis for sampling adequacy and sphericity of variables were performed using Kaiser–Meyer–Olkin and Bartlett's tests. Subsequently, we used the composite variable that explained most of the variance in the spectral measurements to compare spectral characteristics of song among our focal populations through analysis of variance

(ANOVA). We also used the resulting composite variables in paired *t*-tests to compare spectral characteristics of phrase types between pre- and postplayback periods.

We analysed the effects of playback on further aspects of singing behaviour with linear mixed-effects models (GLM). We performed two separate GLMs fitted by Restricted Maximum Likelihood (REML), as the data were unbalanced (Robinson 1987). We included 'playback period' as a fixed effect and 'pair' as a random variable in each of the two models. In the first model, for each variable, we included preplayback and the first postplayback period, regardless of the stimulus type, to evaluate whether playback had an effect on vocal output (i.e. singing rate, phrase type rate, versatility and diversity). The second model for each variable distinguished responses to local and foreign stimulus types. We modelled the effects of population and stimulus type along with playback period and interactions, as fixed effects; pair was included as a random effect, nested within stimulus type. We removed nonsignificant interactions in a stepwise fashion, using Akaike Information Criterion (AIC) to select the best fit model.

We analysed spatial responses to playback (proportion of time that birds spent less than 15 m from the stimulus speaker and proportion of time that pair members were <10 m apart) using generalized estimated equations (GEE), an extension of generalized linear models that allows nonnormal and correlated data or repeated measures (e.g. two post-playback periods for the same pair). We included 'pair' as random effect to account for repeated observations on each pair. A Poisson-gamma distribution (Tweedie with *P* of 1.5 with logit link function) that admits zeroes was used based on the lowest value of Quasi Likelihood Independent Model Criterion (QIC) for the response variables. *SPSS* version 15.0 (SPSS, Chicago, IL, USA) was used for all statistical analyses.

Results

SONG DIVERGENCE

The composite spectral characteristics of the songs were significantly different between populations ($F = 35.23$, d.f. = 2, $P < 0.001$; Fig. 3). The primary differences were

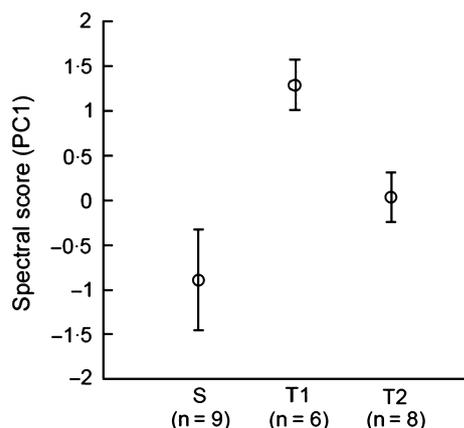


Fig. 3. Comparative mean values (2 SE) of the principal component analysis composite variable representing differential spectral characteristics between source (S) and translocated populations (T1 and T2).

that phrases sung by birds from the source population were typically longer and had lower frequencies than those sung by birds in both translocated populations (Table 1). In most cases, the range of variation for spectral characteristics in translocated populations was similar to or smaller than that of the source population (Table 1).

We determined the phrase repertoire size to be 65 for the source population of Otamatuna ($n = 11$), 40 for the first translocated population (T1-Boundary Stream, $n = 8$) and 27 for the second translocated population (T2-Ngapukeriki, $n = 8$). Our song similarity measure showed that there was high similarity within the source population and within translocated populations, but low similarity among the three populations (Fig. 4). Furthermore, population T2 (later-translocated) showed significantly higher within-population song similarity, in comparison with population T1 (earlier-translocated) and the source population ($\chi^2 = 13.02$, d.f. = 2, $P < 0.01$). We also found that songs from the source population were more similar to the songs from population T2 than to the songs from population T1 ($t = 7.89$, d.f. = 62.85, $P < 0.01$). T1 songs were as similar to the source as they were to T2 songs ($t = -1.00$, d.f. = 9.8, $P = 0.34$). We found a total of five unique phrase types in T1 and 18 shared with T2, whereas just 2 phrase types were only found in T2 (not present in S or T1). From the total repertoire in the Source population, 20 phrases were not present in T1 and 35 were not present in T2.

