Species Collapse via Hybridization in Darwin's Tree Finches

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Submitted May 2, 2013; Accepted September 17, 2013; Electronically published February 5, 2014

Online enhancement: appendix. *Dryad data:* http://dx.doi.org/10.5061/dryad.t6j52.

abstract: Species hybridization can lead to fitness costs, species collapse, and novel evolutionary trajectories in changing environments. Hybridization is predicted to be more common when environmental conditions change rapidly. Here, we test patterns of hybridization in three sympatric tree finch species (small tree finch *Camarhynchus parvulus*, medium tree finch *Camarhynchus pauper*, and large tree finch: *Camarhynchus psittacula*) that are currently recognized on Floreana Island, Galápagos Archipelago. Genetic analysis of microsatellite data from contemporary samples showed two genetic populations and one hybrid cluster in both 2005 and 2010; hybrid individuals were derived from genetic population 1 (small morph) and genetic population 2 (large morph). Females of the large and rare species were more likely to pair with males of the small common species. Finch populations differed in morphology in 1852–1906 compared with 2005/2010. An unsupervised clustering method showed (*a*) support for three morphological clusters in the historical tree finch sample (1852–1906), which is consistent with current species recognition; (*b*) support for two or three morphological clusters in 2005 with some (19%) hybridization; and (*c*) support for just two morphological clusters in 2010 with frequent (41%) hybridization. We discuss these findings in relation to species demarcations of *Camarhynchus* tree finches on Floreana Island.

Keywords: Camarhynchus, *Philornis downsi*, parasite, mate choice, disassortative pairing, asymmetric reproductive isolation.

Introduction

Insights from the past 2 decades have transformed our understanding of the ecological context of processes that underpin speciation (Coyne 1992; Schluter 2000; Mayr and Diamond 2001; Doebeli and Dieckmann 2003; Birand et al. 2012). According to Charles Darwin (1859) and his "principle of divergence," speciation sometimes involves the extinction of intermediate forms via competition and divergent selection, resulting in sympatric species that are separated by a morphological gap. Ernst Mayr's biological species concept (Mayr 1942) focused on the processes that maintain the morphological gap between species, such as prior geographic isolation and the prevention of hybridization through premating and/or postmating isolating mechanisms (see also Mallet 2008). Darwin (1859) was aware of hybrid sterility, but he cautioned that many studies of apparent hybrid sterility were confounded by evidence of inbreeding by hybrids. Furthermore, Darwin showed that hybrid fertility could be markedly increased after crossing with new strains. Thus, for Darwin, crosses between species could increase fertility in hybrid offspring, while in other circumstances these crosses could lead to hybrid sterility. The current view is that ecological conditions correlate with patterns of hybridization, which can lead to individual fitness costs, biological species collapse, adaptive hybridization, and hybrid speciation (Grant and Grant 1996*a*, 1996*b*, 2002, 2010; Fritz 1999; Rieseberg et al. 2003; Mallet 2007).

The persistence of closely related species in sympatry suggests that natural and/or sexual selection are operating to prevent interspecific gene flow and maintain species barriers. But interbreeding between sympatric populations of recently diverged species is common and regularly occurs in ~10% of animal and ~25% of plant species (Mallet 2007). Hybridization is especially well documented in birds (Clarke et al. 1998; Grant 1998; Good et al. 2000; Grant and Grant 2002; Randler 2002; Joseph et al. 2008). Hybridization can drive the formation of new species by increasing genetic variance and favoring novel evolutionary trajectories in changing environments (DeMarais et al. 1992; Dowling and Secor 1997; Brelsford et al. 2011) but can also result in the extinction of a parental species when selection favors the persistence of hybrids (Rhymer and Simberloff 1996; Seehausen 2006; Taylor et al. 2006). Speciation can thus occur "in reverse" when selection for extreme phenotypes is removed and interspecific hybrids

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Am. Nat. 2014. Vol. 183, pp. 325–341.
DOI: 10.1086/674899

maintain or increase fitness of parental types (Gow et al. 2006; Seehausen 2006; Taylor et al. 2006).

It is widely recognized that hybridization is common, but there is still debate about the role of hybridization for speciation and extinction and whether hybrid offspring generally have lower fitness (Mallet 2007, 2008; Grant and Grant 2010). What is the mechanism and function of hybridization? To test hypotheses, we need more examples of hybridization patterns in the field with multiple sources of evidence such as behavioral, molecular, and fitness measures. Randler (2006) outlined five major hypotheses to predict the occurrence and frequency of avian hybridization, which can be summarized as (1) a size-difference hypothesis, with less hybridization when there are mechanical barriers to reproduction; (2) a sexual selection hypothesis, with more hybridization in the absence of sexual dichromatism; (3) scarcity of conspecifics, which predicts hybridization is initiated by the rare species (Grant and Grant 1997); (4) a paternal-care hypothesis, with more hybridization if females choose species that provide more paternal care; and (5) a parapatric distribution hypothesis that predicts stronger female discrimination in sympatry and hence less hybridization in sympatric than in parapatric species. A comparative approach with 65 phylogenetically independent avian hybrid types showed that hybridization was more common in parapatric species pairs, in species pairs with less parental care, and when at least one of the hybridizing species pairs was endangered (Randler 2006).

Another more recent hypothesis for the occurrence and frequency of hybridization is parasite-mediated selection for hybrids, reviewed in Karvonen and Seehausen (2012). According to this hypothesis, hybrid offspring have an immunological advantage through novel genetic combinations against detrimental parasites and experience lower parasite prevalence and/or susceptibility (Tompkins et al. 2006). Several studies support this hypothesis. Hybrid offspring in sticklebacks (Rauch et al. 2006), red-crowned parakeets (Tompkins et al. 2006), collared and pied flycatchers (Wiley et al. 2009), white-crowned sparrows (MacDougall-Shackleton et al. 2005), and mice (Fritz 1999; Moulia 1999) had intermediate or reduced parasite prevalence and susceptibility (Karvonen and Seehausen 2012), though other studies have shown lower hybrid viability in relation to disease (e.g., MacDougall-Shackleton et al. 2002, 2005; Goldberg et al. 2005).

Here we test the mechanism and function of annual patterns of hybridization in sympatric Darwin's finches (*Camarhynchus parvulus*, *Camarhynchus pauper*, *Camarhynchus psittacula*) on Floreana Island. This model system has several key characteristics for testing hybridization hypotheses (Randler 2006; Karvonen and Seehausen 2012): (1) the three tree finch species differ in body size, as evident from their common names: small (*C. parvulus*), medium (*C. pauper*), and large (*C. psittacula*) tree finch (Lack 1947; Bowman 1963; Grant 1999); (2) the three species have the same pattern of parental care and the same pattern of dichromatism, whereby older males have a black head and females are creamy brown (Lack 1947; Grant 1999; Kleindorfer 2007); (3) the species differ in population status: common (small tree finch), critically endangered (medium tree finch), and rare (large tree finch; O'Connor et al. 2010*c*, 2010*d*); (4) an introduced parasite *Philornis downsi* (discovered in finch nests in 1997) is causing 27%–98% annual nestling mortality on Floreana Island in all three species (Fessl et al. 2001; Dudaniec and Kleindorfer 2006; O'Connor et al. 2010*a*, 2010*b*, 2010*d*); and (5) parasite intensity varies with finch body size, with larger-bodied finches having higher *P. downsi* intensity (Dudaniec et al. 2007). Within the small tree finch, larger individuals with larger nests had more parasites, which suggests selection for smaller nest and body size (Kleindorfer and Dudaniec 2009). Several species of Darwin's finches have had population declines of 40%–70% from 2004 to 2008, which may be partly explained by the impacts of *P. downsi* on Santa Cruz and Floreana Islands (O'Connor et al. 2010*c*; Dvorak et al. 2012).

