Avian population trends in *Scalesia* forest on Floreana Island (2004-2013): Acoustical surveys cannot detect hybrids of Darwin's tree finches *Camarhynchus* spp.

KATHARINA J. PETERS and SONIA KLEINDORFER

Summary

Floreana Island has the highest proportion of local land bird extinctions on the Galápagos Archipelago, and is home to the range-restricted and critically endangered Medium Tree Finch *Camarhynchus pauper*. We used acoustic surveys during 2004, 2008 and 2013 to compare the estimated population size of *C. pauper* and other land bird species in a remnant patch of *Scalesia* forest. First, we compared song in *C. pauper* and *C. parvulus* and the recently discovered *Camarhynchus* hybrid group to justify our use of acoustic surveys to detect population trends given contemporary hybridisation between *C. pauper* and *C. parvulus*. Song differed significantly between *C. pauper* versus *C. parvulus* and hybrid birds, but not between *C. parvulus* versus hybrid birds. Second, we compared population size estimates. *Camarhynchus pauper* declined by 52% between 2004 and 2013 (with a 10% increase since 2008); *C. parvulus*/hybrid increased by 45% between 2004 and 2013 (with 28% decrease since 2008). In 2013, there were ~ 419 *C. pauper* males in the *Scalesia* forest (estimate for *Scalesia* habitat only) and ~ 2,537 males on Floreana Island (estimate for the entire available highland habitat). Not all species showed a pattern of decline in the highland *Scalesia* habitat between 2004 and 2013: *Dendroica petechia* (+256%), *Crotophaga ani* (+254%) *Geospiza fuliginosa* (+23%), and *Myiarchus magnirostris* (+11%) increased, while the ground finch *G. fortis* (-76%) decreased. Understanding why *C. pauper* is declining while other land bird species are increasing in the same habitat requires continued inquiry and monitoring efforts.

Introduction

Given the current rate of worldwide habitat and biodiversity loss (Baillie *et al.* 2004, Hails 2008) and the associated rise in the need for population status information, rapid population assessment techniques are becoming increasingly important. Declining populations in vulnerable ecosystems are especially in need of strategic monitoring to make informed decisions about conservation actions (Sinclair *et al.* 2009). Non-invasive survey techniques using reliable signals are a preferred option for population assessment in threatened species (e.g. Karanth and Nichols 2010), whereby species need to be distinguishable. This can be near impossible among some closely related species or in systems with cryptic species and/or hybrid individuals (Dawson and Efford 2009, Lambert and Mcdonald 2014). In such cases, individuals can often only be identified to species using genetic analysis, which requires sampling and sequencing and hence are costly and time-consuming procedures (Hebert *et al.* 2004). Therefore, it is highly desirable to identify traits by which individuals of a species can be clearly and efficiently classified in the field.

Animal vocalisations are widely used to estimate their abundance given the benefit of sound travelling across water and vegetation with little attenuation (Marten and Marler 1977). For this reason, both the presence and distance of an animal can be scored by an observer from afar (Scott *et al.* 1981). Animal vocalisations have been used in ecological surveys assessing species abundance across taxa including amphibians (e.g. Driscoll 1998), cetaceans (e.g. Marques *et al.* 2009) and birds (e.g. Dawson and Efford 2009, O'Connor *et al.* 2010c, Dvorak *et al.* 2012). Sometimes two closely related species may be morphologically similar but produce different vocalisations (Toews and Irwin 2008), but at other times vocal differences may be insufficient to justify their use as a species indicator. Given that 10–16% of bird species regularly hybridise (Grant and Grant 1992, Ottenburghs *et al.* 2015), hybridisation is an additional factor that increases challenges associated with acoustical monitoring.

Darwin's Finches are a model system for evolutionary biology with evidence for evolution and speciation by natural selection in the wild (e.g. Grant and Grant 2014a), but also for species and population decline due to anthropogenic impacts (O'Connor *et al.* 2010c, Dvorak *et al.* 2012). Human activity on the Galápagos has resulted in introduced species and pathogens (Parker *et al.* 2011) as well as habitat loss from increasing human population and agricultural activity (Watson *et al.* 2010). There is consensus about the importance of surveys to monitor endemic populations (Dvorak *et al.* 2004, 2012, O'Connor *et al.* 2010c), but Darwin's Finches are difficult to identify at the best of times given shifts in morphology from interspecific competition (Schluter *et al.* 1985) and rapid evolution (Grant and Grant 2014). Darwin's Finches also regularly hybridise (McKay and Zink 2014), as has been shown in ground finches *Geospiza* spp. (Grant and Grant 1997, Grant *et al.* 2003, 2005a) and tree finches *Camarhynchus* spp. (Kleindorfer *et al.* 2014a). Identifying changes in gene flow between Darwin's finch species and populations is both challenging and necessary to inform our understanding of evolutionary dynamics in this rapidly evolving system.

This study assessed the performance of acoustic survey techniques in a species group with hybridisation. We analyse song in Small Tree Finch *C. parvulus*, Medium Tree Finch *C. pauper*, and the recently discovered *Camarhynchus* hybrid group (Kleindorfer *et al.* 2014a) on Floreana Island, Galápagos Archipelago. While the Small Tree Finch is listed as 'common' and occurs on other islands, the Medium Tree Finch is endemic to Floreana and listed as 'Critically Endangered' (IUCN 2015). Morphological differences between these two parental species are slight (Kleindorfer *et al.* 2014a) which renders visual identification in the field inaccurate. Avian surveys that rely on song activity in the field are now being used in land surveys across the Galápagos Islands (Cunninghame *et al.* 2012). To inform Galápagos land bird surveys, we need to establish that song can be used to distinguish the three tree finch groups on Floreana Island. This study compares song characteristics between *Camarhynchus* tree finches on Floreana Island to test if species and the hybrid birds differ in song. Using these insights, we apply established techniques to assess changes in avian abundance from 2004 compared with 2008 and 2013. In addition to comparing population trends in Darwin's Finches, we analyse population trends in other bird species in our survey of the *Scalesia* forest, which is important habitat for tree finches. The findings across species creates a larger context for considering patterns in the tree finches.

Material and methods

Study site

The study site is situated at the base of the Cerro Pajas volcano on Floreana Island, Galápagos (173 km², 1°28'S, 90°48'W) and consists of humid highland forest dominated by the endemic tree *Scalesia pedunculata*. By 2010, 38% of the *Scalesia* habitat had been degraded through clearing for human settlement and agriculture (Watson *et al.* 2010). Our long-term study of Darwin's finches reveals growing threats from introduced plants, including creeping vines, which could

destabilise the system and require careful monitoring. Despite these challenges, this remnant *Scalesia* forest on Floreana Island is the largest on the Galápagos Islands; it has virtually disappeared from San Cristobal and Isabela Islands and only 1–2% remains on Santa Cruz Island (Watson *et al.* 2010, Dvorak *et al.* 2012). The *Scalesia* forest appears to be the preferred nesting habitat for tree finches (Peters and Kleindorfer 2015, Kleindorfer *et al.* 2016), though tree finches have been observed to sing from tall (> 10 m) non-*Scalesia* trees both within and adjacent to *Scalesia* habitat, and have been observed in the agricultural zones adjacent to the *Scalesia* forest (O'Connor *et al.* 2010b, O'Connor unpubl. data).

Study species

We analysed song characteristics of male Small Tree Finch *C. parvulus*, Medium Tree Finch *C. pauper*, and birds of the recently identified hybrid group, which are the result of pairings between *C. parvulus* and *C. pauper* (Kleindorfer *et al.* 2014a). We aimed to record a comparable number of songs for each of the three genetic groups (for details on genetic analyses see the extended methods in the online supplementary material); this balanced sampling was not achieved because we could only determine genetic assignment after data analysis and after song recordings had been made in the field. In total, we analysed morphology and song recordings from nine *C. parvulus*, 19 *C. pauper*, and 49 hybrid birds. While *C. parvulus* exists on several other Galápagos Islands, the Critically Endangered *C. pauper* only occurs on Floreana Island (Lack 1983, Grant 1986).