PRE- VS. POSTPLAYBACK SINGING

There was a significant effect of playback on singing rate (GLM: $\chi^2 = 7.53$, $P = 0.01$) and phrase type rate (GLM: $\chi^2 = 3.80$, $P = 0.05$) compared with natural song (preplayback). However, versatility (GLM: $\chi^2 = 1.23$, $P = 0.27$) and diversity (GLM: $\chi^2 = 1.04$, $P = 0.31$) were not affected by playback. Effects of playback varied across the study populations; vocal output changed after playback in the source population and in T2 but not in T1 (Fig. 5). The singing rate decreased in the source, and T2 birds considerably decreased their overall vocal output after playback with reduced singing rates and phrase type rates. We did not find any significant changes in the overall performance of the same phrase types from pre- to postplayback (PC1: $t = 0.46$, d.f. = 219, $P = 0.64$; PC2: $t = -0.0004$, d.f. = 219, $P = 0.99$). Similarly, the overall spectral characteristics (e.g. frequency, duration, number of syllables) of the total composition of phrases sung before playback did not change significantly after playback.

RESPONSE TO LOCAL VS. NONLOCAL SONG

We found a lack of discrimination between local vs. nonlocal song within populations, but significant variation between populations in overall response levels (Fig. 5 and 6; see Table S1, Supporting information). Among the song performance variables, only versatility was affected

Table 1. Differences in temporal and spectral song structure between source and translocated populations

Variable	S [†]	T1 [‡]	T2 [¶]	<i>F</i>	<i>P</i>
<i>N</i>	9	6	8		
Number of phrases	22–37 30.00 ± 1.41	18–28 23.5 ± 1.72	18–26 22.25 ± 1.49	8.14	0.003*
Number of syllables	1–8 (2.37 ± 0.09)	1–8 (2.24 ± 0.13)	1–7 (2.07 ± 0.15)	1.53	0.241
Duration of phrases (s)	0.05–1.81 (0.63 ± 0.01)	0.05–1.81 (0.53 ± 0.02)	0.07–1.72 (0.62 ± 0.02)	7.35	0.004*
Peak frequency (Hz)	523.33–2620 (1302.41 ± 31.53)	725–2880 (1408.63 ± 23.18)	655–3010 (1385.05 ± 25.57)	3.94	0.036*
Fundamental frequency (Hz)	380–1921 (869.78 ± 9.83)	620–1370 (967.72 ± 11.41)	555–1390 (913.81 ± 10.93)	19.75	< 0.001*
Peak frequency of the loudest harmonic (Hz)	860–3960 (2145.77 ± 38.47)	1290–5380 (2440.79 ± 68.94)	1135–3780 (2287.59 ± 38.01)	9.58	0.001*
Minimum frequency (Hz)	210–1278 (582.17 ± 8.41)	300–1070 (685.01 ± 13.96)	250–1340 (635.34 ± 11.52)	21.13	< 0.001*
Maximum frequency (Hz)	710–4342 (1483.20 ± 29.45)	860–4170 (1768.81 ± 95.23)	770–4170 (1593.78 ± 56.58)	5.72	0.011*

Values for each population are given as ranges and means ± SE.

[†]Source population.

[‡]First translocated population.

[¶]Second translocated population.

*Significant differences between groups.