The three *Camarhyncus* species on Floreana Island are of special interest because Lack (1947) singled them out as a paradigmatic example of successful speciation in Darwin's finches. The medium tree finch probably originated from a "small morph" of the large tree finch from Isabela Island, which was either followed by (Lack 1947) or preceded by (Grant 1999) separate colonization events of "large morph" large tree finches from Santa Cruz Island and small tree finches from another island. The presence of all three *Camarhynchus* species was confirmed by the work of Ridgeway (1890), and all these species are presumed to have remained in sympatry ever since (Lack 1947; Grant 1999; Grant and Grant 2008; Christensen and Kleindorfer 2009; O'Connor et al. 2010*c*; 2010*d*). Evidence that we present here, however, suggests that these three species may represent a case of evolution in reverse; on Floreana Island, the large tree finch may have become extinct in recent years due to hybridization with other *Camarhynchus* species.

To resolve these and other questions, we examine patterns of hybridization across the three sympatric *Camarhynchus* tree finches during the 2005 and 2010 breeding seasons. We have three main aims: (1) to characterize patterns of hybridization in years that differ markedly in parasite intensity and rainfall; (2) to test predictions about the mechanism and function of hybridization; and (3) to determine whether the large tree finch has become extinct on Floreana Island through a process of reverse evolution. A drought occurred on the Galápagos Archipelago from

2000 to 2007, while there was high rainfall from 2008 to 2010. We have previously shown that two factors correlate positively with *P. downsi* intensity: high rainfall and larger finch body size (Dudaniec et al. 2007; Kleindorfer and Dudaniec 2009). We first test if parasite intensity is higher during a high-rainfall year, as predicted from Dudaniec et al. (2007). If parasite-mediated selection confers hybrid individuals with a fitness advantage, we predict: (1) more hybridization during conditions of high parasite intensity (high-rainfall period: 2010) than during conditions of low parasite intensity (low-rainfall period: 2005); (2) lower *P. downsi* intensity in hybrid nests; and (3) higher recruitment of hybrid individuals than large-bodied individuals into the breeding population in years of high parasite intensity compared with years of low parasite intensity.

Given the hypothesis that hybridization is facilitated by female choice and occurs more frequently in rare species (Wirtz 1999; Randler 2002), we also test the following predictions: (1) disassortative pairing whereby females of the rare (large tree finch) and critically endangered (medium tree finch) species will be paired with males of the common species (small tree finch) and not vice versa; (2) female-to-male size will show a pattern of neutral or positive assortative pairing in the small tree finch, but disassortative pairing in the medium and large tree finches; and (3) there will be a higher proportion of unpaired rare males than common males. Finally, we compare our morphological data collected in 2005 and 2010 with the historical data for the three *Camarhynchus* species collected between 1852 and 1906 and previously analyzed by David Lack (1947; for additional information on Darwin finch historical museum collections, see Parker et al. 2011).

Material and Methods

Species Sampling and Study Sites

The three focal species were Darwin's small tree finch, medium tree finch, and large tree finch (fig. A1; figs. A1–A3 available online), which occur in sympatry in the highland *Scalesia* forest of Floreana Island, Galápagos Archipelago. We collected blood samples during the onset of the breeding season from January to April 2005 and 2010. Our 2005 study occurred during a period of extended drought (2000–2007); our 2010 study occurred during a period of high rainfall (2008–2012; Charles Darwin Foundation Meteorological Database: http://datazone.darwinfoundation.org/climate/; Charles Darwin Foundation 2012). Finches were captured in mist-nets and subsequently banded with a numbered aluminium band and a unique combination of color bands. Mist-netting was along a walking trail (site area = 2.4 km^2) through a native *Scalesia* forest at the base of Cerro Pajas Volcano (1°17′43S, 90°27′23W) between 300 and 400 m elevation (described in O'Connor et al. 2010*a*). Each year we placed six 12-m mist-nets along the walking trail between 0530 and 1100 hours, sampled the location once, and moved all nets farther up the trail into a new adjacent area the next day. The population status for the three focal species on Floreana Island is as follows: the small tree finch is most common (~3,700 individuals), the medium tree finch is endemic to Floreana Island and is critically endangered (International Union for Conservation of Nature red-listed; <1,700 individuals), and the large tree finch is rare (<500 individuals; O'Connor et al. 2010*c*).

Morphology and Age

We measured the following morphological traits per bird: (1) beak-head (beak tip to back of head), (2) beak-naris (beak tip to anterior end of the naris), (3) beak-feather (tip of beak to feather line), (4) beak depth (at the base of the beak), (5) beak width (at the base of the beak), (6) naris diameter (taken from extremes of naris opening), (7) tarsus length, (8) wing length, and (9) body mass. Morphological measurements were taken to the nearest 0.01 mm using dial calipers. Mass was measured to the nearest 0.01 g using Telinga electronic scales. All measurements were taken by S. Kleindorfer in 2005 ($N = 94$) and by both S. Kleindorfer ($N = 23$) and J. A. O'Connor ($N = 84$) in 2010. For the eight morphological traits, reliabilities for morphological measurements (per species) between S. Kleindorfer and J. A. O'Connor in 2010 (r) ranged from 0.91 (for beak-head) to 0.99 (for wing length), with a mean r of 0.96 ($n = 15$ birds). We assigned individuals to a tentative species inferred from body size (small, intermediate, large) in the field using morphological data that we collected from 2004 and in consultation with data tables in Lack (1947). Both researchers agreed on species classifications.

Male tree finches (*Camarhynchus* spp.) can be aged based on the proportion of black plumage on the crown and chin (Kleindorfer 2007). Males become progressively black-headed with each annual molt until attaining a fully black head and crown at age 5+ years. Females remain olive green throughout their lives. We aged males based on the discrete annual plumage categories, whereby males with no black head (black 0) were analyzed as yearling males; males with increasing black (black 1–3) as 1, 2, and 3 years old; males with a fully black head as 4 years old (black 4); and males with fully black head and chin (black 5) as at least 5 years old (precise plumage measurements per age class category are given in Kleindorfer 2007). Because Darwin's tree finches can live up to 14 years, males with a fully black head and chin may be between 5 and

14 years of age (S. Kleindorfer, unpublished data; Grant and Grant 2008).

Historical Morphology

David Lack (1947) measured the museum collections of Darwin finch specimens that were collected between 1852 and 1906. Most of the birds that Lack measured came from the 1905–1906 collection at the California Academy of Sciences. For this study, we use the historical data analyzed by Lack, which has been made available on the website BIRDD: Beagle Investigations Return with Darwinian Data, http://www.bioquest.org/birdd/morph.php (BIRDD). We refer to the D. Lack sample as historical data from the 1900s (although the samples were from 1852 to 1906). We compare our measurements (2005, 2010) with those of D. Lack (1852–1906) using one-way ANOVA. The historical sample size is as follows: small tree finch males ($N = 87$), females ($N = 38$); medium tree finch males ($N = 80$), females ($N = 62$); and large tree finch males ($N = 4$), females ($N = 13$).

Morphological Data Analysis

We used two methods to analyze the contemporary (S. O'Connor and J. A. Kleindorfer, 2005 and 2010) morphological data: (1) a model-based unsupervised clustering method using MCLUST, version 4.0, for R (Fraley et al. 2012); and (2) multivariate analyses (MANOVA) of mean morphological measurements between the three putative species (using SPSS for Mac, ver. 17.0). For the historical data, we used MCLUST only but compared the three morphological traits (beak-naris, beak depth, and wing length) to those of contemporary birds using one-way ANOVAs. Females were excluded from morphological analyses because (1) sample sizes were small and variable by year (10%–37% of N per species per year); and (2) their measurements were significantly smaller compared to males. We used MCLUST software to identify morphological clusters that were present on Floreana Island in each year (2005 and 2010) using principal components scores for beak size (derived from the following variables: beak-head, beak-feather, beak-naris, beak depth, beak width) and body size (derived from wing length and tarsus variables). The program fits the observed frequency distribution to 10 alternative models, and the "best" model is taken to be the one with the highest Bayesian Information Criterion (BIC). Using MANOVA, we examined differences in mean morphological measurements of the remaining seven measured traits with the fixed factors (1) putative species or (2) genetic population (see below), with separate analyses for each year.