Comparing song between tree finches

We recorded the song of birds that had previously been colour-banded, measured and that were later assigned to a genetic group using analysis of nine microsatellite loci following Kleindorfer *et al.* (2014a) and Peters (2016). Darwin's finches do not appear to change their behaviour in the presence of human observers, and we were able to record song at close range (< 10 m) using either a Sony DCD-100 DAT recorder or a Sony WMD6 Cassette Recorder with Sennheiser ME 80 directional microphone in 2006, and a Marantz solid state recorder (model PMD661MKII) with either a Telinga Twin Science parabolic microphone or a Røde Precision broadcast-grade long shotgun microphone (model NTG8) from 2010 onwards. Recordings were made in the years 2006, 2010, 2013 and 2014 during the start of the breeding season between 07h00 and 10h00, which is the time of peak singing activity (Christensen *et al.* 2006). Like the song of other Darwin's finch species, tree finch song is simply structured and consists of one repeated syllable constituting a song (Bowman *et al.* 1983). We recorded up to 15 repetitions of the song of each individual bird. Since the song of tree finches is highly repeatable within individuals (Christensen *et al.* 2006), we selected the five best quality recordings per bird and used the mean of each song parameter for subsequent analysis with Raven Pro Version 1.4 for Mac OS X (http://www.birds.cornell.edu/raven). We measured and analysed the following song parameters: song duration (s), minimum frequency (Hz), maximum frequency (Hz), frequency bandwidth (Hz; calculated by subtracting the minimum frequency from the maximum frequency), dominant frequency, number of syllables, maximum number of syllables per song and trill rate (number of syllables/s). Spectrograms were created using a -24dB cut-off criterion relative to the peak power of the vocalisation with visual adjustment, following Podos (2001) and Goodale and Podos (2010).

Morphological analysis

Birds of the hybrid group have been reported to have intermediate body size between the smaller-bodied *C. parvulus* and the larger-bodied *C. pauper*, but there is much overlap in morphology (Kleindorfer *et al.* 2014a). We examined the following morphological variables across

genetic groups: beak-head (from tip of beak to back of head), culmen length (from tip of beak to base of skull), beak-naris (from tip of beak to naris), beak depth, beak width, tarsus length and wing length (all measurements in mm). We compared morphology across genetic species/ groups using analysis of variance (ANOVA), and in combination with acoustic variables using discriminate function analysis (see below). To graph the relationship between beak and body size, we used morphology factor scores using principal components analysis (PCA). Two components were extracted (Eigenvalues > 1) that explained 82% and 87% of the variance respectively: PC Body Size had high factor loadings for tarsus length (0.91) and wing length (0.91); PC Beak Size had high factor loadings for beak-head (0.95), beak-naris (0.92), culmen length (0.92), beak depth (0.92), and beak width (0.91). Therefore, birds with higher factor scores were larger in beak and body size.

Survey methods

We conducted point count surveys in February 2004, 2008 and 2013 using the variable circular plot method (for details see Martin *et al.* 1997, O'Connor *et al.* 2010c), which has been widely used to census Galápagos birds (e.g. Dvorak *et al.* 2004, 2012, O'Connor *et al.* 2010c). We used a total of 15 point counts separated by 200 m along the walking trail to the inner crater of Cerro Pajas volcano, which covered the longest possible transect through the largest remnant patch of *Scalesia* forest in the archipelago. At each point we conducted a 5-min survey during which we recorded the following: GPS coordinates, species, and estimated distance of bird from observer (to the nearest 5 m). During the survey the observers changed orientation from 0° to 90°, 180° and 270° to ensure 360° coverage. All surveys were conducted early in the breeding season between 06h00 and 11h00. Due to the dense vegetation of the *Scalesia* forest habitat, visual census data are unreliable. Therefore, records of birds were included in the analysis only if they were heard, which also avoided the counting of non-singing females. In 2004 and 2008 small numbers of Large Tree Finches *C. psittacula* had been recorded on Floreana (13 and one, respectively) (O'Connor *et al.* 2010c), but recent genetic and morphological analyses suggest that *C. psittacula* did not occur on Floreana Island in 2004 and is likely locally extinct (Kleindorfer *et al.* 2014a). We therefore reanalysed the survey data from 2004 and 2008, and reclassified the records of *C. psittacula* as *C. pauper* as these two species both produce song with slower trill rate (Bowman 1983), and previously recorded *C. pauper* were likely identified incorrectly as *C. psittacula*. Following song and morphology analysis (see results) we treated *C. parvulus* and birds of the hybrid group as one entity (referred to as *C. parvulus*/hybrid group) for demographic analysis, given that it is not possible to distinguish these groups by song or morphology. Observers were Kleindorfer in 2004, O'Connor in 2008 and Kleindorfer and Peters in 2013, who are all familiar with the resident bird species and their songs; the three observers had spent at least two weeks calibrating distance estimates and bird song identification prior to conducting the surveys. In 2013, both observers conducted the survey at the same time (after training for calibrated field identification in 2012 and 2013).

Male population density calculation

Male population densities were estimated for all bird species. Density estimates (number of birds/km²) and detection probability estimates were explored using DISTANCE 6.0 (Thomas *et al.* 2006) but our dataset did not meet the assumptions required for analysis. Detection numbers were low for all species (all < 60) due to sampling restrictions inflicted by patchy and limited habitat, and minimum detections recommended for calculating reliable density estimates using DISTANCE are 60–100 (Buckland *et al.* 2001). Since we were particularly interested in temporal abundance trends, we wanted to ensure comparability across years and use the same method for all years. We therefore calculated male population density estimates (number of birds/km²) using the inflection-point-per-species method following Reynolds *et al.* (1980). Inflection points

(the distance after which the detection rate steeply descends) varied across years and species as specified in Table S3 in the supplementary material and only birds observed within these ranges were included in population density and size estimate calculations. We obtained the number of birds/km² by dividing the total number of birds observed by the total observation area (area of circle with the inflection point as radius), and then dividing the result by 15 (number of survey points). Because two observers were used in 2013, we calculated bird densities for this year using the average of their two values per species. The density of the Galápagos Flycatcher *Myiarchus magnirostris* needs to be interpreted with caution; due to their curious nature, these birds often follow observers and can be easily double counted (Dvorak *et al.* 2012).The singing activity of the Dark-billed Cuckoo *Coccyzus melacoryphus* can be very low and is not considered a reliable cue to detect cuckoo presence (Dvorak *et al.* 2012), and therefore our calculated density could be an underestimate. We are aware of the large group-size differences of Smooth-billed Ani *Crotophaga ani* which cause problems using point count surveys (Dvorak *et al.* 2012). Given that *C. ani* is a predator of Darwin's finches (O'Connor *et al.* 2010a, Connett *et al.* 2013) we included this species in our analyses but interpreted results with caution.

Avian population size estimates

We estimated the maximum male population size for tree finches only, as their preferred nesting is in *Scalesia* forest, which occurs at Cerro Pajas and Asilo de la Paz, while the other species also breed elsewhere on the island. Estimates were based on the maximum size of the available suitable habitat, 22.5 km² (O'Connor *et al.* 2010c). This area comprises Floreana's entire highland habitat (25 km²) and excludes 2.5 km² that have been cleared for agriculture. Of the 22.5 km² non-agricultural highland area, about 3.71 km² (16.5%) is dominated by *Scalesia,* including the study site at Cerro Pajas (2.4 km²) (O'Connor *et al.* 2010c). Thus, we sampled from 65% of the remnant *Scalesia* forest. We conducted the survey in the Cerro Pajas area and used these data to estimate density for the known *Scalesia* habitat (3.71 km²) as well as the total highland habitat (22.5 km²). We assume that Darwin's finch density will differ across *Scalesia* patches (landscape variation in density was not a focus of this study); it is likely that our total maximum population size estimate will overestimate rather than underestimate tree finch numbers because *Scalesia* dominates just 16.5% of the total highland area.

