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Introduced parasite changes host phenotype, mating signal and hybridization risk: *Philornis downsi* effects on Darwin's finch song

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Introduced parasites that alter their host's mating signal can change the evolutionary trajectory of a species through sexual selection. Darwin's *Camarhynchus* finches are threatened by the introduced fly *Philornis downsi* that is thought to have accidentally arrived on the Galapagos Islands during the 1960s. The *P. downsi* larvae feed on the blood and tissue of developing finches, causing on average approximately 55% in-nest mortality and enlarged naris size in survivors. Here we test if enlarged naris size is associated with song characteristics and vocal deviation in the small tree finch (*Camarhynchus parvulus*), the critically endangered medium tree finch (*C. pauper*) and the recently observed hybrid tree finch group (*Camarhynchus* hybrids). Male *C. parvulus* and *C. pauper* with enlarged naris size produced song with lower maximum frequency and greater vocal deviation, but there was no significant association in hybrids. Less vocal deviation predicted faster pairing success in both parental species. Finally, *C. pauper* males with normal naris size produced species-specific song, but male *C. pauper* with enlarged naris size had song that was indistinguishable from other tree finches. When parasites disrupt host mating signal, they may also facilitate hybridization. Here we show how parasite-induced naris enlargement affects vocal quality, resulting in blurred species mating signals.

1. Introduction

The parasite–sexual selection hypothesis predicts that parasite burden constrains the expression of extravagant secondary sexual characters [1], and choosy females use the presence of secondary characters as honest signals of male quality [2]. Parasitized males generally have less elaborate secondary sexual characters than non-parasitized males [2–9]. For example, parasitized male cichlid fish (*Pundamilia pundamilia*, *P. nyererei*) and greenfinches (*Carduelis chloris*) have duller coloration [10,11], parasitized male wall lizards (*Podarcis muralis*) have altered visual and chemical signals [12], and parasitized mice (*Mus musculus*) have altered chemical signals [13]. When an introduced parasite changes host mating signal [3,14], divergent sexual selection could drive speciation [15]. Parasites may change the target or the direction of sexual selection, which is predicted to increase the speed of divergence and could initiate divergent coevolution of preferences and traits [16–18]. However, current evidence for such effects is scant. Case studies in the wild that identify the effects of parasites on male mating signal and pairing success provide insights into the ecological context of parasite-mediated sexual selection [19–22] and increase our understanding of how parasites could influence divergence or species collapse via sexual selection.

Birdsong is widely regarded as a sexually selected trait in songbirds [23] that provides biological information about individual quality via intraspecific signalling [24–26]. Choosy females prefer males with high-quality song—defined, for example, as having a longer duration or greater element complexity [27–32]. Song can be maintained as an honest signal because developmental stress during the phase of song learning lowers song quality [33–37]. By definition, parasites derive resources from a host. Songbird hosts that have been developmentally stressed by parasites should produce lower quality song as adults. Several studies support this idea: barn swallows (*Hirundo rustica*) with more ectoparasites had lower song output [38], sedge warblers (*Acrocephalus schoenobaenus*) with more haematozoan parasites had lower song complexity [39], and canaries (*Serinus canaria*) experimentally infected with malaria (*Plasmodium relictum*) had smaller brain volume for song nuclei (i.e. higher vocal centre) and produced simpler song as adults [34]. In general, parasitized nestlings are expected to produce lower quality song as adults. Parasite infection may affect song learning, or it may affect physical traits important for song production.