Blood Sample Collection

We collected blood samples in 2005 and 2010 from 94 and 107 tree finches, respectively. The sample size for each species and sex (M = male, F = female, U = unknown sex) per year is 2005: small morph *Camarhynchus parvulus* $N = 62$ ($M = 44$, $F = 18$), intermediate morph *Camarhynchus pauper* $N = 24$ ($M = 19$, $F = 4$, $U = 1$), and large morph *Camarhynchus psittacula* $N = 8$ ($M = 5$, $F = 3$); and 2010: small morph *C. parvulus* $N = 46$ ($M = 32$, $F = 12$, $U = 2$), intermediate morph *C. pauper* $N = 32$ ($M = 27$, $F = 3$, $U = 2$), large morph *C. psittacula* $N = 29$ ($M = 24$, $F = 3$, $U = 2$). We preserved the blood samples using Whatman FTA paper.

Genotyping and Microsatellite Characteristics

DNA was extracted from Whatman FTA paper using the protocol in Galligan et al. (2012). We genotyped 201 individuals at 10 autosomal microsatellite loci: Gf01, Gf03, Gf04, Gf05, Gf06, Gf07, Gf09, Gf11, Gf12, Gf13 (Petren 1998). Polymerase chain reaction amplification followed Galligan et al. (2012). An Applied Biosystems ABI-3770 automated sequencer provided genotypes that were manually scored with Genemapper, version 4.0 (Applied Biosystems). We excluded juvenile finch samples to minimize the use of genetically related individuals. We tested linkage disequilibrium for each locus by putative population using GENEPOP v4.0.10 and assessed significance ($P < .01$) after Bonferroni correction (Rice 1989). Locus pairs in linkage disequilibrium were further assessed using Linkdos software (Garnier-Gere and Dillman 1992; http://genepop.curtin.edu.au/linkdos.html), which estimates the correlation coefficient r_{LD} (Black and Krafsur 1985) and is correlated with the distance between loci (Kaeuffer et al. 2007). An r_{LD} of <0.3 (with $P < .05$) indicates a distance between loci greater than 3 cM, which is sufficient distance that any linkage effect does not bias clustering analyses (Pritchard and Wen 2004). The number of alleles (N_A), expected and observed heterozygosity (H_E, H_O), and pairwise F_{ST} (Weir and Cockerham 1984) were calculated for each locus by putative population and globally for each locus (table A1; tables A1–A5) using GENEPOP v4.0.10 (Raymond and Rousset 1995; Rousset 2008) and GENALEX v6.1 (Peakall and Smouse 2006).

Population Genetic Structure and Hybridization

We determined population structure using the Bayesian clustering method implemented in STRUCTURE v2.3.2 (Pritchard et al. 2000; Falush et al. 2007; Hubisz et al. 2009). We used the correlated allele frequencies option and the admixture model, with the latter allowing indi-

viduals to have partial ancestry in each cluster. We set allele frequency priors according to the data (mean = 0.15, SD = 0.05, $\lambda = 1$) and ran 10 Markov chain Monte Carlo (MCMC) replicates for $K = 1$–10. We expected the degree of admixture to be relatively low, so we set α at 0.5. We tested the suitability of these priors, with attention to α and λ, by comparing convergence dynamics of multiple MCMC chains (1×10^5 MCMC iterations for $K = 1$–6) for a range of priors that included our custom priors and the default STRUCTURE priors. The results supported the use of our custom priors and chains appeared to converge with mixing within 8×10^4 MCMC iterations; therefore, we chose a relatively conservative burn-in of 1×10^5 MCMC iterations for all further runs. Exploration of the data for consistency across longer and shorter chains for a range of K indicated that a chain length of 5×10^5 MCMC iterations was most appropriate. Using our optimized burn-in length (1×10^5 iterations) and MCMC length (5×10^5 iterations), we ran 10 MCMC replicates for $K = 1$–6. We used two methods to infer the correct number of clusters: (1) the mean log likelihood method of Pritchard et al. (2000); and (2) the delta K method of Evanno et al. (2005), which involves calculating a quantity based on the second-order rate of change of the likelihood function with respect to K. As we have relatively few loci, we were concerned that differentiation between populations may be more difficult to detect, so we also implemented the LOCPRIOR model in STRUCTURE, which uses a modified prior distribution for clustering that allows the distribution of cluster assignments to vary by putative population (Hubisz et al. 2009), or in this case, by putative field-assigned species. Assignment probabilities to each cluster were averaged over the 10 MCMC replicates. Due to the close phylogenetic relationship between our three putative species (Petren et al. 1999; Sato et al. 1999), we chose an arbitrary assignment cutoff value of 0.75 to each cluster. Individuals with assignment probabilities <0.75 were considered of hybrid origin. We calculated the percentage of assignments to each cluster ($\pm$ SE) as an average across all runs for the best value of K.

Simulations to Assess Hybrid Assignment Power

The most likely number of clusters was $K = 2$ for both 2005 and 2010 analyses, and clusters corresponded to small and large beak morphs (see "Results"). To validate the use of our 0.75 assignment cutoff to cluster 1 and cluster 2 and therefore our ability to assign individuals to a hybrid class using our data, we ran STRUCTURE analyses using 20 simulated data sets created in HybridLab, version 1.0 (Nielsen et al. 2006), 10 for each year (2005 and 2010). HybridLab draws alleles randomly as a function of their estimated frequency distributions, assuming linkage equilibrium among loci, selective neutrality, and random mating. STRUCTURE runs were identical to those used with the real data, using both the standard admixture and LOCPRIOR models but carried out for $K = 2$ only. These runs differed from the analyses with real data both in terms of the proportional composition of pure and hybrid genotypes and in pure genotypes being in perfect Hardy-Weinberg and linkage equilibria, which is unlikely in real data (Stark et al. 2011). Each data set consisted of 100 parent 1 individuals (simulated from cluster 1), 100 parent 2 individuals (simulated from cluster 2), and 100 F_1 hybrids (with parents as cluster 1 and cluster 2). Assignment probabilities were averaged across runs for each data set, and then averaged across data sets. The proportion of individuals correctly assigned to their simulated class (parent 1, parent 2, F_1 hybrid) with a cutoff of 0.75 was assessed and, therefore, the estimated error of our genetic assignments. The lack of a defined pure parental species and the high levels of admixture across the three putative species precluded us from differentiation among classes of hybrids beyond the F_1 generation, and thus from obtaining meaningful results from other molecular hybridization analysis software.

Rainfall and Philornis downsi *Intensity*

Rainfall on the Galápagos Islands tends to alternate between prolonged La Niña periods of low rainfall (2–11 years) and brief El Niño periods of high rainfall (1–2 years; Snell and Rea 1999). Because we collected data between January to March, we analyze parasite intensity in relation to rainfall between January to March (Charles Darwin Foundation 2012). In our study, 2010 was a high-rainfall year (~600 mm) and 2005 was a low-rainfall year (~300 mm). For comparison, we provide the highland rainfall data from January to March for several years, including 2004 (144 mm), 2005 (332 mm), 2008 (1,340 mm), 2010 (635 mm), and 2012 (672 mm). A previous study showed that *P. downsi* intensity per nest increased with high rainfall (Dudaniec et al. 2007). Therefore, we tested the prediction that *P. downsi* intensity would be higher in 2010 (high-rainfall year) than in 2005 (low-rainfall year). We have a standardized protocol to sample *P. downsi* from finch nests at our study sites: daily transects are searched for active nests; nests are monitored for nesting outcome every 2 days; after a completed nesting attempt, nests are dismantled to collect and count *P. downsi* larvae; chick age at death is noted (Dudaniec and Kleindorfer 2006; Fessl et al. 2006). We retrospectively analyzed *P. downsi* intensity in relation to genetic population. The sample size for *P. downsi* intensity in relation to genetic assignment is: 18 nests assigned to genetic population 1, 17 nests assigned to the hybrid cluster, and 8 nests assigned to genetic population 2. For this anal-

ysis, we used 11 nests sampled in 2012 (high-rainfall year) for which we have data on *P. downsi* intensity as well as parental genotypes, the latter which were sampled (and color-banded) in 2010 and assigned to a genetic population. The sample size per year is as follows: genetic population 1 (2005 = 6, 2010 = 10, 2012 = 2), hybrid cluster (2005 = 5, 2010 = 6, 2012 = 6), and genetic population 2 (2005 = 3, 2010 = 2, 2012 = 2).