Statistical analyses

Male population density estimates were calculated in Microsoft Office Excel following Reynolds *et al.* (1980); all other statistical analyses were performed using IBM® SPSS® Statistics version 22. Graphs were produced using SigmaPlot version 12.0. We analysed distinctiveness of song and morphology between genetic groups using (i) ANOVA and Kruskal-Wallis test; and (ii) a discriminant function analysis (DFA). We examined data for normality before using parametric tests. Because data were collected across years, we first assessed if song and morphology differed across years using multivariate analysis of variance for the interaction effect of 'year' and 'genetic group'. Three variables were transformed to meet assumptions of normality: maximum frequency (reflect and square root transformation), maximum number of syllables (reflect and logarithmic transformation) and beak width (inverse transformation). We checked the data for homogeneity of variance using Levene's test. The variables for minimum frequency, duration, and number of syllables showed homogenous variances and were analysed using ANOVA with Tukey HSD post-hoc test. The variables trill rate, maximum number of syllables, maximum frequency and frequency bandwidth violated the assumption of homogeneity of variance (all $P < 0.05$) and were therefore analysed using Welch's ANOVA with Games-Howell post-hoc tests. The variables - number of syllables, dominant frequency, beak length head, beak length naris and beak depth - violated assumptions of normality and were analysed using Kruskal-Wallis tests for independent samples with pairwise comparisons as post-hoc tests.

Since many variables were strongly correlated (Pearson's correlation > 0.8) violating a key assumption of DFA, we first performed a principal component analysis (PCA) and used PC scores (varimax rotation) instead of raw variables in the DFA. PCA produced one morphology variable accounting for 75.1% of variance, and three acoustic variables accounting for 82.3% of variance in the original dataset. We performed the DFA using all four variables and examined the significance of discriminant models using F-tests (Wilk's Lambda).

Results

Species determination based on morphology and song

There was no significant interaction effect between 'year' and 'genetic group' for morphology (MANOVA: Pillai's Trace = 0.71, $F_{35,310}$ = 1.46, P = 0.05); therefore we pooled data across years for morphological analysis. *Camarhynchus pauper* was significantly larger in all analysed variables (post-hoc tests all P < 0.04, Table 2, Figure 1), but *C. parvulus* and hybrid birds were morphologically indistinguishable (post-hoc tests all P > 0.79, Table 2, Figure 1). These results were consistent with results from discriminant function analysis: cross-validated DFA including vocal and morphological variables was able to correctly assign 93.8% of *C. pauper* males, but could only assign 57.1% of *C. parvulus* and 54.5% of hybrid males (Wilk's Lambda = 0.32, χ^2_8 = 58.07, P < 0.001).

We obtained a total of 325 song recordings from 77 genetically identified Darwin's finches across four years (2006, n = 14; 2010, n = 22; 2013, n = 36; 2014, n = 5; Table 1). There was no significant interaction effect of 'year' and 'genetic group' (MANOVA: Pillai's Trace = 0.464, $F_{30,325}$ = 0.936, P = 0.576) and therefore we pooled the data across years. Tree finch *Camarhynchus* spp. song did not differ significantly between genetic groups for the variables maximum frequency and song duration. However, there were significant differences across genetic groups in minimum frequency, dominant frequency, frequency bandwidth, number of syllables, maximum number of syllables and trill rate (ANOVA: minimum frequency $F_{2,76}$ = 16.75, P < 0.01, Welch's ANOVA: frequency bandwidth $F_{2,76}$ = 8.08, P = 0.003, trill rate $F_{2,20.647}$ = 14.49, P > 0.001, maximum
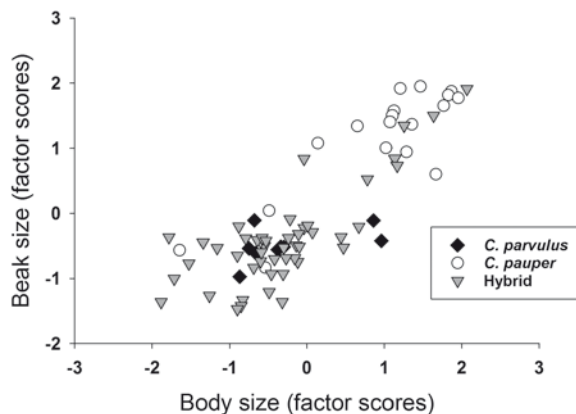

Figure 1. The association between body size and beak size (factor scores form principal component analysis) in 77 Darwin's tree finches that have been genetically assigned and for which we also have song recordings. The three recognised populations on Floreana are *C. pauper*, *C. parvulus*, and the hybrid group that arose from pairings between female *C. pauper* and male *C. parvulus*. The large-bodied *C. pauper* can be identified based on morphology, but the small-bodied *C. parvulus* overlaps with the morphology of hybrid birds. Some hybrid birds appear large like *C. pauper*.
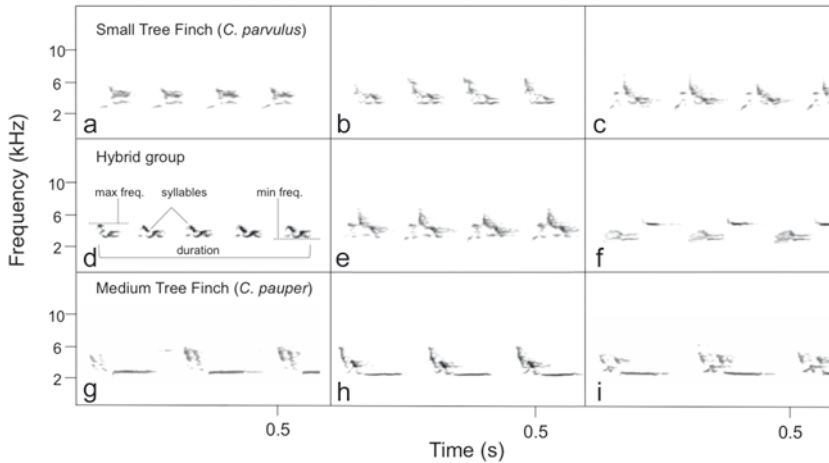
Figure 2. Spectrograms of song in: (a-c) Small Tree Finch *Camarhynchus parvulus*, (d-f) hybrid group, and (g-i) Medium Tree Finch *C. pauper*. Each spectrogram represents ~0.6 seconds of song of one male; we chose this partial representation to include three males per genetic group and visualise the difference in trill rate between *C. pauper* vs. *C. parvulus* and hybrid. The song of *C. parvulus* and hybrid birds could not be statistically distinguished, while the song of *C. pauper* had slower trill rate, fewer syllables and lower minimum frequency (see Table 1). One spectrogram of a complete song per genetic group is provided in the supplementary material.

number of syllables $F_{2,20.077} = 5.04$, $P = 0.016$, Kruskal-Wallis test: dominant frequency $F_2 = 21.81$, $P < 0.001$, number of syllables $F_2 = 9.18$, $P = 0.010$, Table 1). Effect size was calculated using eta squared (minimum frequency = 0.31, frequency bandwidth = 0.11, trill rate = 0.16, maximum number of syllables = 0.06). Post-hoc comparison showed that *C. pauper* had a lower minimum and dominant frequency, a broader frequency bandwidth, fewer syllables, and a slower trill rate than *C. parvulus* and hybrid birds (all $P < 0.04$), but there was no significant difference between the song of *C. parvulus* and hybrid birds (all $P > 0.5$) (Figure 2).

