

1 **Title:** Inferring punctuated evolution in the learned songs of African sunbirds

2

3 **Authors:**

4 Jay P. McEntee<sup>1,2\*</sup>, Gleb Zhelezov<sup>3</sup>, Chacha Werema<sup>4</sup>, Nadjé Najar<sup>5</sup>, Joshua V. Peñalba<sup>6</sup>, Elia  
5 Mulungu<sup>7</sup>, Maneno Mbilinyi<sup>8</sup>, Sylvester Karimi<sup>9</sup>, Lyubov Chumakova<sup>3</sup>, J. Gordon Burleigh<sup>2</sup>, &  
6 Rauri C.K. Bowie<sup>1</sup>

7

8 **Author Affiliations:**

9 <sup>1</sup>Museum of Vertebrate Zoology & Department of Integrative Biology, University of California,  
10 Berkeley, Berkeley, CA 94720 USA

11 <sup>2</sup>Biology Department, University of Florida, Gainesville, FL 32611 USA

12 <sup>3</sup>School of Mathematics, University of Edinburgh, Edinburgh EH9 3FD UK

13 <sup>4</sup>Department of Zoology and Wildlife Conservation, P.O. Box 35064, University of Dar-es-  
14 salaam, Tanzania

15 <sup>5</sup>School of Natural Resources, University of Nebraska, Lincoln. Lincoln, NE 68503 USA

16 <sup>6</sup>Division of Evolutionary Biology, Ludwig-Maximilians-Universität Munchen, Faculty of Biology,  
17 Biocenter, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany

18 <sup>7</sup>P.O. Box 934, Iringa, Tanzania

19 <sup>8</sup>Tanzania Bird Atlas, Iringa, Tanzania

20 <sup>9</sup>National Museums Kenya, Nairobi, Kenya

21

22 \*Corresponding author current address: 901 S. National Ave., Department of Biology, Missouri  
23 State University, Springfield, MO 65897. Phone: 510-388-4065. Email:

24 [jaymcentee@missouristate.edu](mailto:jaymcentee@missouristate.edu). ORCID ID: <https://orcid.org/0000-0002-1213-9734>

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28 landscape

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34

35 **Abstract**

36

37 Signals used in animal communication, especially those that are learned, are thought to be  
38 prone to rapid and/or regular evolution. It has been hypothesized that the evolution of song  
39 learning in birds has resulted in elevated diversification rates, as learned song may be subject to  
40 especially rapid evolution, and song is involved in mate choice. However, we know little about  
41 the evolutionary modes of learned song divergence over timescales relevant to speciation. Here  
42 we provide evidence that aspects of the territorial songs of Eastern Afrotropical sky island  
43 sunbirds *Cinnyris* evolve in a punctuated fashion, with periods of stasis, on the order of  
44 hundreds of thousands of years or more, broken up by strong evolutionary pulses. Stasis in  
45 learned songs is inconsistent with learned traits being subject to constant or frequent change,  
46 as would be expected if selection does not constrain song phenotypes, or if novel phenotypes  
47 are frequently advantageous. Learned song may instead follow a process resembling peak  
48 shifts on adaptive landscapes. While much research has focused on the potential for rapid  
49 evolution in bird song, our results suggest that selection can tightly constrain the evolution of  
50 learned songs over fairly long timescales. More broadly, these results demonstrate that some  
51 aspects of highly variable, plastic traits can exhibit punctuated evolution, with stasis over fairly  
52 long time periods.

53

54 **Introduction:**

55

56 Signal evolution has long been thought to be important to the process of animal speciation, in  
57 part because many closely related species differ strongly in signals while differing little in other  
58 traits (1, 2). In particular, the evolution of signals involved in mate choice has been thought to be  
59 critical to the evolution of pre-mating reproductive isolation, such that correlated evolution of  
60 signals and mating preferences could lead in and of itself to speciation (3, 4). However, there  
61 remain many questions about how signal divergence proceeds over time, which mechanisms  
62 are responsible, and how it contributes to speciation and diversification processes.

63

64 Some signals that may be important to speciation are highly plastic, including those that are  
65 impacted by learning processes (5–7). While divergence in less plastic traits generally requires  
66 genetic divergence, the same is not true for learned signals (even if they have components with  
67 genetic predispositions (8, 9)). Indeed, novel learned signals can arise without genetic mutation,  
68 and spread quickly throughout populations (10). Thus, learned signals are potentially subject to

69 different evolutionary pressures (1, 6), and may exhibit different evolutionary trajectories (11)  
70 than signals that are not learned. West-Eberhard (1) suggested that taxa with learned signals  
71 may be especially subject to regular and rapid evolutionary change because cultural novelties  
72 appear frequently, and because novelty itself may increase the success of new signals.

73  
74 Modeling has supported the hypothesis that learning can result in increased rates of trait  
75 evolution (12). However, selection may not be as effective in driving the evolution of learned  
76 signals as compared to more innate signals (13), as learned signals may generally have low  
77 heritability (14). In some cases, such a depressed response to selection should lead to slower  
78 trait evolution. More generally, modeling of plastic traits has shown that rates of evolution may  
79 be fastest at low (15), high (15, 16), or even intermediate (17) levels of plasticity, depending on  
80 specific conditions. Thus it remains unclear how quickly learned traits should be expected to  
81 evolve relative to traits with less plasticity, and whether learned traits should exhibit similar  
82 evolutionary modes (i.e. gradual versus punctuated evolution) to less plastic, non-learned  
83 phenotypic traits (11, 13).