Darwin's finches (Passeriformes: Thraupidae) of the Galapagos Islands are currently threatened by the accidentally introduced fly *Philornis downsi* (Diptera: Muscidae) whose larvae parasitize developing nestlings [40]. The fly occurs on 13 of 15 islands surveyed to date [40–42] and is considered the greatest risk to the survival of Galapagos land birds [40,43,44]. Adult female *P. downsi* oviposit into the host nest [45]; the fly eggs hatch and 1st instar larvae move to reside inside the nares of nestlings where larvae consume the keratin, tissue and blood [46] of the developing birds [47]. The 2nd and 3rd instar larvae move from inside the naris to feed externally on the developing nestlings, as shown by in-nest video [45,48]. Nearly every highland Darwin's finch nest examined in 1997–2012 had *P. downsi* larvae that caused nestling mortality (approx. 55% of nestlings died) [44,49–51] or naris deformation (2 × larger naris size in nestlings with versus without *P. downsi*) in the few surviving fledglings [52]. Nestling mortality was caused by blood loss [53], and naris deformation was the result of tissue consumption by *P. downsi* larvae [52]. The number of *P. downsi* larvae per nest differs significantly across host species [54,55], and is highest in critically endangered medium tree finch (*Camarhynchus pauper*) on Floreana Island [56] compared with the sympatrically occurring small tree finch (*C. parvulus*) and small ground finch (*Geospiza fuliginosa*) [57]. While most active Darwin's finch nests had *P. downsi* larvae, there were differences in the number of *P. downsi* per nest and individual [52]. There were also differences in the extent of naris deformation caused by *P. downsi*, which ranged from slight to extreme enlargement of naris size [52,58].

This study examines the effect of naris size on male song and pairing outcome in Darwin's finches. The *Camarhynchus* tree finches on Floreana Island are currently hybridizing [59,60]. Female *C. pauper* often pair with male *C. parvulus*, producing hybrid offspring that subsequently pair with *C. parvulus* and other hybrids [59,60]. The song of male *C. parvulus* and the hybrid birds is indistinguishable, whereas the song of male *C. pauper* has slower trill rate [61]. In this study, we (i) report on male naris size per species, (ii) examine the effect of naris size on song characteristics (maximum frequency, minimum frequency, duration, trill rate and frequency bandwidth) within species and (iii) between *C. parvulus*, *C. pauper* and hybrid males, and (iv) examine the effect of naris size and vocal deviation on pairing outcome. We predict that males with enlarged naris size will produce song with higher vocal deviation and have lower pairing success, with the same pattern in *C. parvulus*, *C. pauper* and hybrid birds. At the species level, we ask if males with enlarged naris size maintain species-typical song.

2. Material and methods

(a) Study site and species

This study was conducted on Floreana Island (1°17′60.0″ S, 90°27′09.5″ W), Galapagos Archipelago during February and in some cases March/April across 2004 to 2014. The focal tree finch species are small tree finch (*Camarhynchus parvulus*), medium tree finch (*C. pauper*), and the recently discovered hybrid group that arises from pairings between *C. pauper* females and *C. parvulus* males [59,60]. This study uses song recordings from 77 male Darwin's tree finches that have been colour-banded, morphologically measured and genetically assigned using microsatellite markers (9 *C. parvulus*, 19 *C. pauper* and 49 hybrid birds) [61]. The sample size is larger for hybrid birds, which is the unexpected outcome of *post hoc* genetic assignment after fieldwork. We used nine microsatellite loci to assign adult males to a genetic group [59,60]. Assignment to each genetic group was based on the individual membership coefficient (qi) derived from Bayesian clustering analysis using STRUCTURE, which rates the probability (0–1) of an individual belonging to the *C. parvulus* cluster ($q\text{i} \geq 0.80$ for *C. parvulus*, $q\text{i} \leq 0.20$ for *C. pauper*, and $0.80 > q\text{i} > 0.20$ for the hybrid group).

(b) Morphology

We measured naris size and morphology in 236 adult *Camarhynchus* tree finch males, including the 77 males with song recordings. Birds were mist-netted, banded and measured. Beak and naris size were measured using callipers to the nearest 0.1 mm: (i) beak length from naris (culmen length from tip of upper mandible to anterior edge of naris); (ii) beak length from base of feathers (tip of upper mandible to first feathers); (iii) beak length from head (tip of upper mandible to back of skull); (iv) beak width at base; (v) beak depth at base; and (vi) right and left naris size (mm), measured at the widest point. Morphological traits measured in the same bird across different years did not differ significantly [62]. The method for measuring naris size is described in detail in [52]. We used callipers placed dorsoventrally across each naris; we calculated naris size as the maximum naris size (either left or right), average naris size, and relative naris size (naris size / beak length). Here, we analysed maximum naris size per bird ($n = 77$) as a continuous variable and as a categorical variable. To compare the proportion of birds with average and extremely enlarged naris size, we defined average naris size as within 2 s.d. of the mean and extreme naris size as greater than 2 s.d. of the mean.