Female Choice for Heterospecific Males

There are two common predictions for female preferences in hybridizing populations: (1) females of the rarer species prefer larger heterospecific males, perhaps because they are a super stimulus (Wirtz 1999) or can control more resources (Pierotti and Annett 1993); or (2) females of the rare species have few males to choose as mates and are therefore more likely to pair with males of the common species (Grant and Grant 2002). In this study, rare females are larger bodied and common males are smaller bodied. We test assortative pairing in relation to female body size to test whether rare, large-bodied females are paired with large-bodied rare males or with small-bodied common males. We analyze the data for each putative species (based on the morphology ranges in table A2), and separately for population genetic assignment. We compare assortative pairing outcome based on female attributes given that females choose a male partner in this study system. The sample size for assortative pairing per sampling year is as follows. During the low-rainfall year (2005), we have morphological data for the male and female in 9 pairs from genetic population 1; 2 pairs from the hybrid cluster; and 5 pairs from genetic population 2. During the high-rainfall year (2010), we have morphological data for the male and female in 5 pairs from genetic population 1; 11 pairs from the hybrid cluster; and 3 pairs from genetic population 2. Pairs were identified if both male and female birds at a nest had color bands; we cross-referenced the morphology measurements and genetic assignment for the color-banded birds. To test the prediction that larger females paired with smaller males, rather than smaller females with larger males, we compared the size difference in male and female partners using derived body size scores from principal components (PC) analysis. A positive score indicates that the female in the pair was larger (beak, body), and a negative score indicates that the male in the pair was larger (beak, body). In males, the derived PC beak factor scores explained 83% of the variance and had high factor loadings for beak length (0.89), beak depth (0.92), and beak width (0.92); the derived PC body factor scores explained 78% of the variance and had high factor loadings for male tarsus length (0.88) and wing length (0.88). In females, the derived PC beak factor scores explained 83% of the variance and had high factor loadings for beak length (0.90), beak depth (0.95), and beak width (0.88); the derived PC body factor scores explained 57% of the variance and had high factor loadings for female tarsus length (0.76) and wing length (0.76).

Data and Results

All data used to produce figure and tables for this manuscript are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.t6j52 (Kleindorfer et al. 2014).

Morphology

Our subjective classification into species category, informed by D. Lack's measurements and based on morphological measurements in the field, resulted in three morphologically distinguishable populations and a statistically significant interaction term species × year (MANOVA: $F_{16,\ 236} = 3.93$, $P < .001$; Wilks's $\lambda = 0.62$; fig. 1, table A2). The purported species showed patterns of morphology in accordance with their common name: morphology was smallest in the "small tree finch," intermediate in the "medium tree finch," and largest in the "large tree finch."

In contrast to our subjective analysis of morphological classification, which seemed to identify three species, MCLUST analyses found support for two and three morphological clusters (table A3). There was a morphological gap between the two clusters in 2005, but by 2010 the morphological gap was occupied by individuals with "intermediate" morphology (fig. 2). MCLUST provides BIC values for the top three models that best fitted the data in terms of cluster size, shape, and orientation (but also provides BIC values for all 10 models under 1- to 9-cluster scenarios). In 2005, differences in BIC values ($\Delta \leq 0.49$) for the top three models showed substantial support for both two- and three-cluster models. Specifically, differences in BIC values (ΔBIC) were 0.13 and 0.49 for alternative three-cluster and two-cluster solutions, respectively. In 2010 there was strong support for a two-cluster model, weaker support for one-cluster models ($\Delta > 2.57$), and no support for a three-cluster model ($\Delta > 7$; table A3. As large tree finches, in the period studied by Lack (1888–1906), never constituted more than 3% of the total tree finch population (and represent only 19% of the field-assigned classifications in the modern sample), clustering models will tend to underrepresent three-cluster models, owing to low statistical power.

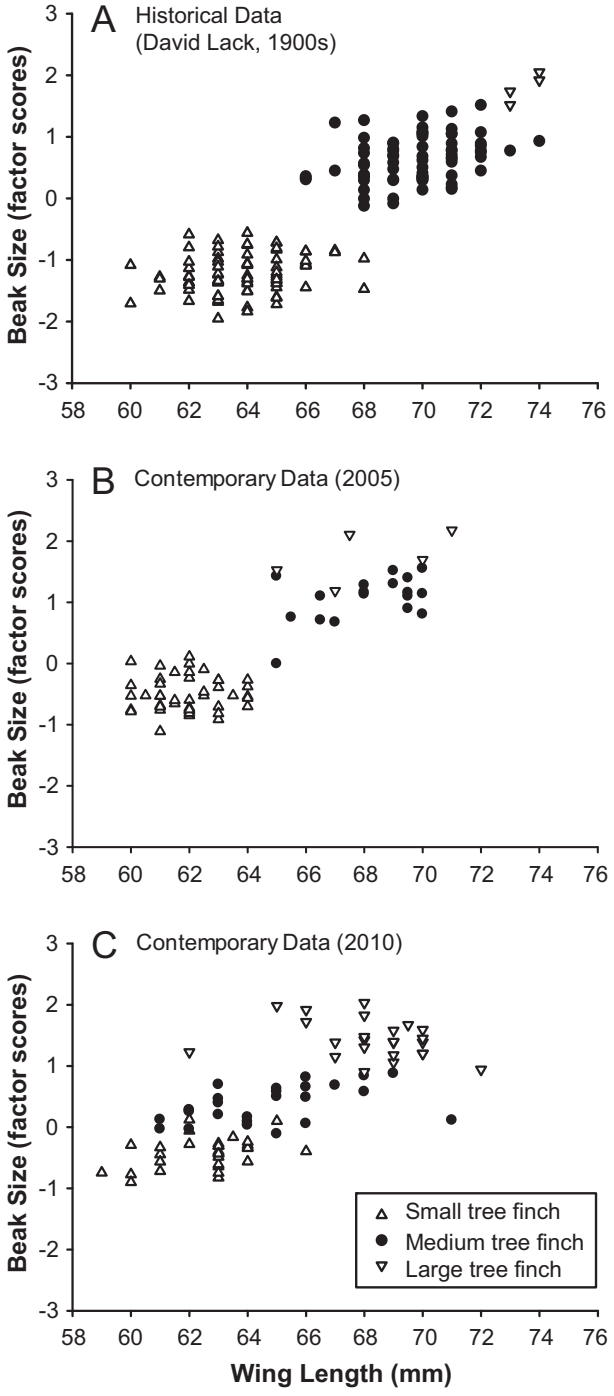

Figure 1: The relationship between wing length (mm) and beak size (principal components factor scores) for individual tree finches on Floreana Island shown per putative species assignment at the time of measurement in the field for data analyzed by D. Lack ($N = 171$ males, 1900s; *A*), S. Kleindorfer and J. O'Connor ($N = 76$ males, 2005; *B*), and S. Kleindorfer and J. O'Connor ($N = 83$ males, 2010; *C*). The confusion over reliable morphological distinctions in the contemporary tree finch populations was the impetus for the subsequent genetic analyses.

Locus Characteristics and Genetic Diversity

Significant departure from linkage disequilibrium was detected for one locus pair (Gf09 and Gf11), although it was only detected in a single putative species in a single sampling period. The r_{LD} for this locus pair was <0.3 ($P < .05$), indicating a probable distance of greater than 3 cM between loci. Therefore, we chose to retain these loci in further analyses. The loci Gf09 and Gf11 also showed deviation from Hardy-Weinberg equilibrium (HWE) across three and two of the putative species, respectively, in 2010 only, with large heterozygote deficit (table A1) and these loci were excluded from further analyses. Six of the remaining eight loci (Gf01, Gf03, Gf04, Gf07, Gf09, Gf11) showed significant departure from HWE ($P < .01$), although three of these loci departed from HWE in only a single putative species (table A1). All six loci showed heterozygote deficiency (table A1). Because we expected our data to contain hybrids that would influence Hardy-Weinberg dynamics, we interpreted these results cautiously and proceeded with analyses using all loci. All loci have previously been used successfully in Darwin's finches (Petren 1998; Petren et al. 1999, 2005; Galligan et al. 2012). Missing data ranged between 1% and 8% across loci. Across all individuals, the number of alleles per locus ranged from 3 to 20 (mean 10.1 ± SE 1.6) and expected heterozygosity ranged from 0.08 to 0.9 (mean 0.56 ± SE 0.09).