84  
85 The songs of oscine songbirds present intriguing cases for the study of learned signal evolution.  
86 In the oscine songbirds, most species learn to perform aspects of songs by imitating  
87 conspecifics (18). The oscine learning process is directed by innate predispositions that result in  
88 selective learning - that is, species only learn or reproduce vocalizations with certain  
89 characteristics (19–21). Song in oscine songbirds typically has two functions - territorial  
90 competition and mate attraction (18). While some birds' vocal repertoires are extensive, a single  
91 song component of the vocal repertoire is often used for both of these functions. The mate  
92 attraction function of oscine bird song has been especially important to arguments about its  
93 relevance to speciation. However, it remains unclear how much of learned song evolution in this  
94 group is driven by its role in mate choice, which is central to most hypotheses regarding the role  
95 of bird song in speciation (22), compared to territorial competition (23) or other functions.

96  
97 If song evolution is critical to speciation processes in birds, the evolution of song learning from  
98 non-learning ancestors may impact diversification rates in birds. The evolution of song learning,  
99 relative to the absence of learning, may decrease the waiting time to speciation (22) and  
100 increase diversification rates (24). Alternatively, however, the evolution of song learning may  
101 slow the speciation process because learning can result in heterospecific copying between  
102 incipient species (25), facilitating hybridization (26). Additionally, song learning is associated

103 with increased within-species variation, which may translate to slower rates in the evolution of  
104 song discrimination in incipient species, as larger evolutionary changes to mean phenotypes  
105 result in less discrimination in learners than non-learners (27). Slower evolutionary rates of  
106 discrimination could result in slower evolutionary rates of prezygotic reproductive isolation, and  
107 thus slower speciation rates. Critically, however, we know very little about the trajectories of  
108 learned song evolution to inform how learned song evolution may differ from the evolution of  
109 less plastic traits.

110

111 Previous reviews (1, 11) have suggested that learned signals should exhibit little conservatism,  
112 with isolated populations typically diverging before any genetic differences have accrued.  
113 Wilkins et al. (11) suggest that non-learned signals, by contrast, may diverge approximately  
114 linearly, for example if signal divergence is a function of mutation-order processes. In this way,  
115 learned signal divergence might outpace the divergence of non-learned signals early in the  
116 divergence process. The strongest evidence for the trajectories of learned song relevant to  
117 speciation likely comes from studies of the greenish warbler *Phylloscopus trochiloides*, which  
118 exhibits nearly continuous variation across geographic space, suggestive of gradual evolution  
119 (28). Gradual evolution has elsewhere been posited to be important in song divergence in birds,  
120 with relevance for speciation (29).

121

122 Here we examine the evolutionary mode of learned songs in the eastern double-collared  
123 sunbird (EDCS) species complex (30), which inhabits mountains of the Eastern Afromontane.  
124 Sunbirds are oscine songbirds, and their territorial songs exhibit all signatures of songs  
125 developed through learning, including striking complexity and variation (18). The geographic  
126 ranges of these species are archipelago-like (Figure 1), with populations occupying discrete,  
127 island-like patches of suitable montane forest and forest edge habitats. There is a broad  
128 spectrum of molecular divergence, from minimal divergence in some neighboring populations to  
129 deep divergences among major lineages.

130

131 This species complex allows us to investigate the temporal trajectories of song divergence.  
132 Here we first analyze song using multivariate approaches. Then we fit univariate evolutionary  
133 models to song traits on phylogenies to examine the tempo and mode of learned song  
134 evolution. We were motivated especially by the question of whether learned songs evolve  
135 gradually, as is suspected in the Greenish warbler and hypothesized generally for learned bird  
136 songs, or via punctuated evolution. This question is rarely posed of signaling phenotypes (31).

137

138 **Results**

139

140 Clustering analyses of 141 multivariate song phenotypes from across the EDCS species  
141 complex found support for six distinct phenotypic clusters in multivariate space (the preferred  
142 model by BIC had 6 components). Visualization by bivariate plotting demonstrated that the set  
143 of phenotypic clusters is separated across different song dimensions - i.e. song divergence  
144 across the species complex is multifarious. Some cluster pairs are predominantly separated by  
145 frequency, others by fine temporal structure, and others by song duration (Figure A1).

146

147 Multi-locus phylogenetic analyses based on the mtDNA gene ND2 and five nuclear intron  
148 sequences revealed molecular lineages that correspond with song phenotype clusters (Figure 1,  
149 Figures A2 - A3). We recovered five major molecular lineages across the species complex that  
150 were similar to those found in previous phylogenetic analyses using only mtDNA sequences  
151 (30), and correspond with the taxonomy proposed in Bowie et al. 2004 (which recommended  
152 elevating *Cinnyris fuelleborni* and *C. usambarica* to species). Additionally, we recovered distinct  
153 clades within three species: *C. mediocris*, *C. fuelleborni*, and *C. moreaui*. In *C. mediocris*, our  
154 samples from the Mbulu highlands in northern Tanzania formed a clade, while those from  
155 Kenyan populations formed a clade sister to it. *C. fuelleborni* also comprised two clades, with  
156 individuals from the Njesi Plateau in northern Mozambique sister to all other *C. fuelleborni*. In  
157 *C. moreaui*, samples from the Nguru Mountains formed a clade nested within a phylogenetic  
158 grade representing samples from all other localities for this taxon (Figure A3). Phylogenetic  
159 analysis using BEAST recovered the same topology for species relationships for the five named  
160 species as our ML analysis, and estimated a divergence time of 3.4 My (HPD Interval: 2.67 -  
161 4.18 My) for the most recent common ancestor of the EDCS species complex (Figure A4). Our  
162 population trees, which we used to fit evolutionary models for song phenotypes (e.g. Figure 2),  
163 recovered the same topology among named taxa as the ML and Bayesian analyses.