Avian population density and population size estimates

Using the respective inflection points per species and year as a threshold for data inclusion, our avian surveys at Cerro Pajas generated 362 bird records from nine species across the three survey years (2004 = 133, 2008 = 152, 2013 = 77; Table 3). As shown in Table 3, *C. pauper* abundance declined by 52% from 2004 to 2013, and *C. parvulus*/hybrid group numbers increased by 45%. Two other species showed patterns of decline: Medium Ground Finch *G. fortis* (-76%) and Dark-billed Cuckoo *Coccyzus melacoryphus* (-95%). Four other highland species showed patterns of increase: Galápagos Flycatcher *Myiarchus magnirostris* (+11%), Small Ground Finch *Geospiza fuliginosa* (+23%), Yellow Warbler *Dendroica petechia* (+256%), and Smooth-billed Ani *Crotophaga ani* (+254%). Neither the Warbler Finch *Certhidea fusca* nor the Large Tree Finch *C. psittacula* were detected.

Discussion

Main findings for song analyses and population estimates

Hybridisation created a considerable obstacle for species detection using acoustic surveys in tree finches due to the similarity between the song of hybrid birds and *C. parvulus*. For this reason,

Table 1. Male song characteristics in three tree finch *Camarhynchus* spp. genetic groups. Data are shown as mean ± SE (95% CI), statistical results are shown for Kruskal-Wallis test* and ANOVA. The sample size per genetic group is shown in brackets. Songs were recorded from colour-banded birds in the field and retrospectively assigned to species/group after laboratory analysis of genetic samples.

Genetic group (n)	Minimum frequency (Hz)	Maximum frequency (Hz)	Frequency bandwidth (Hz)	Dominant Frequency (Hz)*	Song duration (s)	Number of syllables/song*	Trill rate (number of syllables/s)	Maximum number of syllables
Small Tree Finch	2484.3 ± 89.9	5797.6 ± 308.2	3313.2 ± 304.0	4070.3 ± 160.6	1.2 ± 0.1	8.3 ± 0.8	6.8 ± 0.6	9.8 ± 0.9
(*C. parvulus*) (n = 9)	(2277.0 – 2691.6)	(5086.8 – 6508.3)	(2612.1 – 4014.3)	(3700.0 – 4440.6)	(1.0 – 1.4)	(6.4 – 10.1)	(5.5 – 8.3)	(7.7 – 11.9)
Hybrid group	2464.9 ± 33.5	5977.0 ± 111.0	3512.2 ± 117.0	4202.2 ± 86.4	1.2 ± 0.04	7.8 ± 0.4	6.5 ± 0.3	8.9 ± 0.5
(n = 49)	(2397.6 – 2532.2)	(5753.9 – 6200.2)	(3277.0 – 3747.4)	(4028.5 – 4375.9)	(1.1 – 1.3)	(7.0 – 8.6)	(6.0 – 7.1)	(7.9 – 9.8)
Medium Tree Finch	2117.9 ± 44.1	6194.2 ± 87.9	4076.4 ± 97.1	3427.0 ± 103.8	1.3 ± 0.1	6.1 ± 0.4	4.9 ± 0.2	7.7 ± 0.4
(*C. pauper*) (n = 19)	(2025.2 – 2210.5)	(6009.5 – 6378.9)	(3872.5 – 4280.3)	(3849.7 – 4141.3)	(1.1 – 1.4)	(5.4 – 6.9)	(4.6 – 5.3)	(7.0 – 8.5)
df	2, 76	2, 20.440	2, 76	2	2, 76	2	2, 20.647	2, 22.077
F	16.745	0.404	8.077	21.813	0.426	9.180	15.491	5.039
P	< 0.001	0.673	0.003	< 0.001	0.655	0.010	< 0.001	0.016

Table 2. Male morphology shown as mean ± SE (95% CI) per genetic group of tree finches *Camarhynchus* spp. for which we also analysed song recordings. Statistical results are shown for Kruskal-Wallis test* and ANOVA; post-hoc tests showed that *C. parvulus* and birds of the hybrid group were statistically indistinguishable from each other, but smaller than *C. pauper* (see results).

Genetic group (n)	Beak-head*	Beak-naris*	Culmen length	Beak depth*	Beak width*	Tarsus	Wing
Small Tree Finch	26.6 ± 0.1	13.3 ± 0.2	7.5 ± 0.1	7.5 ± 0.1	6.6 ± 0.1	20.7 ± 0.3	64.4 ± 0.9
(*Camarhynchus parvulus*) (n = 9)	(26.3–26.9)	(12.9–13.7)	(7.3–7.7)	(7.3– 7.7)	(6.4 – 6.7)	(20.0–21.4)	(62.3–66.6)
Hybrid group	26.9 ± 0.1	13.5 ± 0.1	7.6 ± 0.1	7.5 ± 0.1	6.6 ± 0.1	20.5 ± 0.3	64.0 ± 0.4
(n = 49)	(26.6–27.2)	(13.2–13.8)	(7.4–7.8)	(7.4–7.6)	(6.5–6.7)	(20.0–21.0)	(63.3–64.8)
Medium Tree Finch	29.1 ± 0.3	15.1 ± 0.2	8.7 ± 0.1	8.4 ± 0.2	7.1 ± 0.1	21.9 ± 0.3	67.5 ± 0.7
(*C. pauper*) (n = 19)	(28.4–29.7)	(14.7–15.5)	(8.5– 9.0)	(8.1–8.7)	(7.0–7.3)	(21.4–22.5)	(66.1–69.0)
df	2	2	2, 76	2	2	2, 76	2, 76
F	25.420	26.208	30.745	21.000	16.109	5.306	10.929
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.007	< 0.001

Table 3. Estimated male population density of bird species in the highlands of Floreana Island during 2004, 2008, and 2013. Data are from singing males monitored using the circular plot method. The highland population estimate [shown in brackets] is given for *Scalesia* forest (3.71 km²) and for total highland habitat (22.5 km²) for males only in both tree finch groups; total population estimates were not calculated for the other species, as they do not predominantly nest in the highlands or in *Scalesia* forest.

	Estimated number of male birds km⁻² (numbers of birds heard) [estimated male population size for *Scalesia* habitat, estimated male population size for all highland habitat]			% Change from 2008 to 2013	% Change from 2004 to 2013
	2004	2008	2013		
Small Tree Finch *Camarhynchus parvulus*[1] and hybrid group[1]	182 (42) [675, 4,095]	365 (43) [1,354, 8,212]	263 (43) [975, 5,917]	−28	+ 45
Medium Tree Finch *C. pauper*[2]	234 (54) [868, 5,265]	102 (12) [378, 2,292]	113 (9) [419, 2,537]	+ 10	−52
Large Tree Finch *C. psittacula*[1]*	0 (0)*	0 (0)*	0 (0)	-	-
Small Ground Finch *Geospiza fuliginosa*[1]	136 (23)	272 (32)	167 (10)	−39	+ 23
Medium Ground Finch *G. fortis*[1]	29 (5)	17 (2)	7 (1)	−59	−76
Yellow Warbler *Dendroica petechia*[1]	189 (8)	623 (47)	483 (21)	−22	+ 256
Galápagos Flycatcher *Myiarchus magnirostris*[1]	0 (0)**	477 (9)	531 (3)	+ 11	-
Smooth-billed Ani *Crotophaga ani*[1]	13 (1)	40 (2)	33 (3)	−18	+ 254
Dark-billed Cuckoo *Coccyzus melacoryphus*[1]	0 (0)	43 (4)	4 (1)	−91	-

Current IUCN status: [1]Least Concern, [2]Critically Endangered.