Genetic Population Assignment

Estimates of the logarithm of probability of the data for $K = 1$–6 were maximal for $K = 2$ under the standard admixture model (F_{ST} between clusters = 0.16, $P < .001$) (fig. A2). Applying the LOCPRIOR model to our data, estimates of the logarithm of probability of the data for $K = 1$–6 were also maximal for $K = 2$ (F_{ST} between clusters = 0.10, $P < .001$) (fig. A3). As the standard admixture and LOCPRIOR models both estimated the most likely number of genetic clusters to be $K = 2$, we conclude that the inclusion of putative population information in the model did not overly influence clustering. Hence we chose to interpret the individual ancestry assignments provided by the LOCPRIOR model, which can often provide more accurate inference of individual ancestry in data sets where the signal of structure is weak (Pritchard et al. 2009). Clusters (defined by the 0.75 cutoff value) were generally representative of groups of putative populations; cluster one (hereafter, genetic population 1) contained the majority of individuals from putative small tree finch (*Camarhynchus parvulus*) from 2005 and 2010, and cluster two (hereafter, genetic population 2) contained the majority of individuals from putative medium tree finch (*Camarhynchus pauper*) from 2005 and putative large tree

finch (*Camarhynchus psittacula*) from 2005 and 2010, while the majority of individuals from putative *Camarhynchus pauper* from 2010 showed intermediate memberships (assigned to the category "hybrid"; table 1). Each genetic cluster and the hybrid cluster contained between 1 and 8 private alleles (table A4). There was a significant association between putative species and genetic population ($\chi^2 = 155.31$, df = 4, 201, $P < .001$).

Validating Hybrid Assignment

Across all simulated data sets ($n = 10$) and runs (5 per data set) without using population information (i.e., no LOCPRIOR) in the program STRUCTURE, the percentage of F_1 hybrids correctly assigned to their class of origin was 70% ± 0.6%. Of the incorrectly assigned individuals, 15.4% ± 0.4% were assigned to parent 1 and 14.4 ± 0.5% were assigned to parent 2 with a probability > 0.75. This suggests that up to approximately 30% of individuals assigned to either of the parental genetic clusters in the STRUCTURE analysis using the real data could potentially be incorrectly assigned and instead warrant hybrid status. For individuals in the parent 1 category (derived from "small morph" genetic cluster), 86.7% ± 0.56% were successfully assigned, and for those in the parent 2 category ("large morph" genetic cluster), 85.0% ± 0.39% were successfully assigned with a probability > 0.75. Just 0.2% and 0.1% of individuals were incorrectly assigned to parent 1 and parent 2, respectively, while the remainder of misassigned individuals were of hybrid status (<0.75). With LOCPRIOR activated in the analyses, all individuals were assigned to their category of origin with 100% success and assignment probabilities >0.9 for parent 1 and parent 2.

Comparing Morphology and Age per Genetic Population

Figure 3 shows the relationship between body size (using PC body factor scores) and beak size (using PC beak factor scores) per genetic population (MANOVA $F_{16, 352} =$

Figure 2: Morphological clusters identified by MCLUST. Shown are morphological data (males only) analyzed by D. Lack ($N = 171$, 1888–1906; *A*), Kleindorfer and O'Connor ($N = 76$, 2005; *B*), and Kleindorfer and O'Connor ($N = 83$, 2010; *C*). The small-bodied finches are represented by filled circles, intermediate-sized finches by crosses, and large-bodied finches by filled triangles. Component means are marked by an asterisk at the center of each ellipse, and ellipses with axes indicate covariances. Wing length (mm) and principal components scores for derived beak size were used as input variables for *A*. Principal components scores for derived beak size and body size were entered as input variable in *B, C*. This method identified three morphological clusters in historical data (*A*) and either two or three clusters in contemporary data, depending on the year sampled (*B, C*).

Table 1: Percentage membership of a field-identified putative species (purported small, medium, and large tree finch) to a genetic population

Model	Small tree finch (% [*N*])	Medium tree finch (% [*N*])	Large tree finch (% [*N*])
LOCPRIOR:			
2005:			
Pop 1	0	92 (22)	100 (8)
Pop 2	61 (38)	0	0
Hybrid	39 (24)	8 (2)	0
2010:			
Pop 1	2 (1)	3 (1)	97 (28)
Pop 2	48 (22)	3 (1)	0
Hybrid	50 (23)	94 (30)	3 (1)
Standard admixture:			
2005:			
Pop 1	31 (19)	4 (1)	0 (0)
Pop 2	5 (3)	58 (14)	38 (3)
Hybrid	65 (40)	38 (9)	63 (5)
2010:			
Pop 1	29 (13)	19 (6)	0 (0)
Pop 2	7 (3)	9 (3)	67 (20)
Hybrid	64 (29)	72 (23)	33 (10)

Note: The genetic population membership percentage was based on mean STRUCTURE assignment (LOCPRIOR model, standard admixture model) across 10 runs at $K = 2$. The assignments were for genetic population 1 (Pop 1), genetic population 2 (Pop 2), or hybrid cluster (probability of assignment < 0.75). N = population size.

20.78, $P = < .001$; Wilks's $\lambda = 0.26$, partial $\eta^2 = 0.49$) and year ($F_{8, 176} = 7.08$, $P = < .001$; Wilks's $\lambda = 0.76$, partial $\eta^2 = 0.24$). The interaction effect was not significant ($F_{16, 352} = 1.37$, $P = .15$; Wilks's $\lambda = 0.89$, partial $\eta^2 = 0.06$). Genetic population 1 predominantly contained individuals with small morphology, whereas large individuals were mostly assigned to genetic population 2 (fig. 3; table 2). Individuals with mixed assignments (hybrid cluster) between the two populations had intermediate morphology (fig. 3; table 2).

There was no significant difference in morphology comparing low- and high-rainfall years (2005, 2010) for genetic population 1 (PC beak: $t = 0.91$, df = 44, $P = .37$; PC body: $t = -1.01$, df = 43, $P = .32$) or genetic population 2 (PC beak: $t = 0.36$, df = 44, $P = .72$; PC body: $t = -0.51$, df = 44, $P = .61$). But birds in the hybrid cluster in 2010 were significantly larger than in 2005 (PC beak: $t = -1.49$, df = 57, $P = .14$; PC body: $t = -2.56$, df = 56, $P = .01$; table 2).

Recruitment into the breeding population was significantly different across years in relation to population genetic assignment (fig. 4). We examined recruitment as the proportion of yearling males that were singing at a nest to attract a female. In 2005, there was no significant difference in the proportion of yearling males and older males (5+ years of age) between genetic population 1, hybrid cluster, and genetic population 2 (see fig. 4; $\chi^2 = 2.48$, df = 2, 13, $P = .29$). But by 2010, there was a significant difference, with more yearling males in genetic population 1 (7.1% vs. 21.1%) and the hybrid cluster (0% vs. 14.6%) and more older males in genetic population 2 (20.8% vs. 61.5%; fig. 4; $\chi^2 = 12.07$, df = 2, 36, $P = .002$). More specifically, yearling hybrids had higher recruitment in the 2010 population than did nonhybrids ($\chi^2 = 4.20$, df=1, 14, $P = .04$).

Size-Assortative Pairing

There were no significant differences in assortative pairing across low- and high-rainfall years for genetic population 1 (PC beak: $t = 0.07$, $P = .94$; PC body: $t = -0.19$, $P = .85$) or the hybrid cluster (PC beak: $t = -0.61$, $P = .56$; PC body: $t = 0.24$, $P = .81$). But for genetic population 2, females were significantly larger than males during the high-rainfall versus low-rainfall years (PC beak: $t = -1.15$, $P = .36$; PC body: $t = -3.05$, $P = .023$; fig. 5). Further evidence that female choice for smaller males is driving our observed pattern of hybridization is that 67.0% (18/27) of males assigned to genetic population 2 (rare birds with large morphology) were unpaired, and just 15.2% (5/33) of males assigned to genetic population 1 (common birds with small morphology) were unpaired ($\chi^2 = 16.67$, $P < .001$).