164

165 Classification of individuals by song phenotype corresponded closely to the assignment of their  
166 populations to major lineages in the molecular phylogenetic tree (with the Mbulu population of  
167 *C. mediocris* included as a separate lineage). In unsupervised classification, 93.6% of 141  
168 individuals were assigned correctly to the corresponding molecular lineage (for populations  
169 where there were no molecular samples, molecular lineage was presumed based on geography  
170 and/or plumage).

171

172 *Inference of tempo and mode of learned song evolution*

173 Using novel model-fitting approaches and a novel model implementation for punctuated  
174 evolution on phylogenetic trees (see Supplementary Information), we compared support for  
175 Brownian motion versus punctuated evolution across different song traits. To compare support,  
176 we calculated AICc values for each model. However, we found that standard model comparison  
177 in this case was biased to favor punctuated evolution (Supplementary Information), such that we  
178 could not report uncorrected  $\Delta$ AICc weights to represent relative model support. Thus, we  
179 performed stochastic simulations under a range of parameters close to the maximum likelihood  
180 parameters (within 5%) estimated under both models, then calculated AICc differences for these  
181 simulations to calibrate relative support for each model, by each trait. Our approach may be  
182 understood as an approximate correction that takes into account phylogenetic correlations  
183 present in the data, as is necessary for BIC (32). We found variation across song traits in the  
184 relative support for Brownian motion and punctuated evolution. We found strong support for  
185 punctuated evolution in four of the fourteen song traits. There was moderate support for  
186 punctuated evolution in three more traits. For the remainder, neither model was strongly favored  
187 over the other. Sensitivity analyses examining the fit of trait evolution models on bootstrap trees  
188 showed that our results were broadly robust to phylogenetic uncertainty (Figure S4).

189

190 Our approach allowed us to compare support for the number and positions of evolutionary  
191 pulses on our phylogenetic tree. Pulses were allowed to occur on any branch. In our modeling  
192 approach, each pulse was considered a parameter, such that more parsimonious models had  
193 fewer pulses. We present the results for our pulse localization approach for all song traits where  
194 pulsed evolution had strong or moderate support (Figure 2, Figures A4-A9, Table S2) to make  
195 evident the degree to which pulses were co-localized across song traits. The pulse  
196 configurations with minimum AICc for the seven traits where punctuated evolution was  
197 supported had a mean of  $1.14 \pm 0.35$  SD pulse positions (Figure S8). Support for the punctuated  
198 evolution model across these traits, coupled with the small number of pulses supported for  
199 them, indicated that evolutionary change is minimal for these traits for long stretches of time,  
200 corresponding to millions of years, on the phylogenetic tree.

201

202 **Discussion**

203

204 *Learned song evolution as peak shifts on adaptive landscapes*

205 Here we have presented evidence that punctuated evolution explains the evolution of multiple  
206 aspects of territorial song better than gradual evolution (Brownian motion), across the EDCS  
207 species complex. Our results indicate that the evolutionary mode of multiple aspects of territorial  
208 songs includes large jumps in mean trait values, and extended periods of highly bounded  
209 evolution, or stasis, in the same aspects. While rapid evolution of animal signals, as in abrupt  
210 pulses here, has often been discussed in the literature on signal evolution, and has frequently  
211 been invoked as a route to pre-zygotic reproductive isolation in speciation, extended periods of  
212 stasis in signals has received comparatively little attention (31). Characterizing the evolutionary  
213 mode of territorial song as we have done here sheds light on the form of signal evolution, how it  
214 may be involved in speciation processes, and what may or may not cause abrupt evolutionary  
215 changes.

216  
217 The learning process in the development of song in oscine songbirds, like the focal sunbirds  
218 here, is a form of phenotypic plasticity (6). As such, our finding that song evolution proceeds as  
219 punctuated evolution can be viewed as an example in a learned trait as well as, more generally,  
220 in a highly plastic phenotype. As in previous studies using phylogenetic comparative methods  
221 (33, 34), we interpret punctuated evolution by visualizing peak shifting on an adaptive  
222 landscape. Authors have suggested that phenotypic plasticity itself could assist peak shifting by  
223 allowing phenotypes to initially approach alternate peaks on an adaptive landscape without  
224 having to wait for novel genetic variation, especially in the case of behavior (35). However, high  
225 levels of plasticity may enable so much variation that phenotypes may take on extremely  
226 different characteristics without underlying genetic change, such that adaptation to a novel peak  
227 does not occur (i.e. plasticity itself is the adaptation). In the case of song, it would seem unlikely  
228 that there is a fixed adaptive landscape (16), in which peaks maintain stable shapes, and  
229 occupy the same positions through time. Instead, because the efficacy of signals can change  
230 depending on environmental variation (e.g. habitat structure (36); population density (37)) or  
231 with the evolution of receiver responses (31)), adaptive peaks for learned song would appear  
232 likely to change shape, move, appear, and/or disappear, over time and across space.

