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**Faster evolution of a premating reproductive barrier is not associated with faster
speciation rates in New World passerine birds**

Benjamin G. Freeman^{1*}, Jonathan Rolland^{1,2}, Graham Montgomery³ & Dolph Schluter¹

¹ Biodiversity Research Centre and Department of Zoology, University of British Columbia,
Vancouver, V6T1Z4, Canada.

² CNRS, UMR5174, Laboratoire Evolution et Diversité Biologique, Université Toulouse 3 Paul
Sabatier, Bâtiment 4R1, 118 Route de Narbonne, 31062 Toulouse, France

³ Department of Ecology and Evolutionary Biology, UCLA, Los Angeles, California, USA,
90095

* corresponding author

14 **Abstract**

15 Why are speciation rates so variable across the tree of life? One hypothesis is that this variation
16 is explained by how rapidly reproductive barriers evolve. We tested this hypothesis by
17 conducting a comparative study of the evolution of bird song, a premating barrier to
18 reproduction. Speciation in birds is typically initiated when geographically isolated (allopatric)
19 populations evolve reproductive barriers. We measured the strength of song as a premating
20 barrier between closely related allopatric populations by conducting 2,339 field experiments to
21 measure song discrimination for 175 taxon pairs of allopatric or parapatric New World passerine
22 birds, and estimated recent speciation rates from a global molecular phylogeny of birds. Taxon
23 pairs with high song discrimination in allopatry failed to regularly interbreed in parapatry,
24 evidence that song discrimination is indeed an important reproductive barrier. However,
25 evolutionary rates of song discrimination were not associated with recent speciation rates, and
26 song discrimination evolves faster in suboscine passerines than their more species-rich sister
27 clade, the oscines. Our findings support the long-held idea that song is a key premating
28 reproductive barrier in birds, but show that faster evolution of this reproductive barrier between
29 populations does not result in faster diversification between species.

30

31 Keywords: behavior, diversification, evolutionary rates, field experiments, reproductive
32 isolation, song evolution, speciation

33 **Introduction**

34 How fast new species form varies tremendously within larger taxonomic groups [1]. For
35 example, speciation rates, defined as the splitting rate on a phylogenetic tree, vary over 30-fold
36 within New World birds [2]. One intuitive expectation is that speciation rates are faster when
37 populations more rapidly evolve reproductive barriers, as speciation is often defined as the
38 evolution of reproductive isolation between related populations [3,4]. However, the sole
39 empirical study testing this “reproductive isolation” hypothesis found no association between
40 speciation rates and evolutionary rates of reproductive barriers in either fruit flies or birds [5].
41 For these taxa, the rate of evolution of the measured reproductive isolation between populations
42 over shorter time scales apparently fails to leave an imprint on speciation rates measured over
43 longer time scales. Alternatives to the reproductive isolation hypothesis are that speciation rates
44 are largely idiosyncratic, or set by demographic and ecological factors that govern rates of
45 population formation, population persistence, and range expansion [6–8]. For example,
46 speciation rates are positively correlated with rates of population differentiation in New World
47 birds [9], and speciation in Himalayan birds appears to be limited not by rates of reproductive
48 isolation but instead by rates of population persistence and range expansion [10].

49 Here, we test these contrasting hypotheses by measuring the association between
50 evolutionary rates of song divergence and recent speciation rates in New World passerine birds.
51 Song is traditionally considered to be a potent premating reproductive barrier in passerine birds
52 because they use song to attract and choose mates and because closely related sympatric species
53 often differ in song [11–15], though this perspective has been challenged by recent work
54 suggesting premating barriers such as song have relatively little impact on speciation [16]. We
55 focus on how song diverges between geographically isolated (allopatric) populations because

56 speciation in birds is typically initiated in allopatry [17,18], and divergence in song between
57 allopatric populations is thought to constitute a barrier to reproduction that promotes speciation
58 [19–21]. The reproductive isolation hypothesis predicts that evolutionary rates of song
59 divergence between allopatric populations are positively associated with speciation rates; other
60 hypotheses predict no such association.