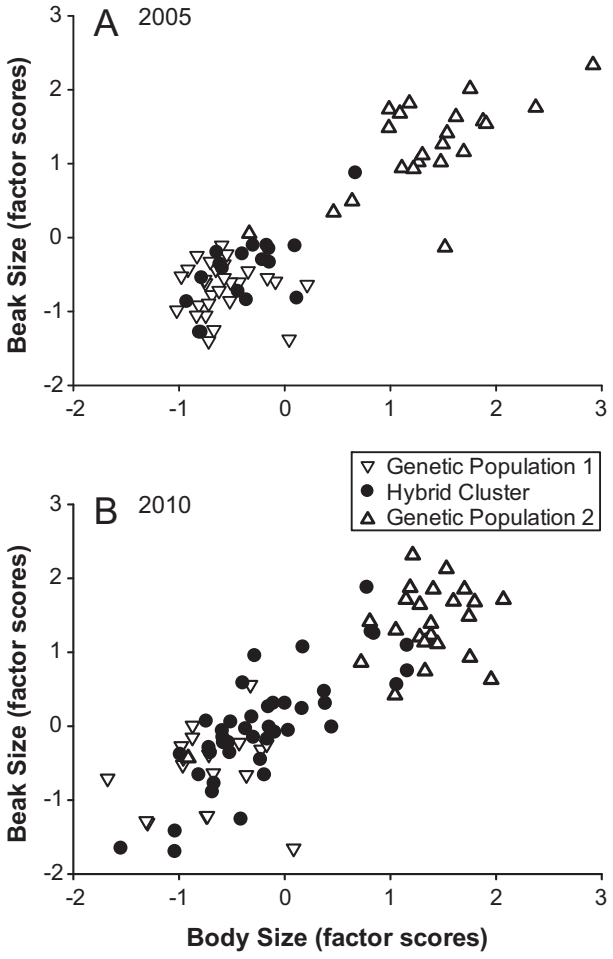

Figure 3: The relationship between body size (principal components factor scores) and beak size (principal components factor scores) for individual tree finches on Floreana Island shown in relation to genetic population for (*A*) 2005 (low-rainfall year; $N = 76$ males) and (*B*) 2010 (high-rainfall year; $N = 83$ males).

Parasite Intensity

Parasite intensity differed significantly in relation to year (ANOVA; year: $F_{1, 42} = 4.42$, $P = .042$) and population genetic assignment (genetic population 1 = small morphology, hybrid cluster = intermediate morphology, genetic population 2 = large morphology; genetic population: $F_{2, 42} = 10.37$, $P < .001$) but not the interaction term year × genetic population (interaction term: $F_{2, 42} = 2.10$, $P = .14$). Using Tukey's HSD posthoc tests, we found that during the low-rainfall year in 2005, genetic population 2 (the largest morph) had the highest parasite intensity, which was significantly greater than genetic population 1 ($P = .038$) and the hybrid cluster ($P = .025$). genetic population 1 (i.e., small morph) did not differ significantly in parasite intensity from the hybrid cluster ($P = .91$; fig. 6). During the high-rainfall years (2010, 2012), genetic population 2 had significantly higher parasite intensity than genetic population 1 ($P = .002$) and the hybrid cluster ($P < .001$), with no significant difference between genetic population 1 and hybrid cluster ($P = .46$; fig. 6). Parasite intensity was higher during 2010 than 2005 for genetic population 1 and genetic population 2 but remained constant across years for the hybrid cluster (fig. 6).

Historical versus Contemporary Morphology

Both medium and large tree finches collected from 1852 to 1906 and later measured by D. Lack were larger than those we measured in 2005 and 2010 (fig. 1; table A4). Notably, despite larger sample sizes for the contemporary data, we did not measure any finches that had beak morphology within the size range of historical large tree finches (Lack 1947, app. 8). MCLUST analyses found evidence for three distinct morphological clusters in Lack's historical data (fig. 2; table A3).

Discussion

The results presented here go to the heart of evolutionary biology: by what criteria do we denote species, and by what criteria do new species form or collapse? Here we present evidence that three sympatric species of Darwin's tree finches in the 1900s have collapsed, under conditions of hybridization, into two species by the 2000s. The proportion of yearling hybrid birds increased from 0% in 2005 to 14.6% in 2010, indicating a potential for elevated hybrid fitness in this system. While hybrid nests had fewer *Philornis downsi* parasites, we do not infer that parasites were the cause of the hybridization. Rather, in a novel environment, hybrid offspring may be favored (Grant et al. 2003, 2004)—especially if their phenotype and/or genotype provide(s) a fitness advantage in the face of a novel parasite. There is widespread agreement that the benefits of hybridization include increased genetic variance that facilitates novel evolutionary trajectories in changing environments (Grant and Grant 1996*a*; Seehausen 2004).

Number of Morphological and Genetic Populations

In addition to species collapse via hybridization, we report on the suspected extinction of the large tree finch (*C. psittacula*) on Floreana Island. In the field, using our putative species categories, we identified three statistically different morphological populations in 2005 and 2010. Unsupervised cluster analysis of morphological data strongly supported the presence of either two or three tree finch populations in 2005 (ΔBIC < 0.49) but only two

Table 2: Male morphological traits (mean ± SE) for each genetic assignment (genetic population 1, hybrid, genetic population 2) in relation to study year

	Population 1		Hybrid		Population 2		Year	Genetic assignment	Year × genetic assignment
Trait	2005 ($N = 28$)	2010 ($N = 18$)	2005 ($N = 18$)	2010 ($N = 44$)	2005 ($N = 22$)	2010 ($N = 24$)	F	F	F
Beak-head (mm)	26.2 ± .1	26.4 ± .1	26.5 ± .2	27.1 ± .2	29.4 ± .2	29.3 ± .2	.81	37.09***	.26
Beak-feather (mm)	13.3 ± .1	13.5 ± .1	13.5 ± .2	14.0 ± .1	15.4 ± .1	15.6 ± .1	3.34	41.75***	.80
Beak-naris (mm)	7.4 ± .1	7.5 ± .1	7.5 ± .1	7.9 ± .1	8.8 ± .1	8.9 ± .1	1.78	45.96***	3.07
Beak depth (mm)	7.1 ± .1	7.4 ± .1	7.4 ± .1	7.7 ± .1	8.4 ± .1	8.6 ± .1	5.765*	34.86***	.30
Beak width (mm)	6.3 ± .1	6.6 ± .1	6.4 ± .1	6.9 ± .1	7.3 ± .1	7.4 ± .1	6.586*	20.69***	.19
Tarsus (mm)	20.3 ± .1	20.4 ± .2	20.5 ± .2	21.1 ± .1	22.3 ± .2	22.5 ± .2	1.05	22.73***	.40
Wing (mm)	61.7 ± .2	62.2 ± .5	62.6 ± .4	64.2 ± .4	67.9 ± .5	68.0 ± .5	.65	22.91***	.18
Mass (g)	13.1 ± .3	12.9 ± .2	13.4 ± .6	13.7 ± .3	17.3 ± .2	17.6 ± .3	.04	32.65***	.11

Note: We used MANOVA to test for the main effects year and genetic assignment and the interaction term. The F values are shown. The interaction term year × genetic assignment was not significant for any morphological variable.

* $P < .05$.

*** $P < .001$.

populations in 2010. Analyses of 2010 data showed weaker support (ΔBIC > 2.57) for a one-cluster solution, which the authors do not perceive to be biologically plausible given our understanding of morphological, behavioral, and now genetic differences within the Floreana tree finch group (Christensen and Kleindorfer 2009; O'Connor et al. 2010*c*, 2010*d*). The historical data showed three morphological populations using both methods (MCLUST and MANOVA analyses).