233 Kirkpatrick (38) and Whitlock (16) showed that even small changes in the slopes and heights of  
234 adaptive peaks alone could trigger peak shifts, suggesting they may occur regularly, especially  
235 for plastic traits (35). Thus, there are two main theoretical obstacles for highly variable learned  
236 song to exhibit peak shifting dynamics over longer timescales. First, song may be so plastic that  
237 it can travel about an adaptive landscape without any underlying genetic change (35, 39), in  
238 which case it would likely be prone to extremely high lability. Secondly, the adaptive landscape

239 for song may change rapidly through time, and vary across environments, such that adaptive  
240 peaks are unlikely to remain in the same shape and position over evolutionary timescales (16,  
241 31). Thus, there was little reason to expect learned songs to be restricted to peaks, because of  
242 their high variability, and little reason to expect adaptive peaks to be stable in position and  
243 shape over time such that they could be observed.

244  
245 Our finding that punctuated evolution better characterizes the evolution of some song traits than  
246 gradual evolution indicates that evolution can be tightly bounded, approaching stasis, over long  
247 periods of time ( $> 10^6$  years). These results suggest that adaptive peaks for song are stable  
248 over time. The stability of adaptive peaks for learned songs suggests that the songs' receivers  
249 mediate stabilizing selection on song traits. There are two sets of receivers, males and females,  
250 that are likely to exert stabilizing selection forces in sunbirds. If narrow female preferences alone  
251 were responsible for stasis, we would expect strong behavioral reproductive isolation where two  
252 species with highly divergent song come into contact. However, *C. moreaui* and *C. fuelleborni*,  
253 which have extremely different songs across many song aspects, hybridize where they come  
254 into contact (40), indicating that female preferences are unlikely to be narrow. Thus, male  
255 receivers are likely to play an important role in the evolutionary stasis of learned song traits, by  
256 exerting quadratic selection itself, by exerting directional selection opposite selection from  
257 females, or perhaps by exerting selection opposite natural selection. An alternate hypothesis for  
258 stasis in some traits is that evolutionary constraints result from limited genetic variance (41–44).  
259 However, limited genetic variance should not strongly constrain evolution over longer  
260 timescales, as examined here, because novel genetic variation will arise over these timescales.

261  
262 If near-stasis occurs over long periods of time in some bird song traits, what explains  
263 evolutionary divergence when it occurs? One prominent hypothesis explaining the evolution of  
264 bird song is that song evolves as a byproduct of morphological evolution. Two aspects of  
265 morphology have been consistently highlighted with respect to such byproduct evolution: beak  
266 morphology and body size. Morphological evolution of the beak may be important as the beak is  
267 a part of the vocal apparatus itself (45). Body size evolution may be important because of  
268 allometric changes in pieces of the vocal apparatus, which could alter song frequency (46).  
269 When song evolves by punctuated evolution, the morphological byproduct hypothesis would  
270 predict that evolutionary pulses are consequences of morphological evolution (which in itself  
271 might be punctuated). In the EDCS, there is limited morphological evolution, with subtle  
272 changes in morphology across the complex, and substantial overlap in morphological



273 characteristics that differ on average between species (30). Thus, there is overall little reason to  
274 suspect that song evolution is tightly connected to morphological evolution. However,  
275 Loveridge's Sunbird *C. loveridgei* is unambiguously the largest member of the species complex,  
276 and has the longest bill. Thus this species represents a test case for the predictions of the  
277 morphological by-product hypothesis for acoustic signal evolution, in which we would predict  
278 that Loveridge's Sunbird should have the lowest frequency songs within the species complex.  
279 We find the opposite of this prediction. Loveridge's Sunbirds sing songs with the highest peak  
280 frequencies of all the members of the species complex, and our analyses evince a pulse of peak  
281 frequency evolution unique to Loveridge's Sunbird. Thus, within the EDCS complex, we see  
282 punctuated evolution that is not associated with substantial morphological evolution generally,  
283 and in the instance where morphological divergence is most pronounced, song evolution  
284 exhibits pulsed change opposite the direction predicted under the by-product hypothesis. Thus  
285 the overall picture from this species complex is that song evolution is not contingent on  
286 morphological evolution, extensive song diversity is generated without great morphological  
287 change, and the impacts of morphological evolution on song evolution are dwarfed by other  
288 processes.

289  
290 Range expansion provides another possibility as a cause for pulses in learned songs. Studies  
291 on North American juncos (47, 48) have suggested that pulses of phenotypic divergence (in that  
292 case, plumage) might take place in association with instances of rapid range expansion. During  
293 range expansion, serial founder effects can induce the fixation of rare genetic variants, providing  
294 a mechanism for rapid genetic change. Moreover, selective forces on signals may be distinct at  
295 the leading front of range expansions. For example, population densities at the leading edges of  
296 range expansions may be low, which could advantage signals that broadcast across further  
297 distances. In the future, genome-wide molecular studies could be used to reconstruct range  
298 expansions to examine correspondence in phenotypic change with range expansion in the  
299 EDCS. Studies of the transmission properties of the different song phenotypes across the  
300 species complex are also desirable, as they could inform hypotheses about song evolution  
301 based on population densities.