61 We tested these predictions by conducting 2,339 field playback experiments to measure
62 song divergence for 175 taxon pairs. Each taxon pair consisted of a pair of related allopatric or
63 parapatric populations; playback experiments simulated secondary contact between these
64 populations. We quantified song divergence as song discrimination, measured as the degree to
65 which birds behaviorally discriminated against allopatric song relative to local song. In some
66 taxon pairs, birds responded just as strongly to allopatric song as to local song, whereas birds in
67 other taxon pairs ignored allopatric song while responding strongly to local song. We assumed
68 that taxon pairs in which territorial individuals completely ignored allopatric song represent
69 cases for which song potentially constitutes a strong reproductive barrier. To test this
70 assumption, we focused on 26 parapatric taxon pairs for which we have information on
71 propensity to interbreed in parapatry. We used these cases to test whether response to simulated
72 secondary contact -- behavioral response to playback experiments, which took place in allopatry
73 -- was correlated with mate choice decisions in real secondary contact. Our study thus tests the
74 hypothesis that the faster evolution of a premating reproductive barrier is associated with faster
75 recent speciation rates, while simultaneously examining the often-held but seldom-tested
76 assumption that song divergence in geographic isolation constitutes a reproductive barrier in
77 birds.

78

79 **Methods**

80 *Taxon pair selection*

81 We studied 175 taxon pairs of New World passerines. Most taxon pairs were allopatric (N =
82 131), with a smaller number that were parapatric (N = 44); all experiments were conducted in
83 allopatric regions of species' ranges in order to measure behavioral response to unfamiliar songs
84 (i.e. to simulate secondary contact). We studied pairs of populations without regard to their
85 taxonomic species status. Taxon pairs included allopatric populations ranked as subspecies (N =
86 89) and as species (N = 86; typically sister species), and came from both major clades of
87 passerines: oscines, which learn their song at young ages from listening and copying adult birds
88 (N = 108) and suboscines, which have innate, genetically controlled song (N = 67) [22]. We
89 selected taxon pairs that met four criteria: (1) there was a population that was common at one of
90 our study sites that uses song in territorial defense; (2) there was a closely related allopatric or
91 parapatric population that also uses song in territorial defense (this population could be classified
92 either as the same species or as a distinct species); (3) good quality recordings for both
93 populations were available on xeno-canto.org or Macaulay Library; and (4) homologous mtDNA
94 sequences for both populations were available on GenBank.

95

96 *Field experiments*

97 We conducted 2,339 experiments (mean of 13.4 experiments per taxon pair, range = 4 to 35) to
98 measure bird behavior in response to two treatments: (1) song from the local population
99 (sympatric treatment) and (2) song from the related, allopatric population (allopatric treatment).
100 We conducted fieldwork at multiple sites within four regions: (1) the Pacific Northwest, in the
101 vicinity of Vancouver, BC, Canada (430 experiments on 32 taxon pairs); (2) southwestern

102 United States: southeastern Arizona (93 experiments on 11 taxon pairs), southern Texas (22
103 experiments on 4 taxon pairs), and southern California (17 experiments on 3 taxon pairs); (3)
104 southern Central America (785 experiments on 59 taxon pairs from Costa Rica and western
105 Panama); and (4) the tropical Andes (1002 experiments on 83 taxon pairs from Ecuador and
106 northern Peru). The number of taxon pairs described for different regions sums to more than the
107 total number of taxon pairs in this study. This is because some taxon pairs were studied in
108 multiple regions. For example, for several taxon pairs with one population found in Central
109 America and the other in the tropical Andes, playback experiments took place in both regions.

110 Prior to fieldwork, we downloaded multiple high quality natural vocalizations for each
111 population for each taxon pair from the online archives of xeno-canto.org and the Macaulay
112 Library of Natural Sounds (mean unique recordings used per population = 6.8; interquartile
113 range = 5-8). In the field, we searched for individuals or pairs of one of our taxon pairs, then
114 initiated an experiment when we detected one or more individuals. We synced a wireless speaker
115 (UE Roll or JBL Charge 2+) to a smartphone with Bluetooth, and placed the speaker at least 15
116 m from the nearest bird (typically 15-25 m). We then retreated to ~ 10 m from the speaker and
117 began the first treatment by broadcasting a song at a natural volume (~80 dB) for two minutes.
118 We alternated the order of the first treatment in an experiment (sympatric or allopatric) between
119 experiments. We then observed behavioral responses during these two minutes and over a
120 subsequent five-minute observation period. The key behavioral response we measured was the
121 distance of the bird's closest approach to the speaker, quantified as the minimum distance in the
122 horizontal plane from the bird, whether perched or in flight, to the speaker. If the bird(s)
123 approached within five meters of the speaker during the initial two minutes of song playback, we
124 stopped playback, as we considered this to represent an obvious strong approach response. At the