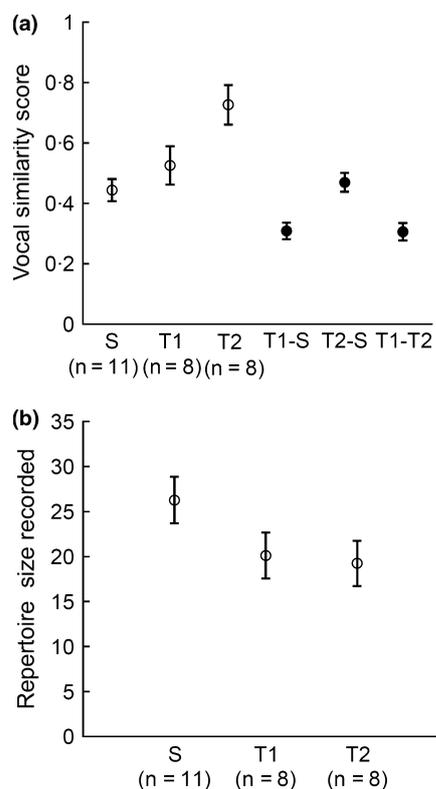


Fig. 4. The vocal divergence of song elements between the source (S) population and two translocated populations (T1 and T2). (a) Mean Jaccard (SE) indices comparing songs shared within and between S, T1 and T2. Open circles indicate within-population song similarity; solid circles indicate song similarity between populations. (b) Mean repertoire size (SE) for the three study populations.

by playback treatment (GLM: $\chi^2 = 6.19$, d.f. = 1, $P = 0.01$; see Table S1, Supporting information), and phrase type rate was affected by the interaction between population and playback treatment (GLM: $\chi^2 = 13.89$, d.f. = 2, $P = 0.001$; see Table S1, Supporting information). In contrast, several aspects of vocal response differed among the populations. T1 presented the highest singing rate and number of phrase types, followed by the source population, in contrast to the low singing rate and reduced number of phrase types of T2 (Fig. 5). Pairs in T2 also sang at a significantly lower rate and used fewer phrase types than pairs in T1 and S in response to both playback stimuli (Fig. 5).

We found significant differences among populations in the number of new phrase types that birds added in response to local and nonlocal song relative to the repertoire recorded before playback (GLM: $\chi^2 = 23.14$, d.f. = 2, $P < 0.0001$; see Table S1, Supporting information). T1 birds incorporated more new phrase types than other populations in response to both playback types, with the difference being greater for nonlocal song playback (Fig. 5c). T2 pairs incorporated relatively few new phrase types into their responses to both playback treatments. The responses by pairs in the source population to local and nonlocal song were somewhat intermediate between T1 and T2 (Figs 5 and 7).

Generalized estimated equations models showed no effects of playback treatment (local vs. nonlocal) on time spent close to the speaker (GEE: Wald $\chi^2 = 0.07$, d.f. = 1, $P = 0.79$) or pair proximity (GEE: Wald $\chi^2 = 0.21$, d.f. = 1, $P = 0.65$). However, there were significant differences among populations (GEE pair proximity: Wald $\chi^2 = 11.89$, d.f. = 2, $P = 0.003$; GEE speaker proximity:

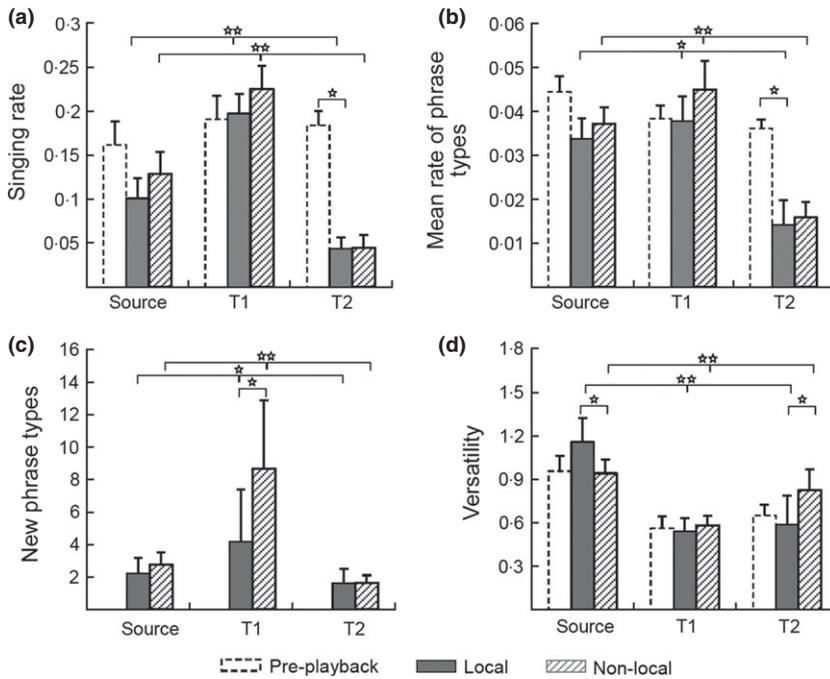


Fig. 5. Mean vocal output (SE) recorded in each population during preplayback, after local stimulus, and after nonlocal stimulus. Significant effects are indicated by stars: (a) singing rate (GLM population effect: $\chi^2 = 23.69$, $P < 0.0001$); (b) phrase type rate (GLM population and treatment effect: $\chi^2 = 13.89$, $P = 0.001$); (c) new phrase types after playback (GLM population effect: $\chi^2 = 23.14$, $P < 0.0001$); (d) versatility (GLM population effect: $\chi^2 = 42.52$, $P < 0.0001$; treatment effect: $\chi^2 = 0.26$, $P < 0.0001$).

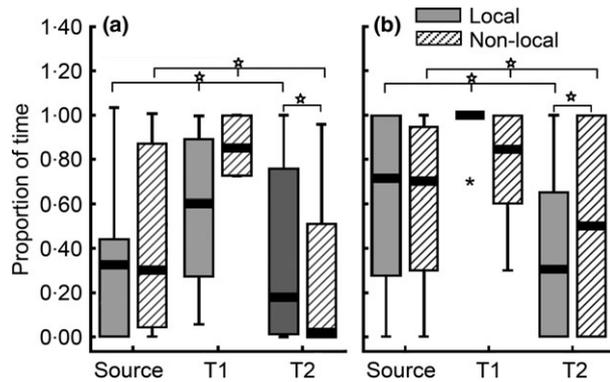


Fig. 6. (a) Proportion of time spent within 15 m of the speaker, and (b) proportion of time pair members spent <10 m apart in each population (Source, S; translocated populations, T1 and T2). Stars over horizontal bars indicate significant response differences between playback type and across populations.

Wald $\chi^2 = 7.49$, d.f. = 2, $P = 0.02$). During the Source treatment, pairs from T2 (later-translocated) spent considerably less time close (<15 m) to the speaker and less time closer to each other, than did pairs in T1 (Fig. 6).

Discussion

We have shown divergence in song and playback response between translocated and source populations of the endangered North Island kōkako. Song elements were of higher frequency and of shorter duration in songs from translocated populations. Furthermore, singing behaviour of birds in two translocated populations differed significantly from their source population in having smaller individual repertoire sizes and higher repertoire sharing among pairs. In our reciprocal playback, kōkako pairs tended to respond

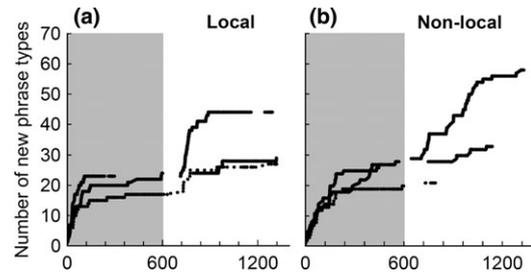


Fig. 7. The cumulative number of new phrase types incorporated by two representative pairs from each population (Source, S; translocated populations, T1 and T2) during the preplayback period (grey interval) through the playback period for (a) local and (b) nonlocal playback treatments.

with equal strength to local and nonlocal songs. However, responsiveness in the translocated populations differed in two ways from the responsiveness in the source population. In the oldest and acoustically most divergent population (T1), pairs spent more time close to simulated intruders and exhibited stronger vocal response (singing rate and phrase type rate). In the more recent and less divergent population (T2), pairs exhibited overall low levels of vocal response and approach to the stimuli.

ACOUSTIC DIVERGENCE IN TRANSLOCATED POPULATIONS

We found differences among translocated and source populations which were reminiscent of differences among fragmented but nontranslocated populations (Valderrama 2012). There are several explanations possible for the kinds of vocal divergence reported here for kōkako. First and maybe most likely, there may be cultural founder effects or

sampling issues (Baker 1996; Parker, Hauber & Brunton 2010). For example, small repertoire sizes in the translocated populations may be determined by the restricted subset of songs sung in the sample of birds that was translocated. Divergence in the number of unique phrase types may to some extent also depend on the current sample sizes for both source and translocated populations; more sampling could have discovered more unique phrase types. Recent evidence shows that there is a positive correlation between population size and song characteristics such as repertoire size and diversity, and a negative effect of population size on song sharing and syntactical structure in longer-isolated surviving populations as well as translocated populations of kōkako (Valderrama, Molles & Waas 2012).