302  
303 As a learned trait, bird song may be prone to evolution via genetic assimilation of phenotypic  
304 novelties without an initial genetic basis (5, 6). Traits showing pulsed evolution - frequency, fine-  
305 scale temporal structure, duration - are traits that have been shown to have underlying genetic  
306 variation in other songbirds (8, 9, 39). Based on this evidence, we suspect that these traits have

307 underlying genetic predispositions in the focal species complex. This hypothesis is supported by  
308 the absence of a cultural bleed of phenotypes across the *C. moreaui* - *C. fuelleborni* contact  
309 zone where the two species interact (40). Genetic assimilation remains a plausible path for  
310 evolutionary divergence (i.e. “genes as followers” (5)), as underlying genetic differences are the  
311 end stage of genetic assimilation. However, peak shifting mechanisms involving substantial  
312 genetic change concomitant with phenotypic change are also plausible in explaining song  
313 evolution here, as we do not find evidence for substantial song divergence in the absence of  
314 genetic differentiation.

315

316 *Relevance of punctuated evolution of learned song for speciation*

317 The EDCS species complex bears hallmarks of speciation by sexual (2), or social (1) selection:  
318 species are strongly divergent for a signal used in social competition, and do not differ strongly  
319 in ecological respects (40). Panhuis et al. (2) suggested that an additional signature of  
320 speciation by sexual selection is the evolution of variation in sexually selected traits among  
321 populations within species, with this variation generating partial premating isolation. Our  
322 sampling of isolated sky island populations, especially within *C. moreaui* and *C. fuelleborni*,  
323 allows us to characterize within-species variation in territorial song. Across most song traits,  
324 variation across populations within species is minimal, including for many traits with strong  
325 differences across species, e.g. CV peak frequency (Figure A5) and median pause duration  
326 (Figure A7). As such, between-species divergence cannot be extrapolated from within-species  
327 variation (49). There are discontinuities in evolutionary processes in learned song that give rise  
328 to the diversity of songs across the species complex. These discontinuities in evolutionary  
329 process appear responsible for species differences.

330

## 331 **Conclusions**

332

333 The effects of learning on evolutionary processes are poorly known. Previous work has  
334 suggested that stabilizing selection on learned traits should be inadequate to prevent the  
335 divergence of genetic predispositions by drift, ultimately facilitating more rapid divergence in  
336 those genes underlying traits (22). Our study shows that multiple song traits can exhibit stasis  
337 for prolonged periods, likely lasting hundreds of thousands of years or more. These results  
338 suggest that learned song in the focal taxa is subject to a combination of sufficiently strong  
339 stabilizing selection and sufficient exposure of the underlying genetic variation to prevent  
340 incremental change for long periods of time. An alternative, that there is insufficient genetic

341 variation underlying these traits, is potentially plausible, but appears less likely given the  
342 evidence that genetic variation for learned song traits is present in captive populations, and the  
343 long span of evolutionary time during which such variation could be generated.

344

## 345 **Materials and methods**

346

### 347 *Song analysis*

348 We made sound recordings of EDCS from 2007-2011 in Kenya, Tanzania, and Mozambique,  
349 using solid-state digital recorders (Marantz PMD models 660, 661, and 670) and shotgun  
350 microphones (Sennheiser ME-67). A small number of recordings were made using a parabolic  
351 dish with an omnidirectional microphone (Sennheiser ME-62). We complemented our field  
352 recordings with additional recordings from the Macaulay Library (<http://macaulaylibrary.org>) and  
353 the British Library of Natural Sounds ([https://www.bl.uk/collection-guides/wildlife-and-](https://www.bl.uk/collection-guides/wildlife-and-environmental-sounds)  
354 [environmental-sounds](https://www.bl.uk/collection-guides/wildlife-and-environmental-sounds)). The vocal repertoires of the focal taxa are complex, including a wide  
355 array of different signal types. Here we measure the acoustic properties of male territorial songs  
356 delivered in bout form, in which consecutive songs are typically separated by a short duration  
357 (<15 s) of silence, or a series of short calls and pauses (50). Sunbirds sing these songs from a  
358 perch in the vegetation, ranging in height from 2 to 30m. These songs function in male-male  
359 territorial interactions (51). Further, as in other passerine birds (18), these songs likely serve to  
360 attract mates. Singing can coincide with, or immediately precede, female wing-fluttering displays  
361 directed at singing males, which has been observed in *C. loveridgei* and *C. fuelleborni* (JPM  
362 pers. obs.).

363

364 Before analyses, recordings were standardized for frequency sampling at 44.1 kHz, and  
365 bandpass filtered at 2 to 10 kHz. More strict filtering, at 2.5 to 9kHz, was then employed for  
366 recordings of *C. mediocris* and *C. usambarica* to allow fine-scale structural analysis of  
367 sonograms, as our recordings of their songs generally had lower signal:noise ratios, and the  
368 lowest frequencies in their songs are >2.5 kHz. Similarly strict filtering could not be applied to *C.*  
369 *fuelleborni* or *C. moreaui* songs because their songs sometimes include peak frequencies below  
370 2.5kHz. Spot filtering was used to remove acoustic signals not emitted by the focal bird. We  
371 selected high-quality field recordings for analyses after sonogram visualization in Raven Pro 1.3  
372 (52). JPM performed all sonogram analysis procedures in the program Luscinia (53).