125 end of the observation period, birds had typically left the area (> 15 m from the speaker) and/or
126 ceased vocalizing at an elevated rate. If not, we waited to start the second treatment until birds
127 were > 15 m from the speaker and had returned to pre-playback vocal activity. In this
128 experimental design, the sympatric treatment is a positive control, and we therefore included
129 only experiments where birds approached to within 15 m of the speaker in the sympatric
130 treatment. Our logic was that a bird defending a territory should approach the speaker in
131 response to playback of local conspecific song. Indeed, the mean closest approach in response to
132 sympatric song was 4 m.

133 For each taxon pair, we quantified song discrimination as the proportion of tested
134 territories where birds ignored allopatric song. We defined “ignoring” allopatric song as failing
135 to approach within 15 m of the speaker in response to allopatric song. Responses to allopatric
136 song were symmetric within a taxon pair (see Figure S1; Pearson’s $r = 0.90$; $df = 34$, $t = 11.93$, p
137 < 0.0001 for 36 taxon pairs with five or more experiments for each population).. We therefore
138 included all experiments performed on both populations when calculating the song
139 discrimination score for each taxon pair. Our experiments measured the response of territorial
140 birds, typically mated pairs or males alone, to allopatric song rather than their mate choice
141 response to allopatric song. However, territorial responses to allopatric song are consistently
142 stronger than mate choice responses to allopatric song (reviewed by 22). Thus, a population that
143 ignores allopatric song in a territorial context is expected to also ignore allopatric song in a mate
144 choice context.

145

146 *Genetic divergence*

147 We calculated genetic divergence for all taxon pairs using homologous sequences of
148 mitochondrial genes downloaded from GenBank (typically cytochrome b or ND2), and
149 quantified mtDNA divergence as uncorrected p-distances. Mitochondrial DNA divergence
150 evolves in a roughly clock-like fashion in birds, with approximately 2% divergence per million
151 years in isolation [24]. Here, we follow many previous comparative studies by using
152 mitochondrial divergence as a proxy for the amount of time since the two populations in a taxon
153 pair last shared a common ancestor.

154

155 *Statistical analysis*

156 We followed previous studies and modeled the evolution of song discrimination by fitting
157 Michaelis-Menten models [25,26]. We fit the Michaelis-Menten model used by Weir & Price
158 [26] where song discrimination = genetic distance / (β + genetic distance). In this formulation, β
159 is the value of genetic distance at which song discrimination reaches 0.5, meaning that half of
160 territorial birds ignore song from their allopatric relative. Larger values of β indicate slower
161 evolution of song discrimination; we thus term β the “song discrimination waiting time”. We
162 tested our hypotheses by fitting a model that included the variable of interest (e.g., recent
163 speciation rate) as a modifier to β , and compared the fit of this full model with a reduced model
164 that did not include the variable of interest. We compared models by conducting F tests using the
165 “anova” function in R. All statistics were conducted in R [27].

166 First, we tested the assumption that song discrimination in allopatry indicates song is a
167 reproductive barrier. If so, taxon pairs with strong song discrimination in allopatry should show
168 strong mate discrimination in parapatry. We tested this prediction using the 44 parapatric taxon
169 pairs whose distributions closely approach one another or narrowly overlap. We inspected the

170 primary literature and The Cornell Lab of Ornithology's Birds of the World
171 (birdsoftheworld.org) to find previous field studies that have measured whether these taxon pairs
172 regularly interbreed with one another where they come into contact. We found relevant data for
173 26 taxon pairs, and compared the fit of a full model that included regular interbreeding as a
174 binary predictor variable to a reduced model that did not include this covariate.

175 Second, we tested whether evolutionary rates of song discrimination between populations
176 in passerine birds are associated with macroevolutionary rates. Oscines and suboscines are sister
177 clades that comprise 99% of the passerine radiation; a third clade, the New Zealand wrens,
178 contains two extant species. However, despite their equivalent evolutionary age, oscines have
179 nearly four times more species than suboscines. We tested whether evolutionary rates of song
180 discrimination were associated with this diversity disparity by comparing model fit between a
181 full model with clade (oscine versus suboscine) as a predictor variable with a reduced model that
182 did not include clade as a predictor variable.