The mean morphological trait values of the largest tree finch comparing historical (1852–1906) versus contemporary (2005/2010) samples showed a significant reduction or absence of large-bodied tree finches. Globally, there are many examples of bird species that are indistinguishable by morphology and differ only in song. In these cases, song is an effective premating barrier (reviewed in Kroodsma 2005; Price 2008; Toews and Irwin 2008). Therefore, it is possible that there are three sympatric tree finch species on Floreana Island that are morphologically indistinguishable but differ in gene flow due to premating reproductive barriers such as female choice for male song. When we tested for evidence of reproductive isolation among three populations, we found two genetic clusters and hybrid individuals in both study years (2005, 2010). Birds with small beak and body size were assigned to genetic population 1,whereas birds with large beak and body size were assigned to genetic population 2. Individuals with mixed genetic assignment, the hybrid cluster, occupied novel intermediate morphological space in 2010.

There were twice as many hybrid individuals during the high-rainfall year (2010, $N = 44/107 = 41\%$ hybrid birds) than the low-rainfall year (2005, $N = 18/94 = 19\%$ hybrid birds). During 2005, the hybrid males were mostly older (38.9% black 4; 27.8% black 5) with 0% yearling males in the population, whereas there were fewer older males in genetic population 1 (11% black 5) and genetic population 2 (21% black 5; fig. 4). These data suggest that the 2005 hybrids had survived from a previous period of hybridization—perhaps the 1998 El Niño high-rainfall year. In contrast, in 2010, there were more yearling males for the hybrid cluster (14.6%) and none for genetic population 2 (0%) (fig. 4), showing hybrid recruitment following the high-rainfall conditions beginning in 2008.

Comparison with the Historical Data: Is the Large Tree Finch Locally Extinct?

The historical data analyzed by D. Lack (published in 1947) further informs our interpretation of the tree finch morphs on Floreana Island. Compared with what Lack found, our largest field-identified large tree finches were 18% smaller. In the 2000s, mean beak-naris length in large tree finches was 9.0 mm compared with 9.9 mm in the 1900s (Lack 1947). In the 2000s, the large tree finch beak-naris size (9.0 mm) was comparable to Lack's medium tree finch (9.0 mm), the medium tree finch (8.3 mm) was smaller than Lack's medium tree finch (9.0 mm), but the small tree finch beak-naris size remained very similar (7.3 and 7.4 mm, respectively). These comparisons elucidate our observations, and raise several scenarios. Scenario 1: the large tree finch is so rare that we did not capture any. Scenario 2: the large tree finch is extinct, with just small and medium tree finches being extant (i.e., we incorrectly believed that we had sampled large tree finches). Scenario 3: the large tree finch and medium tree finch have both experienced directional selection for smaller body size and still persist today. The absence of genetic and morphological differentiation provides only weak support for sce-

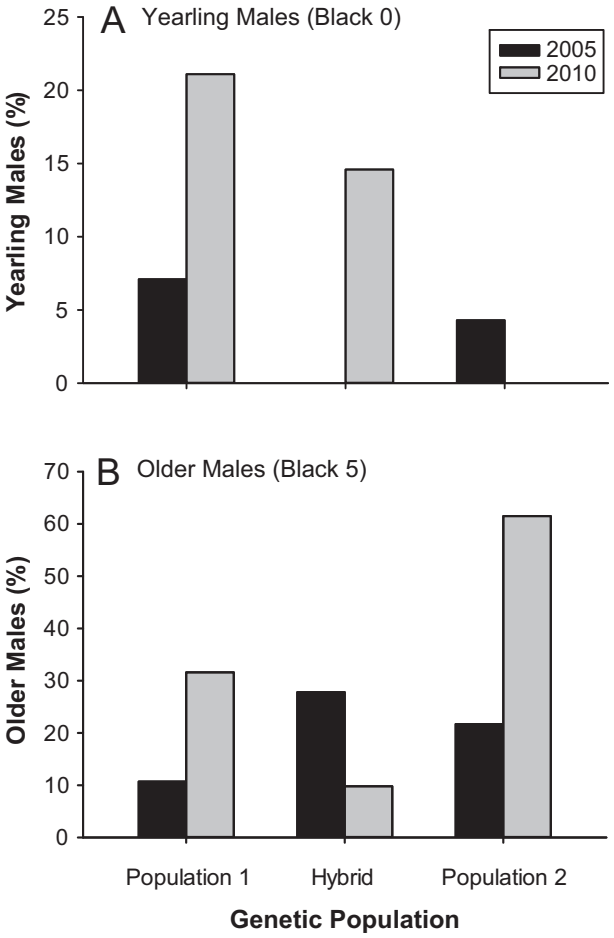

Figure 4: The proportion of (*A*) yearling ($N = 14$) and (*B*) older (5+ years of age; $N = 39$) males in the tree finch population at the time of mist-netting during 2005 and 2010. In 2005, the sample size was as follows for yearling and older males: hybrid cluster ($N = 0$, 5), genetic population 1 ($N = 2$, 3), and genetic population 2 ($N = 2$, 5). In 2010, the sample size was as follows for yearling and older males: hybrid cluster ($N = 6$, 4), genetic population 1 ($N = 4$, 6), and genetic population 2 ($N = 0$, 16).

nario 3, unless there is a plastic species assemblage that appears and reappears. Despite most reference books listing the large tree finch as breeding on Floreana Island (see also Grant and Grant 2008), Peter Grant and Rosemary Grant have both indicated doubt as to the long-term persistence of large tree finches on Floreana Island (personal communication, P. Grant and R. Grant). Notably, the large tree finch has not been found in fossilized form on Floreana Island (Steadman 1986); but fossils have been collected only from the lowlands of Floreana Island where large tree finches rarely occur. Thus, the absence of the large tree finch in Steadman's fossil series is not unexpected, as noted by Steadman (1986). Based on our long-term field observations since 2004, we suggest that scenarios 1 or 2 are most plausible—namely, that the large tree finch is either very rare or extinct from our sampling sites on Floreana Island. We will argue from this point forward that the contemporary "large" tree finch we identified is actually the extant medium tree finch and that the medium tree finch we identified is now bimodal with the intermediate morph finches arising through hybridization with small tree finches. We have no evidence for the persistence of the large tree finch on Floreana Island (K. J. Peters et al., unpublished manuscript), despite having previously published results on three morphological categories (labeled as the three recognized species) that differed in foraging behavior (Christensen and Kleindorfer 2009). We suspect that our observed shift from three to two morphological clusters occurred after the high-rainfall period from 2008 onward. Christensen and Kleindorfer (2009) reported on foraging behavior during 2005 and 2006 in small tree finch, medium tree finch, and large tree finch, but the new evidence provided here suggests they were more likely reporting on small tree finch, hybrid, and medium tree finch individuals. Therefore, this foraging data requires reanalysis for color-banded and genotyped birds.

Evidence for Hybridization

Our genetic data were strikingly congruent with our morphological cluster analysis, showing evidence for species

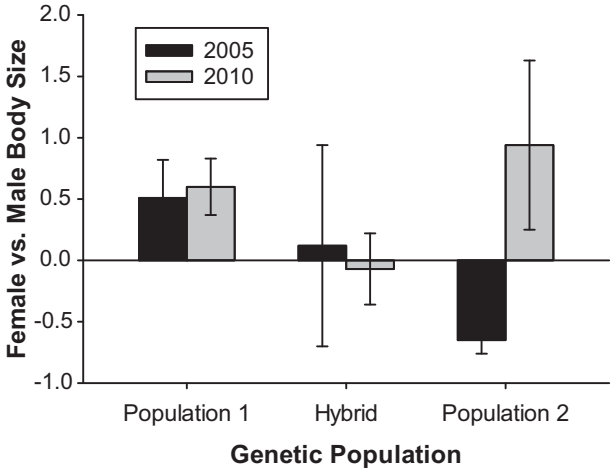

Figure 5: The difference (mean ± SE) in body size (principal components body factor scores) in male and female pairs shown for each genetic population. A value close to zero indicates size-assortative pairing, whereas high positive values indicate that the pair female was larger than the pair male. Genetic population 1 ($N = 14$) and the hybrid cluster ($N = 13$) had size assortative pairing during both years. In genetic population 2 ($N = 8$), the pair female had larger body size than the pair male during 2010 (high-rainfall year).