373 Sonograms were produced in Luscinia with the following settings: maximum frequency: 10 kHz;  
374 frame length 5 ms; time step: 1 ms; spectrograph points: 221; spectrograph overlap: 80%; echo

375 removal: 100%; echo range: 100; windowing function: Hann; and high pass threshold: 2 kHz.  
376 Signals within sonograms were detected using Luscinia's automated signal detection. Results of  
377 automatic signal detection procedures were checked by eye and ear, with recordings slowed for  
378 playback to 1/8 speed. Automated signal detection errors were corrected using the *brush* tool.  
379 Measurements were made for each sonogram trace (hereafter 'elements'), separated by pauses  
380 from other elements.

381  
382 From the set of measurements of each element, we calculated summary statistics at the song  
383 level. For each individual sunbird, we then calculated the mean values of a set of summary  
384 statistics across songs. We calculated the following summary statistics for each song, based on  
385 values for each element: median pause duration between elements (ms), coefficient of variation  
386 (cv) of pause duration (ms), median peak frequency (Hz), cv peak frequency, maximum peak  
387 frequency (Hz), minimum peak frequency (Hz), range peak frequency (difference between  
388 maximum and minimum peak frequencies), number of elements, median frequency bandwidth  
389 (Hz), cv frequency bandwidth (Hz), median frequency change (Hz), cv per-element frequency  
390 change (Hz), song duration (ms), and median element duration (ms). Peak frequency is defined  
391 as the frequency window with the highest amplitude for a given portion of the sonogram. We  
392 took the natural log of the number of elements, median frequency change, median frequency  
393 bandwidth, and song duration to improve downstream analyses with respect to assumptions of  
394 normality. To generate estimates of song phenotypes at the level of the individual bird, we took  
395 the arithmetic mean of the values for each variable across songs. These procedures resulted in  
396 a data set comprising song phenotype estimates for 142 individuals from measurements of 419  
397 songs. A mean of  $2.95 \pm 1.01$ SD songs were measured per individual.

398  
399 We used Gaussian finite mixture modeling (GFMM) to perform cluster analyses on the 14 song  
400 traits measured for each individual. GFMM was performed using the package Mclust (54) in R  
401 3.5.2 (55). We built models with the number of mixture components varying from 1 to 9, and  
402 interpreted each of these components as a cluster in multivariate phenotypic space (56). For  
403 each number of specified components, we built six different types of models representing  
404 different parameterizations of the covariance matrix. These parameterizations allow for flexibility  
405 in the volume and shape of the mixture components. We examined relative support for the 54  
406 total models using the Bayesian Information Criterion (BIC).

407

408 *Molecular phylogenetics*

409 We performed phylogenetic analyses using DNA sequence data for samples collected from the  
410 field (see the Appendix for details on sampling for molecular analyses and for further detail on  
411 phylogenetic methods, see SI Appendix 2 for specimen details). First, to investigate whether  
412 song phenotypes generally correspond to phylogenetic lineages across the species complex,  
413 we built a multi-locus phylogenetic tree from a concatenated alignment of DNA sequences for  
414 three mtDNA genes (ND2, ND3, and ATP6) and six nuclear autosomal introns (MB, CHDZ,  
415 11836, 18142, TGFb2, and MUSK), for 256 in-group individuals and 12 outgroup species. The  
416 alignment had 5,313 base pairs. We estimated our phylogenetic tree using a maximum  
417 likelihood approach (57), and assessed support for nodes by bootstrapping. Secondly, we built  
418 population-level trees to investigate the history of song and beak length divergence over the  
419 focal species complex. We constructed these trees by calculating mean population distances for  
420 16 and 15 populations, for analyses of song and beak phenotypes respectively. For these trees,  
421 we used sequences of the mtDNA genes ND2 (440 bp) and ND3 (362 bp), for 134 and 128  
422 individuals for song and beak analyses, respectively. Topological relationships between named  
423 species were the same in the mtDNA population trees as in the multi-locus phylogenetic tree  
424 with species as tips.

425  
426 Third, we sought to estimate the age of the most recent common ancestor of the species  
427 complex. We performed a species-level phylogenetic analysis using a Bayesian coalescent-  
428 based method (BEAST, (58, 59)) with DNA sequence data from two mtDNA genes (ND2 and  
429 ATP6, coded as a single locus) and four nuclear DNA sequences (ATP6, TGFb2, MB, and  
430 CHDZ). This analysis included 16 species as tips, including the five named species in the focal  
431 species complex (*C. mediocris*, *C. usambarica*, *C. loveridgei*, *C. moreaui*, and *C. fuelleborni*),  
432 eight other sunbird species, and three species of flowerpecker (Dicaeidae). We dated the most  
433 recent common ancestor (MRCA) of the focal species complex by implementing a normal prior  
434 distribution (mean = 18 My, SD = 2) on the node age of the MRCA of sunbirds and  
435 flowerpeckers, based on a recent dating analysis of a family-level phylogenetic tree of the  
436 Passeriformes (60). We used a GTR-gamma substitution model and a relaxed lognormal  
437 molecular clock, with substitution rate prior distributions based on divergence rate estimates for  
438 the Hawaiian honeycreeper radiation (an oscine passerine radiation, like sunbirds) (61).