183 To more rigorously test the association between rates of song discrimination and
184 macroevolutionary rates, we tested whether evolutionary rates of song discrimination were
185 associated with recent speciation rates. We estimated recent speciation rates for the clades
186 containing each taxon pair using the diversification rate statistic. The diversification rate statistic
187 infers recent speciation rates for each tip of the phylogeny based on the distribution of nodes and
188 branch lengths leading to it [28,29], and is more reflective of recent speciation than
189 diversification (speciation minus extinction). We computed recent speciation rates for taxon-
190 pairs using species-level molecular phylogenies. For suboscines, we used the recently published
191 suboscine tree [30]. There is not a single recent species-level tree for oscines. The majority of
192 New World oscines are in the clade Emberizoidea: for these, we used the Emberizoidea tree [31].

193 For the remaining non-emberizoid passerines, we used a consensus phylogenetic tree built using
194 TreeAnnotator [32] from a pseudo-posterior distribution of 10,000 phylogenies downloaded
195 from birdtree.org [28]. For taxon pairs classified as two distinct species, we used the average
196 recent speciation rate of the two species as our estimate of recent speciation rate for the taxon
197 pair. We tested the importance of recent speciation rate by comparing model fit between a full
198 model with clade (oscine versus suboscine) and logged recent speciation rate as predictor
199 variables with a reduced model that did not include recent speciation rate as a predictor variable.

200 Last, we tested whether latitude and elevational zone were linked to song evolution and
201 recent speciation rates, as both song evolution [26,33] and species formation [34] have been
202 reported to occur at faster rates in high latitudes in New World birds than in the tropics. We
203 coded the latitudinal zone as temperate (N = 47) or tropical (N = 128) based on the locations
204 where playback experiments were conducted. For elevational zone, we restricted our analysis to
205 110 tropical taxon pairs that reside in humid forest, and categorized each of these taxon pairs as
206 lowland (midpoint elevation 0-1,000 m), mid-mountain (midpoint elevation 1,000-2,000 m) or
207 high elevation (midpoint elevation > 2,000 m) using a reference volume [35]. We tested the
208 relationship between the evolution of song discrimination and geography by fitting full models
209 that included clade and the geographic variable (latitudinal zone or elevational zone) as predictor
210 variables to reduced models that lacked the geographic variable. In addition, we tested whether
211 recent speciation rates differed between latitudinal zones using a t-test, and between elevational
212 zones by fitting a linear model with elevational zone (lowland, mid-mountain or high mountain)
213 as a predictor variable.

214

215 **Results**

216 *Song discrimination is a metric of reproductive isolation*

217 For parapatric taxon pairs, song discrimination in allopatry is lower in taxon pairs that routinely
218 interbreed with one another in parapatry (t-test; $df = 7.85$, $t = 3.89$, $p = 0.0050$, Figure 1a). This
219 result holds when taking evolutionary age into account (F test; $df = 1$, $F = 10.53$, $p = 0.0034$,
220 Figure 1b). We estimate that taxon pairs that do not regularly interbreed in parapatry have a song
221 discrimination waiting time that is eight times smaller (i.e., is attained eight times faster) than for
222 taxon pairs that regularly interbreed in parapatry (2.4 ± 1.3 versus 19.1 ± 9.6).

223

224 *Song discrimination evolution is faster in suboscines*

225 Suboscines, which have innate song, have faster evolutionary rates of song discrimination than
226 do oscine taxon pairs that learn their songs (Figure 2). A full model that included clade as a
227 predictor variable was a better fit than a reduced model that lacked clade (F -test; $df = 1$, $F =$
228 34.41 , $p < 0.0000001$). We estimate that suboscine taxon pairs have a song discrimination
229 waiting time that is three times smaller (i.e., is attained three times faster) than for taxon pairs
230 that regularly interbreed in parapatry (2.7 ± 0.40 versus 8.4 ± 1.1).