*O'Connor *et al.* (2010) noted 13 (2004) and one (2008) singing *C. psittacula*, but findings by Kleindorfer *et al.* (2014a) suggested the *C. psittacula* was locally extinct; in this study, the *C. psittacula* males heard in 2004 and 2008 are considered to be *C. pauper* males.

**Galápagos Flycatchers were seen (but not heard) in 2004; therefore the relative increase from 2004 to 2013 is due to the occurrence of vocalising flycatchers and likely does not represent changes in population size.

song cannot be used to estimate the abundance of *C. parvulus* and hybrid birds separately but could detect the combined 'C. parvulus/hybrid' group. Song of the critically endangered *C. pauper* differed from that of the *C. parvulus*/hybrid group in several variables, and therefore song can be used to monitor its abundance. However, there is one caveat: cross-validated discriminant function analysis including vocal and morphological variables only correctly assigned 93.8% of *C. pauper* males (and incorrectly assigned 6.3% of males to *C. pauper* when in fact they were hybrid birds). Inspection of Tables 1 and 2 and Figure 1 shows how this error could arise given some cases of overlap between *C. pauper* and hybrid birds in morphology and song. The statistical results of this study (combined with our unpublished field calibration trials) support the view that overall, morphology and song can be used to distinguish ~ 94% of genuine cases of *C. pauper*. Aware of these caveats, using acoustic surveys, *C. pauper* declined by 52% across the decade from 2004 to 2013 (with 10% increase since 2008), while the *C. parvulus*/hybrid group increased by 45% (with 28% decrease since 2008). These results underscore the warranted conservation concern for the critically endangered *C. pauper*. Because we cannot distinguish *C. parvulus* from hybrid birds using song or morphology, only genetic analysis can reveal the population trends for *C. parvulus* relative to the hybrid group.

Differences in song and morphology in tree finches

Compared with *C. parvulus*/hybrid birds, song of *C. pauper* had slower trill rate (fewer syllable/s), fewer syllables per song, broader frequency bandwidth, lower minimum frequency, and lower dominant frequency. *Camarhynchus pauper* was significantly larger in all analysed morphological variables, but differences occurred across a gradient. Inspection of Figure 1 shows that some hybrid birds were indistinguishable from *C. pauper* in morphology, and *C. parvulus*/hybrid birds could not be distinguished morphologically.

The lack of difference in song between *C. parvulus* and hybrid birds has several possible explanations including small sample size (9 vs. 49), lack of time or selection for divergence, and the role of vocal tutors for learning of song type. Despite our efforts to sample equally from all three tree finch groups, post-hoc genetic assignment revealed the high relative abundance of birds of the hybrid group on Floreana Island, which explains the higher number of recorded hybrid songs (n = 49). Using morphology (Kleindorfer *et al.* 2014a) and genetics (Peters 2016), the observed tree finch hybridisation is largely the result of *C. pauper* females pairing with *C. parvulus* males. Darwin's Finches learn song from a male tutor which is usually their father (Grant and Grant 1996), therefore it is likely that hybrid sons would have learned their song from their *C. parvulus* fathers and would sing a *C. parvulus* song. A similar pattern of song learning, and hence a possible mechanism for backcrossing in favour of the paternal genetic lineage, was previously shown in *Geospiza* hybrids (Grant and Grant 1997, 2014b). These possibilities require further investigation.

Survey results for tree finches

Our survey results show fluctuations in numbers in both *C. parvulus*/hybrids (overall 45% increase from 2004 to 2013 but 28% decline from 2008 to 2013) and *C. pauper* (overall 52% decline from 2004 to 2013 but 10% increase from 2008 to 2013). The Galápagos Islands provide compelling case studies for novel evolutionary trajectories as the result of climate impacts in a single year (e.g. Grant and Grant 1993). A four-year drought prevailed on the Galápagos from 2003 to 2007, and then followed years with high rainfall conditions in 2008, 2011 and 2012 (CDF Meteorological Database, http://www.darwinfoundation.org/datazone/climate/). Therefore, the decline in *C. pauper* from 2004 to 2008 may be the result of drought-induced mortality, and the post-2008 increase may the result of rainfall-induced nesting recruitment. It is also possible that we are detecting more unpaired singing *C. pauper* across the years, rather than an increase in *C. pauper* males and females (Kleindorfer *et al.* in review) The pattern is more

difficult to interpret in the *C. parvulus*/hybrid group, because they are indistinguishable using acoustic surveys. It is likely that hybridisation increased post-rainfall, but perhaps hybrids are unable to establish – which remains to be tested.

According to criteria established by IUCN, *C. parvulus* is classified as 'Least Concern' (The IUCN Red List of Threatened Species. Version 2014.3; www.iucnredlist.org). However, hybridisation among tree finches makes its actual status on Floreana Island uncertain. To date we have insufficient information on the makeup of the hybrid group, but unpublished data suggest that the hybridisation extends well beyond F1. Genetic introgression from *C. pauper* to *C. parvulus* was previously suspected (Kleindorfer *et al.* 2014a) and ongoing analyses show evidence for extensive asymmetrical gene flow towards *C. parvulus* (Peters 2016). The acoustic and morphological similarity of *C. parvulus* and the hybrid group presented here supports the scenario that backcrossing has already occurred and the hybrid group does not consist of first generation hybrids but rather comprises later generation hybrids and introgressed individuals (see also Derégnaucourt *et al.* 2001). A reliable classification of the conservation status of the Floreana *C. parvulus* population will depend on results of detailed genetic analyses.

Floreana Island has the longest history of human settlement and activity (Lack 1983, Steadman 1986, Watson *et al.* 2010) and the highest number of species extinctions across the Galápagos Archipelago. Three bird species (Large Ground Finch, *Geospiza magnirostris*; Sharp-beaked Ground Finch, *G. difficilis* and Floreana Mockingbird, *Nesomimus trifasciatus*) have become locally extinct over the past 200 years (1835–2005) (Grant *et al.* 2000, 2005b, Merlen 2013). The Warbler Finch *Certhidea fusca* (Grant *et al.* 2005b), the Vermilion Flycatcher *Pyrocephalus rubinus* (O'Connor *et al.* 2010c), and *C. psittacula* (Kleindorfer *et al.* 2014a) are either currently locally extinct or likely to become locally extinct in the near future (discussed below). The Vegetarian Finch *Platyspiza crassirostris* was only heard once by our group in the highlands in 2010, and hence could also be considered very rare (Kleindorfer pers. obs.).

Habitat fragmentation, habitat loss, introduced species and pathogens (Wiedenfeld and Jiménez-Uzcátegui 2008) can be particularly problematic for small and range restricted populations (Simberloff 1995). Less than 62% of the original *Scalesia* forest persists on Floreana Island given land clearing for human activities. The remaining *Scalesia* habitat is under increasing pressure from introduced flora (Mauchamp 1997, Rentería *et al.* 2012) and fauna (Whiteman *et al.* 2005, Jiménez-Uzcátegui *et al.* 2008), such as black rat *Rattus rattus* and Norwegian rat *R. norvegicus* (Grant *et al.* 2005b), domestic cats *Felis catus* (Jiménez-Uzcátegui *et al.* 2008), Smooth-billed Ani *C. ani* (Connett *et al.* 2013) and the introduced dipteran *Philornis downsi*.

Philornis downsi is considered the biggest threat to Darwin's finch survival and to breeding success in Galápagos land birds in general (Kleindorfer and Dudaniec 2016). Both parasite intensity and Darwin's finch mortality have increased across the past decade (Kleindorfer and Dudaniec 2016). The available data on impacts of *P. downsi* suggest that low annual recruitment in *C. pauper* is the main explanation for its critical decline (O'Connor *et al.* 2010d, Peters 2016). Given the 45% increase in numbers of the *C. parvulus*/hybrid group from 2004 to 2013, another factor contributing to the *C. pauper* decline could be selection favouring hybridisation with *C. parvulus*. If hybrid birds have higher fitness (Kleindorfer *et al.* 2014a, Peters 2016) and if hybrid offspring backcross with other hybrids or *C. parvulus*, this will increase recruitment of the *C. parvulus*/hybrid group rather than the *C. pauper* group.