Alternatively, divergence of vocal repertoire composition among source and translocated populations could have developed over the 5–8 years of separation. Random changes that occur independently of population size or that may be attributable to the small number of individuals can accumulate over time (e.g. Lynch & Baker 1993; Lachlan & Slater 2003). It seems likely that such changes related to individual song learning plasticity and copying accuracy are also apparent in kōkako. A study on natural populations with low population connectivity, but uninhibited dispersal, suggested that post-dispersal song learning and drift may accelerate vocal divergence (Valderrama 2012).

In addition to cultural bottlenecks and cultural evolution, it is theoretically also possible that the specific genotypes of translocated birds determined acoustically divergent subsets of singing abilities and associated song features. However, although we cannot exclude this explanation, genetic differentiation seems at least not essential to get significant song variation among kōkako populations (c.f. Ellers & Slabbekoorn 2003). There was no obvious relationship between genetic and song variation among natural populations in fragmented habitat, which showed little genetic differentiation but similar patterns of song divergence as in the current study with translocated populations (Valderrama 2012).

Studies on the Dupont's lark *Chersophilus duponti* (Vieillot 1824) in Spain (Laiolo & Tella 2005; Laiolo *et al.* 2008) also showed that fewer males in smaller habitat fragments led to an increase in repertoire sharing among neighbours and smaller individual and population repertoire sizes, which is similar to the two translocated kōkako populations. Laiolo & Tella (2005) argued that the increased value of vocal interactions for mediating conflicts over limited resources could have resulted in higher levels of repertoire sharing within population fragments. We believe that the kōkako may be comparable to the larks in that a lack of vocal interactions and connectivity may explain divergence among population fragments in vocal repertoire composition, while limited resources may play a particularly strong role in one of the translocated populations (T1). The reduced repertoire size in birds from both translocated populations relative to birds in the source population may therefore be related to increased levels of territorial interac-

tions or just a reduced number of long-term neighbours (as found in other species: Koetz, Westcott & Congdon 2007; Rivera-Gutierrez *et al.* 2010).

Not only how kōkako sing in translocated populations, but also what they sing differs from kōkako in the source population. Geographic variation in acoustic structure may reflect ecologically neutral features related to random changes yielding patterns of isolation-by-distance (reviewed in Podos & Warren 2007), variation in the impact of sexual selection (Prumm 1998; Price & Lanyon 2002) or variation in environmental selection pressures related to sound transmission and noise interference (Richards & Wiley 1980; Slabbekoorn & Smith 2002). The sites that now support the two translocated study populations were chosen by the Department of Conservation because they lie within the former distribution of kōkako (Lavers 1978) and comprise habitat highly similar to the habitat of the source population (Leathwick, Overton & McLeod 2003). We therefore believe that the higher and shorter notes in the translocated populations are unlikely to be due to differences in environmental selection pressures.

RESPONSE VARIATION IN RECIPROCAL PLAYBACKS

Despite differences in the nature of the playback response between one of the translocated populations and the source population and relatively low response levels in the other translocated population, there was little evidence for discrimination against foreign population songs in any of the populations. Many studies have shown reduced response levels to nonlocal population songs associated with geographic distance (Searcy, Nowicki & Hughes 1997; Nelson & Soha 2004) or ecological divergence (Dingle *et al.* 2010; Ripmeester *et al.* 2010). The kōkako from our recorded populations all originated from the same geographic area (actual translocated individuals or first-generation offspring) and although songs sung may have diverged, songs memorized may still overlap more, which may explain the lack of discrimination. Changes in songs over time within the same population may also yield a reduced response level as reported for a species with shorter generation time than kōkako; the white-crowned sparrow *Zonotrichia leucophrys* (Forster, JR 1772) tested with playback of current and historical recordings (20 years old; Derryberry 2007). In two surviving populations of kōkako separated for longer (>100 years), a recent study did find a stronger response to local vs. foreign song (Bradley, Molles & Waas 2012). Also, a study on New Zealand's North Island Saddleback *Philesturnus carunculatus* (Lesson 1828), which is closely related to the kōkako, revealed discrimination between local and nonlocal song playback (21–40 years after separation; Parker, Hauber & Brunton 2010).