231

232 *Song discrimination evolution is not associated with speciation rate*

233 Evolutionary rates of song discrimination are not statistically associated with higher recent
234 speciation rates (Figure 3). A full model that included recent speciation rate and clade as a
235 predictor variable was not a better fit than a reduced model that lacked recent speciation rate (F -
236 test; $df = 1$, $F = 0.57$, $p = 0.45$). We illustrate this result by fitting a model where we coded
237 recent speciation rate as a categorical variable with three levels (slow, average and fast; each
238 level contained one-third of the data). Results were similar between this categorical model and

239 the model where recent speciation rate is a continuous variable; the categorical model facilitates
240 visualization of model results (Figure 3). Taxon pairs with fast recent speciation rates have
241 similar song discrimination waiting times than taxon pairs with slow recent speciation rates
242 (Figure 1c). Oscines and suboscines in this dataset do not consistently differ in their recent
243 speciation rates (mean values = 0.28 and 0.27 species produced per million years, respectively; t-
244 test; $df = 166.4$, $t = -0.27$, $p = 0.79$).

245

246 *Song discrimination evolution is not explained by geography*

247 Song discrimination evolves at similar rates in the tropics and the temperate zone (Figure 4; F
248 test; $df = 1$, $F = 0.015$, $p = 0.70$) and at similar rates across different elevational zones within the
249 tropics (Figure S2). In our dataset, recent speciation rates were unrelated to latitudinal zone ($df =$
250 146.4 , $t = 0.37$, $p = 0.71$) or elevation (estimate and standard error for elevational zone in a
251 univariate linear model = -0.0081 ± 0.022 ; $t = -0.37$, $p = 0.71$).

252

253 **Discussion**

254 A recent review declared “*The connection between metrics of diversification and reproductive*
255 *isolation remains in its infancy, but it is one of the most urgent questions in speciation biology*”
256 [36]. Here we addressed this urgent question by conducting thousands of field experiments to
257 measure the strength of an important premating barrier—bird song—in hundreds of comparisons
258 of passerine birds. We provide evidence that the song discrimination we experimentally
259 measured is indeed a reproductive barrier that explains observed mating decisions in secondary
260 contact. However, faster evolutionary rates of song discrimination were not associated with
261 faster recent speciation rates. That is, rates of evolution of a barrier to gene flow between

262 population, measured over shorter periods of time, are not linked to rates of speciation, which are
263 measured over deep time-scales [1].

264

265 *Song is a reproductive barrier in passerine birds*

266 Our results directly support the idea that song discrimination between allopatric populations
267 constitutes a barrier to reproduction. We find that behavioral responses to song playback
268 experiments in allopatry are associated with observed mate choice in parapatry—taxon pairs that
269 ignore allopatric song also fail to regularly interbreed in parapatry, while those that respond to
270 allopatric song commonly interbreed in parapatry. That is, observed responses to simulated
271 secondary contact in our playback experiments aligns with observed responses in real world
272 secondary contact. This concordance supports our assumption that the song discrimination
273 measured using playback experiments quantifies the strength of a reproductive barrier. More
274 generally, this constitutes broadscale comparative evidence that song is a potent reproductive
275 barrier in birds. We also provide testable predictions for 16 further cases where we have
276 measured song discrimination in allopatry but it is not yet known how populations behave in
277 parapatry (Table S1). Future work testing our predictions for these case examples will comprise
278 independent tests of our results.

279

280 *Faster evolution of song discrimination is not associated with faster macroevolutionary rates*

281 We found no association between evolutionary rates of song discrimination and recent speciation
282 rates. Supporting evidence for this lack of association comes from an examination of sister clades
283 of passerines, the oscines (song learners) and suboscines (innate song). Oscines have nearly four
284 times as many species as suboscines (~ 4700 versus ~1300), and a traditional explanation for this

285 discrepancy is that faster rates of song evolution associated with song learning promote
286 speciation in oscines [37–39]. This is a special case of the more general hypothesis that
287 phenotypic plasticity leads to faster rates of evolution and speciation [40]. We reject this
288 explanation for passerine birds in the Americas. Instead, we find that allopatric taxon pairs of
289 suboscines (innate song) evolve song discrimination much faster than do allopatric taxon pairs of
290 oscines (song learners). We previously reported the same result when analyzing an earlier
291 version of our song playback dataset that contained 69 taxon pairs, versus the current dataset of
292 175 taxon pairs. Our best explanation for this counterintuitive result is that plasticity increases
293 trait variation, and that increased within-population variation in signal makes it more difficult to
294 discriminate against foreign signals. Supporting this interpretation, within-population variation in
295 acoustic traits of songs is much greater for oscines than suboscines [25]. It remains the case that
296 song learning clades have more species than related clades with innate song, but this does not
297 result from faster song evolution in song learners in allopatry that spurs speciation.