(c) *Philornis downsi* and naris size

We used a standardized protocol to extract and count the number of *P. downsi* larvae and pupae per nest [62]; at the end of a nesting event, the nest was collected and sealed, and the number of *P. downsi* parasites contained in the nest was determined within 6 h of nest collection. The sample size for nests with known *P. downsi* intensity per nest at the time of male song recording was 6 *C. pauper*, 9 *C. parvulus* and 22 hybrid birds. Naris deformation caused by *P. downsi* occurs during the nestling phase and we do not have information on the nestling

phase of the 77 adult males analysed here. To calculate the effect of *P. downsi* intensity on naris size, we used data from 37 nestlings previously measured at day 6 in the nest ($r = 0.67$, $n = 37$, $p < 0.0001$).

(d) Song

Song recordings were made using 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 2008 to 2014. Darwin's finch song is structurally simple and consists of several repetitions of the same syllable [61,63]. Given the tameness of Darwin's finches, song recordings were made at close range (often less than 10 m). Digital recordings were transferred to a computer and single vocal clips extracted using Amadeus Pro v. 1.3.2 (HairerSoft, Switzerland). We selected recordings to create spectrograms using Raven Pro v. 1.5 for Mac OS X (http://www.birds.cornell.edu/raven).

We created spectrograms following Podos [64] and Goodale & Podos [65]. We used the Hann algorithm, a −24 dB cut-off criterion relative to the peak power of the vocalization and visual adjustment to measure the following song parameters: (i) minimum frequency (Hz); (ii) maximum frequency (Hz); (iii) frequency bandwidth (Hz) (maximum frequency − minimum frequency); (iv) peak power; (v) song duration (s); (vi) number of syllables and (vii) trill rate (number of syllables/song duration [s]). We used 1–5 song recordings per male to analyse song characteristics; we used the average per male when we had two or more recordings. The number of song recordings per male per genetic group is as follows: 5 recordings from 49 males (6 *C. parvulus*, 11 *C. pauper*, 30 hybrid), 4 recordings from 12 males (2 *C. parvulus*, 4 *C. pauper*, 6 hybrid), 3 recordings from 9 males (1 *C. parvulus*, 2 *C. pauper*, 6 hybrid), 2 recordings from 6 males (2 *C. pauper*, 4 hybrid), and 1 recording from 3 males (all hybrid).

(e) Vocal performance

We measured vocal performance by calculating the vocal deviation according to methods outlined by Podos [66]. Specifically, we calculated the orthogonal deviation for each song from the upper bound regression line ($y = -1.24$ trill rate $(x) + 7.55$); the distance from the regression line is referred to as the 'vocal deviation' [64,67–69]. A higher vocal deviation indicates lower vocal performance.

(f) Number of days singing to pairing

Male tree finches build a display nest and sing at the display nest until being chosen by a female for nesting [70]. We measured pairing outcome across genetic groups and in relation to vocal deviation score. Our annual fieldwork begins in February, which coincides with the onset of the rainy season and breeding activity. In this study, we only included observations of males that began nest building after the study began. We cannot guarantee that we discovered males on the first day of singing; but we only included males that were observed to sing at the onset of building a new display nest and nest building is a conspicuous activity. Each male display nest was visited for 20 min continuous observation between 07.00 and 10.00. We monitored each nest every day until pairing outcome was known and analysed the number of days the male sang before being chosen by a female. A nest was considered 'not chosen' (unpaired) if a male had not attracted a female within 14 days of first observation. A male was assessed as 'chosen' (paired) when we observed at least one of the following: (i) reciprocated courtship behaviour (i.e. male feeding a female, mutual preening); (ii) female lining the nest; (iii) female seen inside the nest; (iv) egg laying; (v) incubation. The sample size for pairing outcome in relation to male vocal deviation in genetically assigned birds is 52 (7 *C. parvulus*, 15 *C. pauper*, 30 hybrid).