Camarhynchus psittacula has always been rare on Floreana Island (discussed in Grant *et al.* 2005b, Kleindorfer *et al.* 2014a). Our repeated survey and nest monitoring efforts support the view that *C. psittacula* is locally extinct on Floreana Island (Kleindorfer *et al.* 2014a). Because we only surveyed at one location, it is possible that this species exists elsewhere on the island. However, we have traversed the island widely for various reasons, and have not heard *C. psittacula* song. We tested this in the field by comparing our recordings with historical 1960s recordings from Robert Bowman on Floreana and Santa Cruz Islands (Kleindorfer unpubl. data), and no Floreana tree finch had a strong response to historical *C. psittacula* song.

Implications for conservation and survey techniques

Given that the majority of songbird species learn song from an adult tutor which is usually their father (Catchpole and Slater 2003), hybrids are generally likely to sing the song of their paternal species, and therefore other systems with contemporary hybridisation may show the same pattern we present here. In other systems, frequent hybridisation resulted in genetic and demographic swamping of one or both of the parental species by the hybrids (Rhymer and Simberloff 1996, Roberts *et al.* 2010). But rare species can benefit from hybridisation, as it increases their often depleted genetic diversity and possibly their fitness and adaptive potential (Baskett and Gomulkiewicz 2011, Hamilton and Miller 2016). *Camarhynchus parvulus* could constitute an important source of genetic variation for the critically endangered *C. pauper*. The hybrid group could serve as a genetic reservoir preserving the genes of an endemic and declining species, in which case all three genetic groups and their habitat should be conserved (López-Pujol *et al.* 2012).

Because hybridisation usually occurs between already closely related species, detection of hybrids is complicated as it often relies on molecular analyses. Backcrosses and later generation hybrids in particular cannot be determined using morphological characters alone (Allendorf *et al.* 2001). Characterising hybridisation patterns may therefore require genetic analyses, such as in grey wolf *Canis lupus* and domestic dog *C. familiaris* (Andersone *et al.* 2002, Vilà *et al.* 2003), and Hawaiian Ducks *Anas wyvilliana* and introduced Mallard *A. platyrhynchos* (Fowler *et al.*, 2009). Hybridisation therefore makes rapid population assessment practically impossible in many species, which is especially problematic when threatened species are involved that require regular monitoring. In the case of the Floreana tree finch group, the distinct song of *C. pauper* means that acoustical identification can be retained for surveys, which is a significant finding given the critically endangered status of this endemic and declining species.

Survey results for other bird species

While this study focused on the *Camarhynchus* tree finches, we present the findings for other bird species in Table 3. We provide comment here on the introduced, and the very rare or possibly locally extinct species known for Floreana Island. *Crotophaga ani* was introduced to the Galápagos Archipelago in the 1960s to consume the ticks on cattle; but analysis of gizzard contents found Darwin's Finch remains instead (Olivares and Munves 1973, O'Connor *et al.* 2010a, Connett *et al.* 2013). Therefore, the increase in *C. ani* could be a threat to populations of songbirds. The extreme drought across the Galápagos from 2003 to 2007 (CDF Meteorological Database, http://www.darwinfoundation.org/datazone/climate/) is suspected to have negatively influenced insectivorous and frugivorous species in particular. The Vegetarian Finch and Vermilion Flycatcher used to be relatively common in the Floreana highland forest, although there is no information about former population size, and abundance has mainly been inferred from statements made by locals and the previously high numbers of collected specimens (*P. crassirostris*: 48 in 1905/06, three in 1962, one in 1974, *P. rubinus*: seven in 1888–1891, 133 in 1898–1906 and 10 in 1962) (Wiedenfeld 2006, O'Connor *et al.* 2010c, Merlen 2013). We have only one sighting of *P. crassirostris* since 2004, and individuals of *P. rubinus* have not been seen since 2008 (Walter Cruz, K. J. Peters pers. comm.). In the case of the Warbler Finch, several targeted surveys by Grant *et al.* (2005b) during the breeding season in 1979, 1983, 1997, 1999 and 2004 using species-specific playback to stimulate a response, failed to locate any *C. fusca* on Floreana Island; but O'Connor *et al.* (2010c) reported hearing a male *C. fusca* singing at Asilo de la Paz in 2008. This species is suspected to be locally extinct or at least extremely rare on Floreana, and the fact that this study did not observe any *C. fusca* supports this view. Several species increased across the decade including *Dendroica petechia* (+256%), *C. ani* (+254%) *G. fuliginosa* (+23%), and *Myiarchus magnirostris* (+11 %); however, the Ground Finch *G. fortis* (-76%) decreased in the highland *Scalesia*. In summary, the strong decline observed in *C. pauper* surveyed in *Scalesia* forest is historically paralleled by three

local extinctions in Darwin's finch species on Floreana Island, and warrants concern. Understanding why five land bird species have apparently increased in the same habitat, including two Darwin's finch species (*C. parvulus*/hybrid, *G. fuliginosa*), requires focused inquiry.

Conclusion

Acoustic survey techniques could not reliably detect tree finch hybrids. Song can be used to distinguish the critically endangered *C. pauper*, but song was the same in common *C. parvulus* and birds of the hybrid group. Using a comparable survey approach at three sampling times across the decade, the data suggest that *C. pauper* did not recover from its dramatic decline in abundance following the drought years of 2003 to 2007; rather, it maintained its 52% decline across the decade in spite of modest population stabilisation since 2008. Recent evidence suggests substantial introgression from *C. pauper* into the *C. parvulus* population in the Cerro Pajas region (Kleindorfer *et al.* 2014a, Peters 2016), many *P. downsi* parasites per nest (O'Connor *et al.* 2010b), naris malformation from *P. downsi* parasites (Kleindorfer and Sulloway 2016), and no observed nesting success since 2010 (Kleindorfer *et al.* 2014b, Peters 2016). Our second major finding that *C. parvulus* and hybrid birds generally increased across the decade (but declined by 28% since 2008) requires further investigation as we cannot ascertain actual size estimates for each respective population without genetic analysis, and it could mask an undercurrent of decline in *C. parvulus*. Repeated bird surveys across the decade show a range of patterns in populations: several species showed a marked increase (including an introduced avian predator), other species showed a noticeable decline (including the locally endemic *C. pauper*). Floreana Island has a history of local extinctions, which warrants concern for existing species and underlines the need for regular monitoring. Hybridisation may be a driver of biodiversity and adaptive capacity if alleles from rare species are introgressed into common species, but hybridisation can hamper reliable population estimates of common species when the two groups become acoustically and visually indistinguishable. This study highlights the need for urgent conservation measures for the tree finch group on Floreana Island.

Supplementary Material

To view supplementary material for this article, please visit https://doi.org/10.1017/S0959270916000630

Acknowledgements

References

Allendorf, F. W., Leary, R. F., Spruell, P. and Wenburg, J. K. (2001) The problems with hybrids: Setting conservation guidelines. *Trends Ecol. Evol.* 16: 613–622.

Andersone, Ž., Lucchini, V. and Ozoliņš, J. (2002) Hybridisation between wolves and dogs in Latvia as documented using mitochondrial and microsatellite DNA markers. *Mammal. Biol. - Z. Säugetierk.* 67: 79–90.

Baillie, J. E. M., Hilton-Taylor, C. and Stuart, S. N. (2004) *2004 IUCN Red List of Threatened species. A global species assessment.* Cambridge. UK: IUCN Press.