439  
440 *Phylogenetic comparative method approach*

441 To investigate the tempo and mode of song divergence, we compared phylogenetic trait  
442 evolution models fit to population-level data. We fit models to each song trait individually. We

443 built and fit phylogenetic trait evolution models representing 1) strongly bounded evolution  
444 (stasis or near-stasis) punctuated by pulses and 2) gradual evolution (Brownian motion), by  
445 maximum likelihood. These models are described in the Supplementary Information. Models  
446 including pulses were fit under the condition that there were a maximum of four pulses across  
447 the population tree. We compared support for fitted models using AICc values (62). To  
448 characterize the uncertainty in model selection due to phylogenetic uncertainty, we built ten  
449 bootstrap population trees, and fit both trait evolution models on each of the bootstrap  
450 phylogenies for each song trait.

451  
452 For the traits where punctuated evolution models were a better fit than Brownian motion, we  
453 estimated the location of pulses on the population tree. Our method for fitting pulsed  
454 evolutionary models involves fitting the maximum likelihood parameters for all potential pulse  
455 configurations on the tree, given a maximum of four pulses (corresponding to  $\sim 4.6 \times 10^4$  pulse  
456 configurations). To quantify the strength of the evidence for an evolutionary pulse on a given  
457 branch of the phylogenetic tree, we calculated the sum of the AICc weights of those pulse  
458 configurations that include a pulse on the given branch, and divided this by the sum of the AICc  
459 weights of all computed pulse configurations (63). Our approach treats each  $n$ -pulse  
460 configuration as an independent mode We present maximum likelihood parameters for the  
461 maximum likelihood configuration (Tables S1 and S2).

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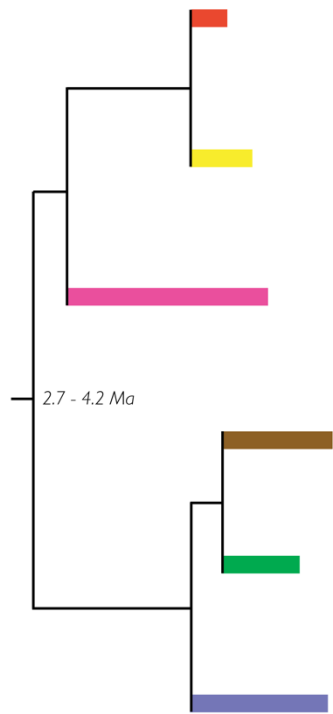
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## 500 **Figures**

501 Figure 1. An overview of the Eastern Double-collared Sunbird species complex (EDCS). a) A  
502 phylogenetic tree trimmed to include named species and one within-species division that  
503 corresponds with a major song divergence. Estimated age of the most recent common ancestor  
504 is shown at the node. b) Depictions of typical adult male plumage for the six lineages  
505 represented. c) Sonograms showing representative songs for the six lineages shown. d)  
506 Ranges of the six lineages in eastern Africa across Kenya, Tanzania, Malawi, and Mozambique.

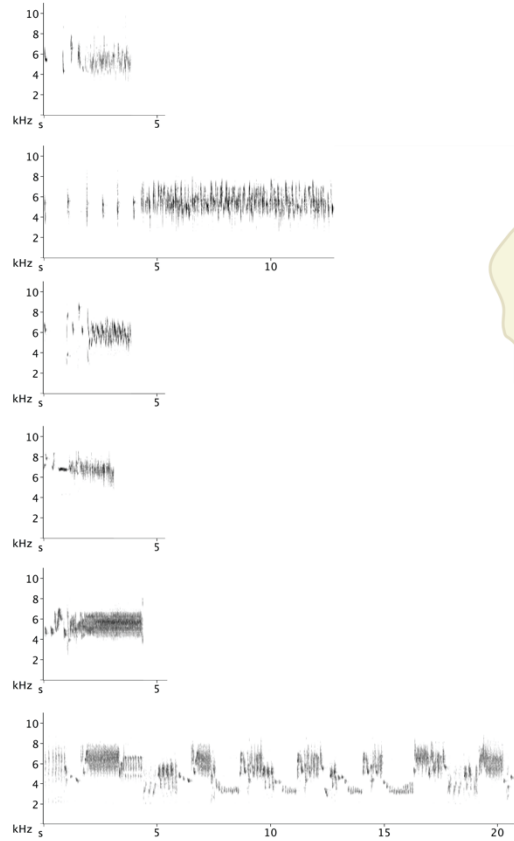
a)