(g) Statistical analysis

Data were analysed using SPSS Statistics v. 23.0 (IBM, Chicago). Data were inspected for assumptions of normality and homogeneity of variance. Male pairing outcome (number of days a male sings to attract a female) was ln-transformed to satisfy requirements of normality; maximum naris size was normally distributed per genetic group but was ln-transformed when graphically compared with male pairing outcome. As shown previously [52], modern Darwin's finches are 1.9 times more likely to manifest extreme naris size than are historical specimens. As we previously reported, most of this difference is attributable to birds having enlarged nares, with modern specimens being a striking 19.8 times more likely to fall into this category [52]. In this study, we compared maximum naris size per bird in relation to song characteristics and pairing outcome, and also analyse naris size as a categorical variable (±2 s.d. of mean = average, greater than 2 s.d. from mean = extremely enlarged). We examined vocal deviation as a continuous variable and as a categorical variable (low deviation, high deviation). Birds in the lowest 50% of vocal deviation scores were scored as 'low vocal deviation' (good vocal performance); birds in the top 33% of vocal deviation were scored as 'high vocal deviation' (poor vocal performance).

Given small sample size, and to reduce over-parametrization of models when comparing morphology in genetically assigned birds, we calculated derived beak size and song variables using principal components analysis. For beak size, we extracted one component with an eigenvalue of 2.62 that explained 87% of the variance. The derived factor score 'PC Beak' had positive factor loadings for beak length (0.92), beak depth (0.95) and beak width (0.93). The derived song variable 'PC Song' explained 63.1% of the variance and had an eigenvalue of 2.53. This variable had high factor loadings for maximum frequency (0.90), minimum frequency (−0.44), trill rate (−0.77), and frequency bandwidth (0.97).

We used path analysis to estimate the effects of *P. downsi* intensity on naris size, naris size on vocal deviation, and vocal deviation on pairing success. We also computed a path model for the effects of hybridization on pairing success. To calculate the path coefficient between *P. downsi* intensity and naris size, we used unpublished data for birds previously measured at day 6 in the nest ($r = 0.67$, $n = 37$, $p < 0.0001$). A path model for standardized selection coefficients was computed using PROC CALIS (SAS 9.4, Cary, NC). Owing to some missing data for pairing outcome (32%), we used the full information maximum-likelihood method for estimating path coefficients. This method of analysis yields results that are nearly identical to those obtained by performing multiple imputation of the missing data with SAS's PROC MI.

3. Results

(a) Male naris size

Male naris size (mean ± s.e.) was highest in *C. parvulus* (2.5 ± 0.2 mm) and *C. pauper* (2.4 ± 0.1 mm) and lowest in hybrid birds (2.1 ± 0.1) (ANOVA: $F_{2,76} = 6.32$, $p = 0.003$). Tukey *post hoc* tests showed a significant difference in naris size between *C. parvulus* and hybrid birds ($p = 0.033$) and *C. pauper* and hybrid birds ($p = 0.012$) but not between *C. parvulus* and *C. pauper* ($p = 0.940$). The percentage of

males with extremely enlarged naris size differed, and was highest in *C. pauper* (7/19; 37%) and *C. parvulus* (3/9; 33%) compared with hybrids (2/49; 4.1%) ($\chi^2 = 13.61$, d.f. = 2, $p = 0.001$). The mean ± s.e. number of *P. downsi* per nest was highest in the two parental species *C. pauper* (37.6 ± 4.3, $n = 9$) and *C. parvulus* (24.5 ± 3.6, $n = 6$) and lowest in hybrid birds (20.0 ± 2.6; $n = 22$; ANOVA: $F_{2,36} = 7.06$, $p = 0.003$). Tukey *post hoc* tests showed a significant difference in number of *P. downsi* between *C. pauper* and hybrid birds ($p = 0.002$), but no statistically significant difference between *C. parvulus* and *C. pauper* ($p = 0.10$) or *C. parvulus* and hybrid birds ($p = 0.69$).

(b) Naris size and song

Naris size was negatively correlated with 'maximum frequency' of song in *C. parvulus* and *C. pauper* but not in hybrid birds. Male *C. parvulus* and *C. pauper* with larger naris size had song with lower maximum frequency (*C. parvulus:* maximum frequency $r = -0.73$, $t = -2.82$, $n = 9$, $p = 0.026$; *C. pauper* maximum frequency: $r = -0.53$, $t = -2.45$, $n = 19$, $p = 0.027$), while minimum frequency and song duration were not affected (minimum frequency: $r = 0.11$, $t = 0.41$, $n = 19$, $p = 0.69$; duration: $r = 0.17$, $t = 0.68$, $n = 19$, $p = 0.50$). We found no such correlation in hybrid birds (maximum frequency: $r = 0.06$, $t = 0.39$, $n = 49$, $p = 0.70$; minimum frequency: $r = -0.22$, $t = -1.51$, $n = 49$, $p = 0.14$; duration: $r = -0.02$, $t = -0.14$, $n = 49$, $p = 0.89$).