298

299 *Geography does not drive song evolution*

300 Accumulating evidence suggests that evolutionary rates tend to be fastest in the temperate zone
301 [43]. However, we do not recover this result in our dataset. Instead, we find that song
302 discrimination evolves at similar rates in the tropics and temperate zone, and also at similar rates
303 within different elevational zones in the tropics. This contrasts with a recent report that song
304 discrimination evolves faster in temperate North America than in the Amazon basin [26]. There
305 are at least two reasons that could explain these different results. First, our tropical field
306 experiments did not include sites in the lowland Amazon basin, but instead took place in Central
307 American and the tropical Andes; song discrimination may evolve particularly slowly in the

308 Amazon. Second, differences in experimental design of field playbacks may impact estimates of
309 song discrimination.

310

311 *Concluding remarks*

312 Speciation is often defined as the evolution of reproductive isolation. The fast evolution of
313 reproductive isolation underlies rapid speciation in adaptive radiations in taxa including three-
314 spined sticklebacks [4], Lake Victoria cichlids [44] and Hawaiian crickets [45]. Here, we provide
315 evidence that evolutionary rates of a premating barrier, song, fail to have cascading effects on
316 recent speciation rates in New World passerine birds. It is perhaps noteworthy that the case
317 examples cited above occur on recently formed lakes or oceanic islands, environments that may
318 feature unusually high levels of ecological opportunity. In continental systems, it appears that
319 faster rates of evolution of reproductive isolation often do not seem to leave a clear imprint on
320 longer-term macroevolutionary processes, though studies remain few [5,8]. One alternative
321 possibility is that speciation rates may be better explained by demographic and ecological factors
322 that set rates of population formation, population persistence, or range expansion. However, we
323 suggest a simpler explanation: that evolutionary rates of reproductive isolation may seldom be
324 intrinsic properties of lineages. In this conception, while the rapid evolution of reproductive
325 barriers between populations (such as the rapid evolution of song discrimination in birds)
326 promotes a single speciation event. However, the newly-formed daughter species may not also
327 rapidly evolve reproductive barriers that lead to additional cycles of speciation. If so, estimated
328 evolutionary rates of divergence between populations are unlikely to be associated with estimates
329 of speciation rate, which reflect multiple rounds of speciation events. Testing the reasons why

330 metrics of diversification are only loosely connected with reproductive isolation remains an
331 urgent question in speciation research.