Baskett, M. L. and Gomulkiewicz, R. (2011) Introgressive hybridization as a mechanism for species rescue. *Theor. Ecol.* 4: 223–239.

Bowman, R. I. (1983) The evolution of song in Darwin's finches. In R. I. Bowman, M. Berson and A. E. Leviton, eds. *Patterns of evolution in Galápagos Organisms*. San Francisco, California, USA: Pacific Division AAAS.

Bowman, R. I., Berson, M., Leviton, A. E. and American Association for the Advancement of Science. Pacific Division. (1983) *Patterns of evolution in Galapagos organisms*. San Francisco, California: Pacific Division, AAAS.

Buckland, S. T., Anderson, D. R., Burnham, K. P., Laake, J. L., Borchers, D. and Thomas, L. (2001) *Introduction to distance sampling*. New York, USA: Oxford University Press.

Catchpole, C. K. and Slater, P. J. (2003) *Bird song: biological themes and variations*. Cambridge, UK: Cambridge University Press.

Christensen, R., Kleindorfer, S. and Robertson, J. (2006) Song is a reliable signal of bill morphology in Darwin's small tree finch *Camarhynchus parvulus*, and vocal performance predicts male pairing success. *J. Avian Biol.* 37: 617–624.

Connett, L., Guézou, A., Herrera, H. W., Carrión, V., Parker, P. G. and Deem, S. L. (2013) Gizzard contents of the smooth-billed ani *Crotophaga ani* in Santa Cruz, Galápagos Islands, Ecuador. *Galápagos Research*, 68. Published online, 15 November 2013.

Cunninghame, F., Ortiz-Catedral, L. and Fessl, B. (2012) Landbird conservation plan: Strategies for reversing the decline of passerine birds on the Galapagos Islands. In *Secondary report of the workshop: Searching for solutions for the control of the avian parasite,* Philornis downsi, *Puerto Ayora, Santa Cruz, Galápagos*. Vol. 31. Puerto Ayora, Santa Cruz, Galapagos: Charles Darwin Foundation and Galapagos National Park Service.

Dawson, D. K. and Efford, M. G. (2009) Bird population density estimated from acoustic signals. *J. Appl. Ecol.* 46: 1201–1209.

Derégnaucourt, S., Guyomarc'h, J. C. and Richard, V. (2001) Classification of hybrid crows in quail using artificial neural networks. *Behav. Process.* 56: 103–112.

Driscoll, D. A. (1998) Counts of calling males as estimates of population size in the endangered frogs *Geocrinia alba* and *G. vitellina*. *J. Herpetol.* 32: 475–481.

Dvorak, M., Fessl, B., Nemeth, E., Kleindorfer, S. and Tebbich, S. (2012) Distribution and abundance of Darwin's finches and other land birds on Santa Cruz Island, Galápagos: evidence for declining populations. *Oryx* 46: 78.

Dvorak, M., Vargas, H., Fessl, B. and Tebbich, S. (2004) On the verge of extinction: a survey of the mangrove finch *Cactospiza heliobates* and its habitat on the Galápagos Islands. *Oryx* 38: 171–179.

Fowler, A. C., Eadie, J. M. and Engilis Jr, A. (2009) Identification of endangered Hawaiian ducks (*Anas wyvilliana*), introduced North American mallards (*A. platyrhynchos*) and their hybrids using multilocus genotypes. *Conserv. Genet*, 10: 1747–1758.

Goodale, E. and Podos, J. (2010) Persistence of song types in Darwin's finches, *Geospiza fortis*, over four decades. *Biol. Lett.* 6: 589–592.

Grant, B. R. and Grant, P. R. (1993) Evolution of Darwin's finches caused by a rare climatic event. *Proc. R. Soc. Lond. B Biol. Sci.* 251: 111–117.

Grant, B. R. and Grant, P. R. (1996) Cultural inheritance of song and its role in the evolution of Darwin's finches. *Evolution* 50: 2471–2487.

Grant, P. R. (1986) *Ecology and evolution of Darwin's finches*, Princeton, USA: Princeton University Press.

Grant, P. R. and Grant, B. R. (1992) Hybridization of bird species. *Science* 256: 193–197.

Grant, P. R. and Grant, B. R. (1997) Hybridization, sexual imprinting, and mate choice. *Am. Nat.* 149: 1–28.

Grant, P. R. and Grant, B. R. (2014a) Synergism of natural selection and introgression in the origin of a new species. *Am. Nat.* 183: 671–681.

Grant, P. R. and Grant, B. R. (2014b) Synergism of natural selection and introgression in the origin of a new species. *Am. Nat.* 183: 671–681.

Grant, P., Curry, R. and Grant, B. (2000) A remnant population of the Floreana mockingbird on Champion Island, Galápagos. *Biol. Conserv.* 92: 285–290.

Grant, P. R., Grant, B. R., Keller, L. F., Markert, J. A. and Petren, K. (2003) Inbreeding and interbreeding in Darwin's finches. *Evolution* 57: 2911–2916.

Grant, P. R., Grant, B. R. and Petren, K. (2005a) Hybridization in the recent past. *Am. Nat.* 166: 56–67.

Grant, P. R., Grant, B. R., Petren, K. and Keller, L. F. (2005b) Extinction behind our backs: the possible fate of one of the Darwin's finch species on Isla Floreana, Galápagos. *Biol. Conserv.* 122: 499–503.

Hails, C. (2008) *Living planet report 2008*. Zürich, Switzerland: WWF International.

Hamilton, J. A. and Miller, J. M. (2016) Adaptive introgression: A resource for management and genetic conservation in a changing climate. *Conserv. Biol.* 30: 33–41.

Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H. and Hallwachs, W. (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proc. Natl. Acad. Sci.* 101: 14812–14817.

IUCN (2015) *The IUCN Red List of Threatened Species. Version 2015.1*. www.iucnredlist.org.

Jiménez-Uzcátegui, G., Carrión, V., Zabala, J., Buitrón, P. and Milstead, B. (2008) Status of introduced vertebrates in Galápagos. Pp. 136–141 in *Galápagos Report 2006–2007*. Puerto Ayora, Galapagos, Ecuador: GNPS, GCREG, CDF and GC.

Karanth, K. U. and Nichols, J. D. (2010) Non-invasive survey methods for assessing tiger populations. Pp. 241–261 in R. Tilson and P. J. Nyhus, eds. *Tigers of the world: The science, politics and conservation of Panthera tigris*. London, UK and Burlington, MA: Academic Press.

Kleindorfer, S. and Dudaniec, R. Y. (2016) Host-parasite ecology, behavior and genetics: a review of the introduced fly parasite *Philornis downsi* and its Darwin's finch hosts. *BMC Zoology* 1: 1–19.

Kleindorfer, S. and Sulloway, F. J. (2016) Naris deformation in Darwin's finches: experimental and historical evidence for a post-1960s arrival of the parasite *Philornis downsi*. *Global Ecol. Conserv.* 7: 122–131.

Kleindorfer, S., Custance, G., Peters, K. J. and Sulloway, F. J. (In review) Parasites change pre-mating signal and blur species boundaries: *Philornis downsi* and Darwin's finches (*Camarhynchus* spp.). *PNAS*.

Kleindorfer, S., O'Connor, J. A., Dudaniec, R. Y., Myers, S. A., Robertson, J. and Sulloway, F. J. (2014a) Species collapse via hybridization in Darwin's tree finches. *Am. Nat.* 183: 325–341.

Kleindorfer, S., Peters, K. J., Custance, G., Dudaniec, R. Y. and O'Connor, J. A. (2014b) Changes in *Philornis* infestation behavior threaten Darwin's finch survival. *Curr. Zool.* 60: 542–550.