To test if naris malformation affects mating signal at the species level, we compare song (PC Song) between the two parental species and hybrid birds in males with normal or enlarged naris size (electronic supplementary material, table S1). When males had normal naris size, song differed between *Camarhynchus* tree finches (ANOVA: PC Song $F_{2,42} = 3.71$, $p = 0.033$). Tukey *post hoc* tests showed significant differences between the song of *C. pauper* and hybrid males ($p = 0.026$). As previously found [55], *C. parvulus* and hybrid song did not differ statistically ($p = 0.97$). When males had enlarged naris size, song did not differ between *Camarhynchus* tree finches (ANOVA: PC Song $F_{2,33} = 2.54$, $p = 0.10$). None of the Tukey *post hoc* comparisons was statistically significant (*C. pauper* versus *C. parvulus*: $p = 0.17$; *C. pauper* versus hybrid birds: $p = 0.11$).

(c) Naris size and vocal deviation

Overall, there was a negative linear relationship between trill rate and frequency bandwidth, which supports the hypothesis of a performance trade-off between these two variables during song ($r = -0.59$, $t = -6.33$, $n = 77$, $p < 0.001$; electronic supplementary material, figure S1). We calculated vocal deviation scores from the upper bound orthogonal regression line. Male *C. parvulus* and *C. pauper* with larger naris size produced song with greater vocal deviation (*C. parvulus:* naris size: $r = 0.67$, $t = 2.41$, $n = 9$, $p = 0.047$; *C. pauper*: naris size: $r = 0.47$, $t = 2.22$, $n = 19$, $p = 0.041$; figure 1*a*) while hybrid birds did not (naris size: $r = -0.14$, $t = -0.97$, $n = 49$, $p = 0.34$; figure 1*b*).

(d) Vocal deviation and pairing outcome

Pairing outcome differed across the three genetic groups (likelihood ratio 15.60, $n = 52$, $p < 0.001$). More males remained unpaired in either parental species (*C. parvulus* 4/7 not chosen, 47%; *C. pauper*: 8/15 not chosen, 53%) compared with hybrid males (2/30 not chosen, 7%). There was no significant association between naris size and number of days a male sang before being chosen by a female ($r = 0.16$, $t = 0.87$, $n = 30$, $p = 0.39$). But vocal deviation tended to be associated with the number of days a male sang before being chosen by a female ($r = 0.33$, $t = 1.82$, $n = 30$, $p = 0.08$), which we explore in more detail per genetic group.