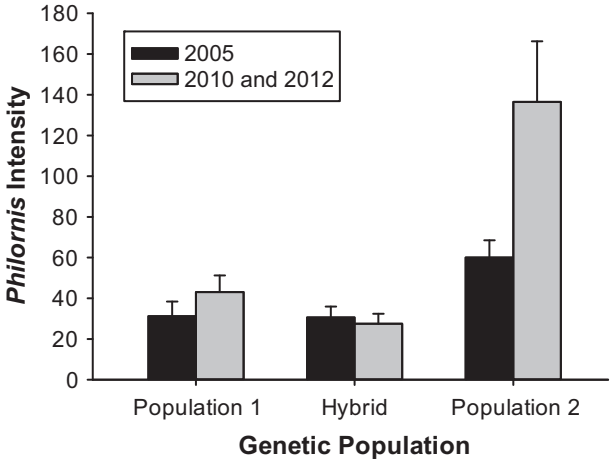

Figure 6: Mean parasite intensity (mean ± SE) from *Philornis downsi* larvae per Darwin's tree finch nest on Floreana Island. The data are shown for nests that have been assigned to a genetic population. On average, genetic population 1 ($N = 18$) corresponds to small-bodied tree finches, hybrid cluster ($N = 17$) to intermediate size, and genetic population 2 ($N = 8$) to larger-bodied tree finches. For this analysis, data were analyzed for 2005 (low-rainfall year), and 2010 and 2012 (high-rainfall years).

collapse via hybridization rather than species-biased extinction (see also Taylor et al. 2006). Our simulations show that approximately one-third of our sampled individuals may be incorrectly assigned to hybrid origin using our data. This error is likely due to the low genetic differentiation and close phylogenetic relationship between our small- and large-morph parental populations, which decreases the efficiency to detect hybrids using Bayesian assignment methods (Vähä and Primmer 2006). Despite this, we were able to detect hybrids reliably in 70% of individuals simulated from our real data. Although the power of our data did not enable us to evaluate other hybrid detection methods, software such as NEWHYBRIDS and STRUCTURE are similar in their efficiency to correctly detect hybrids (Vähä and Primmer 2006).

One pattern that invites comment is that in 2005 the hybrid individuals were morphologically similar to birds in genetic population 1. We suggest that this pattern arose as hybrid individuals may have introgressed back into genetic population 1 following the peak hybridization behavior that may have occurred during the 1998 high-rainfall year (Grant et al. 2004). Introgression coupled with strong natural selection against hybrids during low-rainfall years could explain the rapid change in morphology and the disappearance of birds with intermediate morphology between 1998 and 2005. Evidence supporting introgression of hybrid individuals into genetic population 1 during low-rainfall years is that we find ~15% higher genetic diversity in genetic population 1 compared with genetic population 2 in 2005 (see also Anderson 1968; Cox 2004). Additionally, the existence of private alleles in the hybrid cluster in 2005 and 2010 (table A4) possibly indicates introgression from unsampled parental populations, perhaps from other islands, or previously extant large tree finches on Floreana Island (Lack 1947; summary data in table A5).

Female Choice Driven by the Rare Species

Hubbs (1955) originally suggested that hybridization would be higher in areas where one of two species was rare (Randler 2002). Wirtz (1999) suggested that this pattern arises because females (the choosy sex) of the limiting species prefer heterospecific males, perhaps because of preference for "super stimuli" or larger male size. Pierotti and Annett (1993) predicted that females should prefer bigger males, as these males are more likely to confer better resource holding potential. More evidence for the onus of successful hybridization to be dependent on female choice comes from genetic studies of interspecific sterility, which is often asymmetrical (Mallet 2008). There is often differential hybrid fitness in crosses between interspecific males and females, depending on whether the father or mother is from species 1 or species 2 (Darwin 1859; Turelli and Moyle 2007; Lowry et al. 2008). For these reasons, we tested whether females of the rare species are more often paired with large heterospecific males (Randler 2002). Our results showed disassortative pairing for body size between females of the rare large morph and smaller heterospecific common males (Alipaz et al. 2005; Svensson et al. 2007; Jiang et al. 2013). If large-bodied finches have lower fitness due to increased parasitism (Dudaniec et al. 2007), then small-bodied males should prefer to pair with small-bodied females, and avoid larger females. The opportunity for mutual mate choice under conditions of novel and lethal parasitism remain to be tested. What we show here is a shift in host phenotype that correlates with a change in female pairing pattern.

Outcompeting Parasites: A Possible Function of Hybridization

The Red Queen hypothesis predicts that genetic variance keeps organisms just one step ahead of an evolutionary dead-end, in a never-ending cycle of competition (Bell 1982). In this light, introgression via hybridization can offer novel genetic solutions in a host-parasite cycle (Loker 2012). Traditionally, hybridization was thought to accrue hybrid offspring with reduced fitness (Templeton 1986), and there is much evidence to support this contention in a range of organisms (Holtsford 1996; Hatfield and Schluter 1999; Fenster and Galloway 2000; Goldberg et al. 2005).

Grant et al. (2003) showed variation in fitness patterns in two interbreeding *Geospiza* ground finch species under different social and ecological parameters. In this study, we show that high-rainfall periods correlate with high *P. downsi* intensity; and we therefore argue that the novel genetic combinations through hybridization may confer an immunological advantage (Huber et al. 2010) or parasite resistance (Loker 2012).

Conclusion

Hybridization patterns at different temporal scales are informative for testing ideas about the effects of changing environments on natural selection and evolution. Here, we show evidence for species collapse via hybridization over a short contemporary time period. There is growing evidence that hybridization can facilitate adaptive radiations that span millions of years (Mallet 2007; Sternkopf et al. 2010; Masello et al. 2011; Campagna et al. 2012). Here we show hybridization over the last decade that offers a snapshot opportunity to test ideas about the mechanism and function of hybridization under known population and ecological conditions. In support of predictions by Grant and Grant (1996*b*) and Randler (2006), the rare and endangered species drove the observed hybridization: large rare females paired with small common males. The outcome of disassortative pairing across species was the formation of a hybrid cluster. Our findings are particularly intriguing because asymmetric reproductive isolation is far less common than reproductive isolation arising from positive assortative pairing (Jiang et al. 2013). The role of mate choice in sympatry has often been invoked for reproductive isolation (Arnold et al. 1996) but has rarely been shown to change in contemporary populations. Finally, there are compelling reasons to acknowledge that the large tree finch (*Camarhynchus psittacula*) is locally extinct on Floreana Island: (1) we found only two genetic populations on Floreana Island; and (2) contemporary morphological data for the large-bodied finches correspond with historical data for the medium tree finch but not for those of a historical large tree finch.

Acknowledgments

This research was supported by the Australian Research Council, the Max Planck Institute for Ornithology, the Mohamed bin Zayed Species Conservation Fund, Rufford Small Grants for Nature Conservation, the Winifred Violet Scott Trust, the American Bird Conservancy, the Conservation International, the Australian Federation for University Women, and the Royal Society for the Protection of Birds/Birdfair. TAME airlines provided reduced airfares. We thank the Galápagos National Park Service and the Charles Darwin Research Station for logistical support and for the opportunity to work in the Galápagos. We thank S. Cisneros, C. Cruz, W. Cruz, E. Wittmer, and the community of Floreana Island for logistical support; and C. Charlton, T. Clark, and J. Forwood for field work to capture and observe the finches and collect *Philornis* samples from nests. We thank D. Arango, C. Evans, S. Gantefoer, K. Peters, M. Schmidt, and R. Schubert for dedicated field work and data collection on *Philornis* intensity in relation to genetic population. Special thanks to A. Fitch for assistance with laboratory techniques, methodology, and interpretation of the genetic data. We thank L. Beheregaray and M. Gardner for additional advice on molecular methods and P. R. Grant for insightful comments on an earlier version of this manuscript. This publication is contribution 2,080 of the Charles Darwin Foundation for the Galápagos Islands.

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Associate Editor: Erik Svensson
Editor: Troy Day

Small tree finches (*Camarhynchus parvulus*) from Floreana Island. Left, a yearling (photo credit: Frank Sulloway); right, a small tree finch about 4 years old (photo credit: Jeremy Robertson).