Kleindorfer, S., Peters, K. J., Hohl, L. and Sulloway, F. J. (2016) Flight behaviour of an introduced parasite affects its Galápagos Island hosts: *Philornis downsi* and Darwin's finches. Pp. 158–179 in J. S. Weis and D. Sol, eds. *Biological invasions and behaviour*. Cambridge, UK: Cambridge University Press.

Lack, D. L. (1983) *Darwin's finches*. Cambridge, UK: Cambridge University Press.

Lambert, K. T. A. and Mcdonald, P. G. (2014) A low-cost, yet simple and highly repeatable system for acoustically surveying cryptic species. *Austral. Ecol.* 39: 779–785.

López-Pujol, J., Garcia-Jacas, N., Susanna, A. and Vilatersana, R. (2012) Should we conserve pure species or hybrid species? Delimiting hybridization and introgression in the Iberian endemic *Centaurea podospermifolia*. *Biol. Conserv.* 152: 271–279.

Marques, T. A., Thomas, L., Ward, J., DiMarzio, N. and Tyack, P. L. (2009) Estimating cetacean population density using

fixed passive acoustic sensors: an example with Blainville's beaked whales. *J. Acoust. Soc. Am.* 125: 1982–1994.

Marten, K. and Marler, P. (1977) Sound transmission and its significance for animal vocalization. *Behav. Ecol. Sociobiol.* 2: 271–290.

Martin, T. E., Paine, C. R., Conway, C. J., Hochachka, W. M., Allen, P. and Jenkins, W. (1997) *BBIRD field protocol*. Montana, USA: University of Montana Cooperative Wildlife Research Unit, US Geological Survey.

Mauchamp, A. (1997) Threats from alien plant species in the Galápagos Islands. *Conserv. Biol.* 11: 260–263.

McKay, B. D. and Zink, R. M. (2014) Sisyphean evolution in Darwin's finches. *Biol. Rev.* 90: 689–698.

Merlen, G. (2013) Gone, gone… going: The fate of the Vermilion Flycatcher on Darwin's Islands. Pp. 180–188 in *Galápagos Report 2011–2012*. Puerto Ayora, Galápagos, Ecuador: GNPS, GCREG, CDF and GC.

O'Connor, J. A., Dudaniec, R. Y. and Kleindorfer, S. (2010a) Parasite infestation and predation in Darwin's small ground finch: contrasting two elevational habitats between islands. *J. Trop. Ecol.* 26: 285–292.

O'Connor, J. A., Robertson, J. and Kleindorfer, S. (2010b) Video analysis of host-parasite interactions in nests of Darwin's finches. *Oryx* 44: 588–594.

O'Connor, J. A., Sulloway, F. J. and Kleindorfer, S. (2010c) Avian population survey in the Floreana highlands: is Darwin's medium tree finch declining in remnant patches of *Scalesia* forest? *Bird Conserv. Internatn.* 20: 343–353.

O'Connor, J. A., Sulloway, F. J., Robertson, J. and Kleindorfer, S. (2010d) *Philornis downsi* parasitism is the primary cause of nestling mortality in the critically endangered Darwin's medium tree finch (*Camarhynchus pauper*). *Biodivers. Conserv.* 19: 853–866.

Olivares, A. and Munves, J. A. (1973) Predatory behaviour of the smooth-billed ani. *The Auk* 90: 891.

Ottenburghs, J., Ydenberg, R. C., Van Hooft, P., Van Wieren, S. E. and Prins, H. H. (2015) The Avian hybrids project: gathering the scientific literature on avian hybridization. *Ibis* 157: 892–894.

Parker, P. G., Buckles, E. L., Farrington, H., Petren, K., Whiteman, N. K., Ricklefs, R. E., Bollmer, J. L. and Jimenez-Uzcategui, G. (2011) 110 years of Avipoxvirus in the Galápagos Islands. *PLoS One* 6: e15989.

Peters, K. J. (2016) Unravelling the dynamics of hybridisation and its implications for ecology and conservation of Darwin's tree finches. PhD Thesis, Flinders University, Adelaide, South Australia.

Peters, K. J. and Kleindorfer, S. (2015) Divergent foraging behavior in a hybrid zone: Darwin's tree finches (*Camarhynchus* spp.) on Floreana Island. *Curr. Zool.* 61: 181–190.

Podos, J. (2001) Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* 409: 185–188.

Rentería, J. L., Gardener, M. R., Panetta, F. D., Atkinson, R. and Crawley, M. J. (2012) Possible Impacts of the invasive plant *Rubus niveus* on the native vegetation of the *Scalesia* forest in the Galápagos Islands. *PLoS ONE* 7: e48106.

Reynolds, R. T., Scott, J. M. and Nussbaum, R. A. (1980) A variable circular-plot method for estimating bird numbers. *Condor* 82: 309–313.

Rhymer, J. M. and Simberloff, D. (1996) Extinction by hybridization and introgression. *Annu. Rev. Ecol. Syst.* 27: 83–109.

Roberts, D. G., Gray, C. A., West, R. J. and Ayre, D. J. (2010) Marine genetic swamping: hybrids replace an obligately estuarine fish. *Mol. Ecol.* 19: 508–520.

Schluter, D., Price, T. D. and Grant, P. R. (1985) Ecological character displacement in Darwin's finches. *Science* 227: 1056–1059.

Scott, J. M., Ramsey, F. L. and Kepler, C. B. (1981) Distance estimation as a variable in estimating bird numbers from vocalizations. *Stud. Avian Biol.* 6: 334–340.

Simberloff, D. (1995) Habitat fragmentation and population extinction of birds. *Ibis* 137: S105–S111.

Sinclair, A. R., Fryxell, J. M. and Caughley, G. (2009) Wildlife conservation and management. Pp. 217–383 in A. R. Sinclair and J. M. Fryxell, eds. *Wildlife ecology, conservation and management*. Carlton: Blackwell Publishing.

Steadman, D. W. (1986) *Holocene vertebrate fossils from Isla Floreana, Galápagos*, Washington DC: Smithsonian Institution Press.

Thomas, L., Buckland, S. T., Burnham, K. P., Anderson, D. R., Laake, J. L., Borchers, D. L. and Strindberg, S. (2006) Distance sampling. Pp. 687–697 in A.-H. El-Shaarawi and W. Piegorsch, eds. *Encyclopedia of environmetrics*. Chichester, UK: John Wiley and Sons, Ltd.

Toews, D. P. L. and Irwin, D. E. (2008) Cryptic speciation in a Holarctic passerine revealed by genetic and bioacoustic analyses. *Mol. Ecol.* 17: 2691–2705.

Vilà, C., Walker, C., Sundqvist, A.-K., Flagstad, Ø., Andersone, Z., Casulli, A., Kojola, I., Valdmann, H., Halverson, J. and Ellegren, H. (2003) Combined use of maternal, paternal and bi-parental genetic markers for the identification of wolf–dog hybrids. *Heredity* 90: 17–24.

Watson, J., Trueman, M., Tufet, M., Henderson, S. and Atkinson, R. (2010) Mapping terrestrial anthropogenic degradation on the inhabited islands of the Galápagos Archipelago. *Oryx* 44: 79–82.

Whiteman, N. K., Goodman, S. J., Sinclair, B. J., Walsh, T., Cunningham, A. A., Kramer, L. D. and Parker, P. G. (2005) Establishment of the avian disease vector *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae) on the Galápagos Islands, Ecuador. *Ibis* 147: 844–847.

Wiedenfeld, D. A. (2006) Aves, the Galápagos Islands, Ecuador. *Check List* 2: 1–27.

Wiedenfeld, D. A. and Jiménez-Uzcátegui, G. (2008) Critical problems for bird conservation in the Galápagos Islands. *Cotinga* 29: 22–27.

KATHARINA J. PETERS, SONIA KLEINDORFER*
School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide 5001, Australia.

*Author for correspondence; e-mail: Sonia.kleindorfer@flinders.edu.au