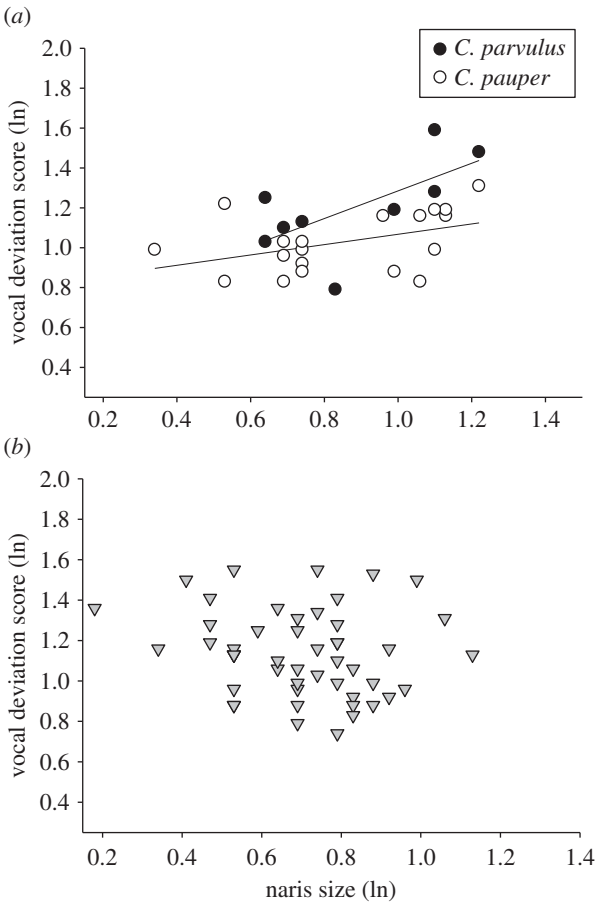

Figure 1. The association between naris size (ln-transformed) and vocal deviation (ln-transformed). Naris size is measured using callipers placed dorsoventrally across each naris. Vocal deviation is calculated as the distance from the upper bound regression between trill rate and frequency bandwidth. The data are shown for (*a*) the two *Camarhynchus* parental species (*C. parvulus*, *C. pauper*) and (*b*) hybrid birds that are the result of pairings between female *C. pauper* and male *C. parvulus*.

Male *C. parvulus* with low vocal deviation sang for 4.5 ± 0.5 days ($n = 2$) and males with high vocal deviation sang for 6.5 ± 0.9 days ($n = 4$) before being chosen by a female. We found a positive correlation between 'vocal deviation score' and 'number of days a male sang before being chosen by a female for nesting' (Pearson correlation analysis: $r = 0.91$, $t = 4.32$, $n = 6$, $p = 0.013$). *Camarhynchus parvulus* males that produced song with less vocal deviation were paired more quickly (figure 2*a*).

Male *C. pauper* with low vocal deviation sang for 5.9 ± 1.3 days ($n = 7$) and males with high vocal deviation sang for 12.7 ± 0.3 days ($n = 3$) before being chosen by a female for nesting. We found a positive correlation between 'vocal deviation score' and 'number of days a male sang before being chosen by a female for nesting' (Pearson correlation analysis: $r = 0.73$, $t = 3.05$, $n = 10$, $p = 0.016$). *Camarhynchus pauper* males that produced song with less vocal deviation were paired more quickly (figure 2*a*).

Male hybrid birds sang for 8.3 ± 1.3 days ($n = 14$) before being chosen by a female, singing 8.4 ± 2.3 days with low vocal deviation ($n = 7$) and 8.0 ± 1.3 days with high vocal deviation ($n = 7$). There was no significant correlation between 'vocal deviation score' and 'number of days a male sang before being chosen by a female for nesting' in hybrid males ($r = 0.20$, $t = 0.72$, $n = 14$, $p = 0.49$; figure 2*b*).

(e) Path analyses

Using SAS's PROC CALIS and the method of full information maximum likelihood to deal with birds for which pairing outcome was not known, we obtained a standardized path coefficient of -0.10 for the indirect effects of *P. downsi* on pairing success among non-hybrids, via its influence on naris size and vocal deviation (95% CIs = −0.25, 0.04, $t = -1.51$, $n = 28$, $p = 0.13$) and a total effect (direct and indirect) that was considerably larger (−0.36, 95% CIs = −0.76, 0.04, $t = -1.77$, $p = 0.08$). For the full sample, the effect of *P. downsi* on pairing success was −0.32 (95% CIs = −0.62, −0.02, $t = 2.11$, $n = 77$, $p = 0.04$), which includes the significant indirect effects (−0.24) of naris size and vocal deviation (95% CIs = −0.45, −0.03, $t = -2.20$, $n = 77$, $p = 0.03$), as well as a non-significant trend (−0.09) for the direct effect (95% CIs = −0.48, 0.31, $t = -0.043$, $n = 77$, $p = 0.67$). We also computed a structural equation model to determine the effects of hybridization on pairing success, including its indirect effects via naris size and vocal deviation. Hybridization's total effect on pairing success was 0.52 (95% CIs = 0.71, 0.33, $t = 5.39$, $n = 77$, $p < 0.001$), with the indirect effect caused by naris size and vocal deviation being 0.07 (95% CIs = 0.18, −0.04, $t = 1.19$, $n = 77$, $p = 0.23$).