The evidence on population divergence and discrimination of population-specific songs in various species, as reported above, indicates that responsiveness to territorial songs is expected to fade with divergence over time at

some point. However, our study shows that there is also a minimum time required to allow divergence and that just song divergence is not sufficient. The kōkako in our study have been separated by translocation for <10 years, and recognition and/or discrimination among kōkako could be affected by the memory of a number of translocated birds, despite some dramatic social changes inherent to translocation (capture and forced divorce, differing periods of captivity, and resettlement). While there is no indication yet of a reduction in mutual response levels, we believe that given the current divergence in song features, it is a matter of time also for the translocated kōkako to develop population-dependent song discrimination.

IMPLICATIONS FOR CONSERVATION

Geographic variation in songs used for territorial defence and mate attraction can be important for conservation in several ways (Håkansson & Jensen 2005; Laiolo 2010). Regardless of the causes of change, reduced repertoire sizes and increased repertoire sharing rates may have consequences for individual reproductive success, possibly leading to lower efficiency in deterring competitors or in attracting and stimulating mates. Two key aspects in reintroduction management are population establishment and population persistence (Armstrong & Seddon 2008). Along with translocation factors such as number of individuals released, post-release dispersal and mortality, ineffective vocal communication may substantially reduce the effective size of translocated populations, thus reducing the likelihood of establishment. To ensure long-term population persistence, post-release adaptive management may require multiple transfers of birds to increase genetic diversity (Armstrong & Seddon 2007) while maintaining some level of cultural homogeneity, which may be necessary to maintain behavioural compatibility at a metapopulation level.

Another potential concern for management is that lower vocal activity levels in translocated populations may lead to reduced immigration or retention of birds into already small clusters of active breeders (Laiolo *et al.* 2008). Recent reports have also confirmed that the numbers of birds present in translocated populations of mixed origin may not reflect numbers of active breeders as birds seem to pair assortatively, potentially based on acoustic recognition of source population (Rowe & Bell 2007; Bradley 2012).

Consequently, we believe that kōkako translocations are more likely to succeed if source populations are sufficiently large or close together to provide a culturally homogenous reservoir of birds for translocation and comprise habitat similar to that at the release site. Small translocated populations may require an influx of new individuals through subsequent translocations to further supplement the genetic diversity. Our playback results indicate that periods of separation of up to a decade are unlikely to impose vocal restrictions. However, supplemental translocations after longer periods of isolation may be less likely to succeed as we did find significant

divergence in song characteristics. Obviously, we still only have very limited insight and more studies are required to investigate rates of acoustic divergence, emergence of discrimination tendencies and the impact on individual reproductive success and population viability of heavily endangered species such as the North Island kōkako (Laiolo *et al.* 2008; Parker, Hauber & Brunton 2010).

CONCLUSION

Our study highlights the potential importance of bioacoustic analyses during translocation efforts. The reduced variability in vocal behaviour, reported here for two translocated kōkako populations after less than a decade of separation, has the potential to undermine population viability but also to signal critical stages of establishment to conservationists. The divergence of recognition in translocated saddlebacks after more than two decades of separation (Parker, Hauber & Brunton 2010) and the lack of discrimination in our translocated kōkako provides us some insight into the pace at which song-dependent behavioural barriers arise, which may also play a critical role in population viability through the impact on connectivity among different translocated and natural populations. However, more studies are required in terms of both the number of translocated populations within species and the number of different species to form a better picture of the optimal conservation approach. Nevertheless, we believe that bioacoustic analyses of song divergence could provide an important and relatively noninvasive tool for monitoring and predicting success of extreme conservation efforts such as translocation of threatened bird species.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Comparative significance of response variables to stimuli between populations.