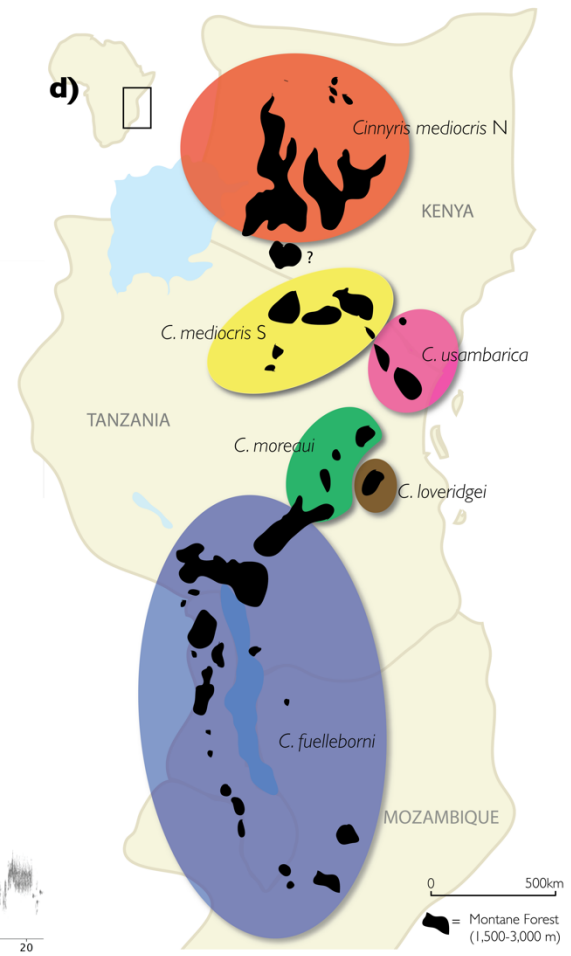
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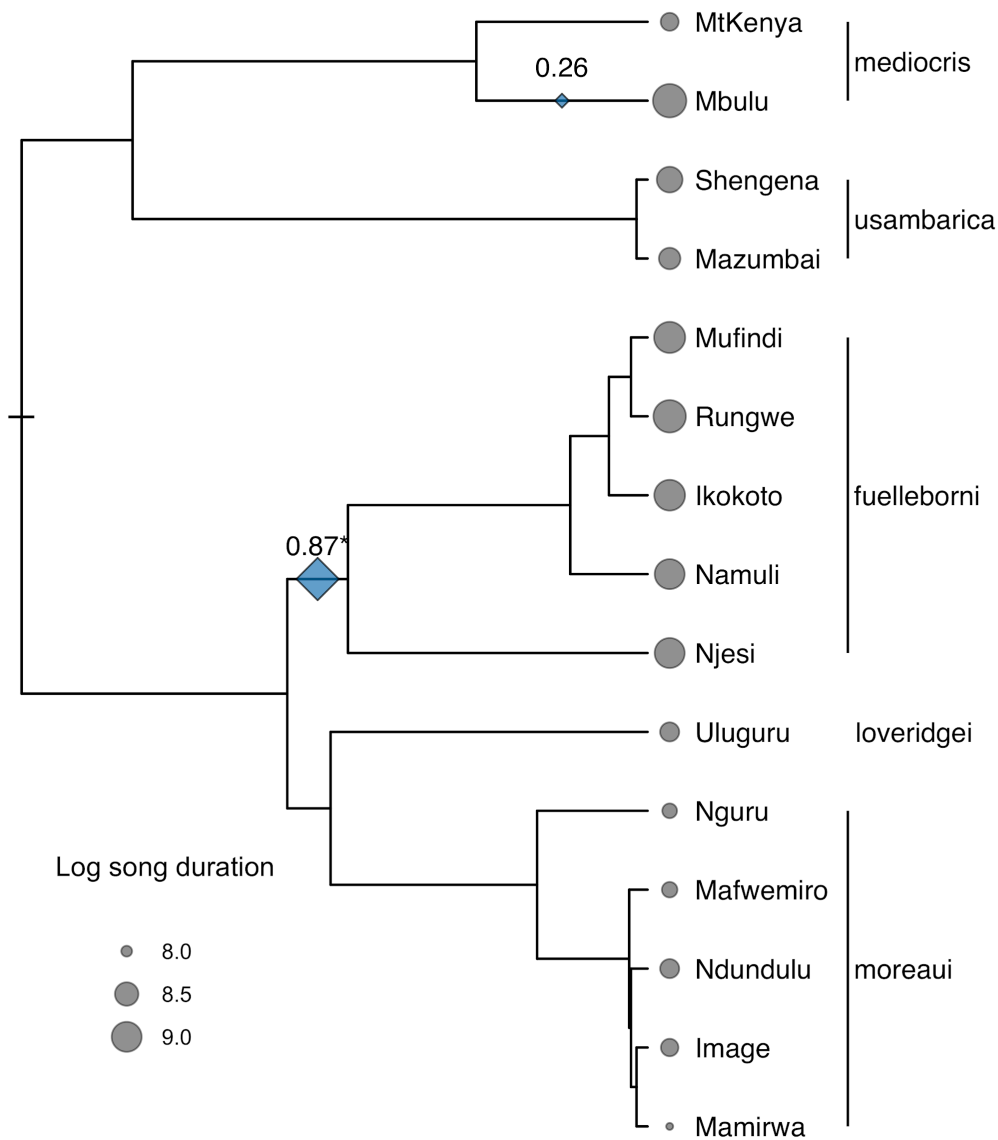


d)





508 Figure 2. Pulse localization for the evolution of (log) song duration. Blue diamonds are found on  
509 branches where pulse localizations had support values > 0.2, with diamond size reflecting  
510 support, and support value shown above. The asterisk signifies a pulse that occurs in the pulse  
511 configuration with the minimum AICc value. Sizes of gray circles correspond to mean phenotype  
512 values at tips, which each represent a geographically discrete sky island population. Species  
513 epithets are indicated at far right.



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