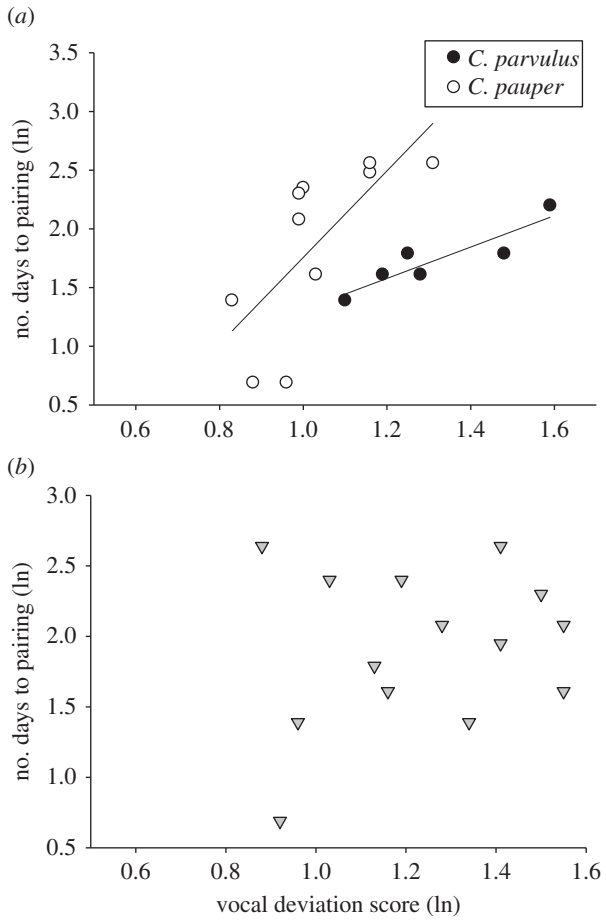

Figure 2. The association between vocal deviation (ln-transformed) and pairing outcome (ln-transformed). Vocal deviation is calculated as the distance from the upper bound regression between trill rate and frequency bandwidth. Pairing outcome is calculated as the number of days a male sings to attract a female. The data are shown for (*a*) two *Camarhynchus* parental species (*C. parvulus*, *C. pauper*) and (*b*) hybrid birds.

4. Discussion

The introduced *P. downsi* fly has been wreaking havoc on the survival of Galapagos land birds since its larvae were first discovered in a Darwin's finch nest in 1997 [54,71]. Identified as the biggest threat to the survival of all Galapagos land birds [43], the larvae of *P. downsi* kill more than half the nestling finches [44,57] and leave the remaining surviving birds with various degrees of naris malformation [45,58]. In-nest video recordings reveal *P. downsi* larvae moving in and out of finch nares and bodies [44,45]. An individual that survives consumption by *P. downsi* can be left with such severe naris malformation that the upper nasal cavity is missing and the beak is laterally open from one side to the other [52]. In less extreme cases, the naris is enlarged and/or plugged up, often with a residual larva that failed to emerge [44]. *Philornis downsi* has changed the beak of the finch.

This study shows that parasite-induced naris deformation changes the song of Darwin's finches. In two parental species, *C. parvulus* and *C. pauper*, males with enlarged naris size produced song with lower maximum frequency and greater vocal deviation. As in previous studies, we found more *P. downsi* per nest in *C. pauper* and *C. parvulus* than hybrid nests [57,59], as well as fitness costs from high vocal deviation [69]. Male *C. parvulus* and *C. pauper* with high vocal deviation sang for 36–73% more days before attracting a female for nesting. In fact, pairing success was rather low, as 47% of *C. parvulus* and 53% of *C. pauper* sang at a display nest [70] but failed to attract a female. In contrast, naris size did not predict the song of hybrid birds, whose nests had fewer *P. downsi* [72], and hybrid males had the smallest average naris size and most pairing success. Only 7% of hybrid males sang without attracting a female within two weeks.

We used path analysis that included the effects of parasite intensity on naris size (sampled from nestlings), effects of adult naris size on vocal deviation and effects of vocal deviation on pairing success. From the resulting structural equation model we obtained a path coefficient of −0.32 for the total effects of *P. downsi* on pairing success, and −0.24 for the indirect effects via naris size and vocal deviation. These results are reasonably close to the median value (0.18) obtained from meta-analysis of prior sexual selection studies [86]. We can conclude that males with parasite-induced naris deformation had lower song quality and lower pairing success, and that *P. downsi* plays a role in male pairing success via its documented effects on naris size.

Males with enlarged naris size failed to produce a species-typical song. Specifically, *C. pauper* with enlarged naris size produced song that was indistinguishable from that of *C. parvulus* and hybrid males. As Peters and colleagues showed, the one-way pattern of introgression in the *Camarhynchus* hybridization is the result of female *C. pauper* pairing with male *C. parvulus*, with evidence for subsequent backcrossing with *C. parvulus* and other hybrids [60,72]. The

results of this study suggest that the observed 'reverse speciation' could occur because of the lack of a reliable pre-mating signal as the consequence of parasite-induced malformation of the birds' vocal production apparatus.

From the perspective of assortative pairing, theory predicts that low-quality female *C. pauper* should pair assortatively with low-quality male *C. pauper* [22,73,74]. The song of low-quality *C. pauper* was indistinguishable from the song of hybrid males (and *C. parvulus*) and therefore, compared with high-quality female *C. pauper*, low-quality female C. *pauper* may be more likely to erroneously pair with hybrid birds or *C. parvulus*. We would expect that high-quality female *C. pauper* pair with high-quality male *C. pauper*, which remains to be tested. Given that *C. pauper* females pair with *C. parvulus* or hybrid males, and not vice versa [59,60], *C. parvulus* and hybrid males risk hybridizing with low-quality *C. pauper* females. The health of the population may be diminished with an influx of low-quality cross-paired offspring.

In some cases, hybridization may be favoured when rapid genetic introgression facilitates novel evolutionary pathways [75,76]. As Lamichhaney and colleagues have recently observed, a hybrid pairing can be the starting point for a new species *in situ* [77,78]. In the recently documented hybrid speciation on Daphne Major, a resident female *G. fortis* paired with an immigrant male *G. conirostris* and, within three generations, produced a lineage of reproductively isolated birds [77]. The cause of hybridization can differ from the selective pressures that favour the survival of the hybrid offspring, and therefore it is useful to disentangle the mechanisms and fitness benefits of hybridization events [79]. In the current study, hybridization could be the result of blurred mating signals as the consequence of parasitism. From a fitness perspective, hybrid offspring may have fewer parasites than the parental species as the result of novel genetic combinations and introgression [80,81]. Perhaps genetic admixture in the host promotes tolerance of a parasite's microbiome [82] or confers a genetic benefit to sustain other parasite-mediated effects [83,84].

We are aware that the sample sizes for some of the populations examined in this study are relatively small, especially in the case of recorded vocalizations. However, in order for the findings in this paper to attain statistical significance, they must entail correspondingly larger effect sizes than would be required with much bigger sample sizes. For instance, the correlations in this study between vocal deviation scores and number of days before a male was chosen for pairing were 0.90 ($n = 6$), 0.81 ($n = 10$), and -0.09 ($n = 14$) for the three different populations. These findings yield a mean-weighted r of 0.49 (95% CIs = 0.16, 0.72), which is considerably larger than the median effect size (0.18) for studies of the strength of sexual selection in the wild [85,86]. Similarly, the mean-weighted correlation for the relationship between naris size and vocal deviation was 0.43 (95% CIs = 0.23, 0.60). Even allowing for the possibility that these correlations err somewhat on the high side, they still support the argument that *Philornis*'s ecological disruptions of normal mating relationships in Darwin's finches are biologically substantial. This conclusion should be of particular cause for concern for the fate of Darwin's finches.

5. Conclusion

Parasites can drive evolution through processes of natural and sexual selection [16,87,88]. Traditionally, the effects of parasites have been studied in relation to individual fitness [89–92]. When species-level outcomes have been considered, research has focused on host mortality and extinction (e.g. [49,93–96]), phylogenetic analysis (e.g. [97]), or the genetics of host resistance and immunity [98,99]. The findings of this study offer a new perspective for understanding evolutionary change from introduced parasites given that parasites can alter pre-mating signals and thus blur species boundaries.

Ethics. The work was approved by the Flinders University Animal Welfare Committee (E393).

Data accessibility. Data reported in this paper is available from the Dryad Digital Respository: https://doi.org/10.5061/dryad.46qf24q [100].

Authors' contributions. S.K. designed the research and wrote the paper; S.K. and K.J.P. collected data; all authors analysed the data; all authors provided critical analysis and feedback.

Competing interests. The authors declare no conflict of interest.

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