332

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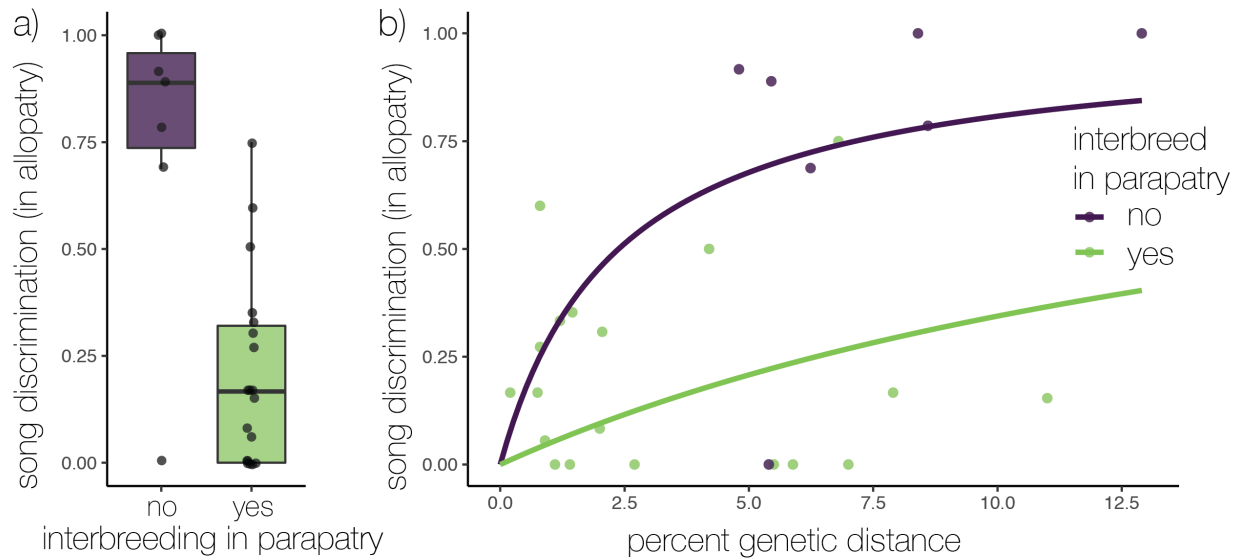
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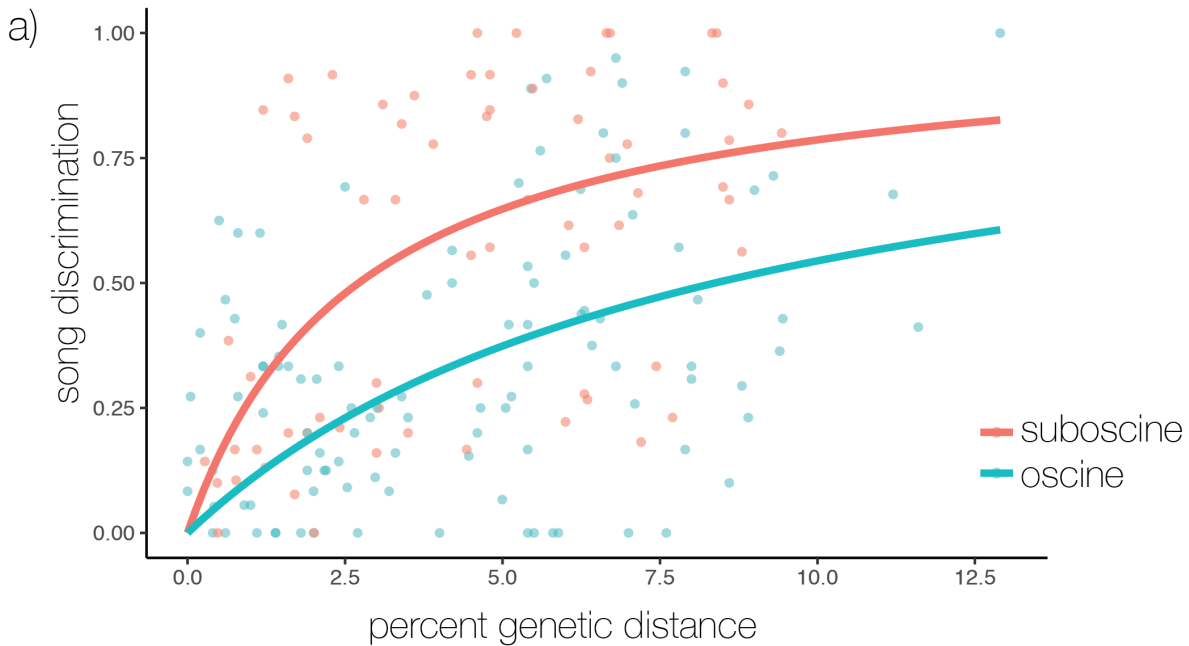
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458

459 Figure 1. Greater song discrimination in allopatry is associated with reduced interbreeding in
 460 parapatry. This analysis is based on 26 taxon pairs that have narrow zones of parapatry; all
 461 playback experiments were conducted in the allopatric portion of the range. Song discrimination
 462 in allopatry is lower in taxon pairs that routinely interbreed with one another in parapatry (panel
 463 a; $df = 7.85$, $t = 3.89$, $p = 0.0050$). This result holds when accounting for the fact that taxon pairs
 464 with strong song discrimination are older and have larger genetic distances (panel b). Predictions
 465 from the full Michaelis-Menten that included regular interbreeding in parapatry are plotted as
 466 trendlines in panel b; raw data is shown as points. This full model was a much better fit
 467 compared to a reduced model that did not include regular interbreeding in parapatry as a
 468 covariate (F test; $df = 1$, $F = 10.48$, $p = 0.0036$).

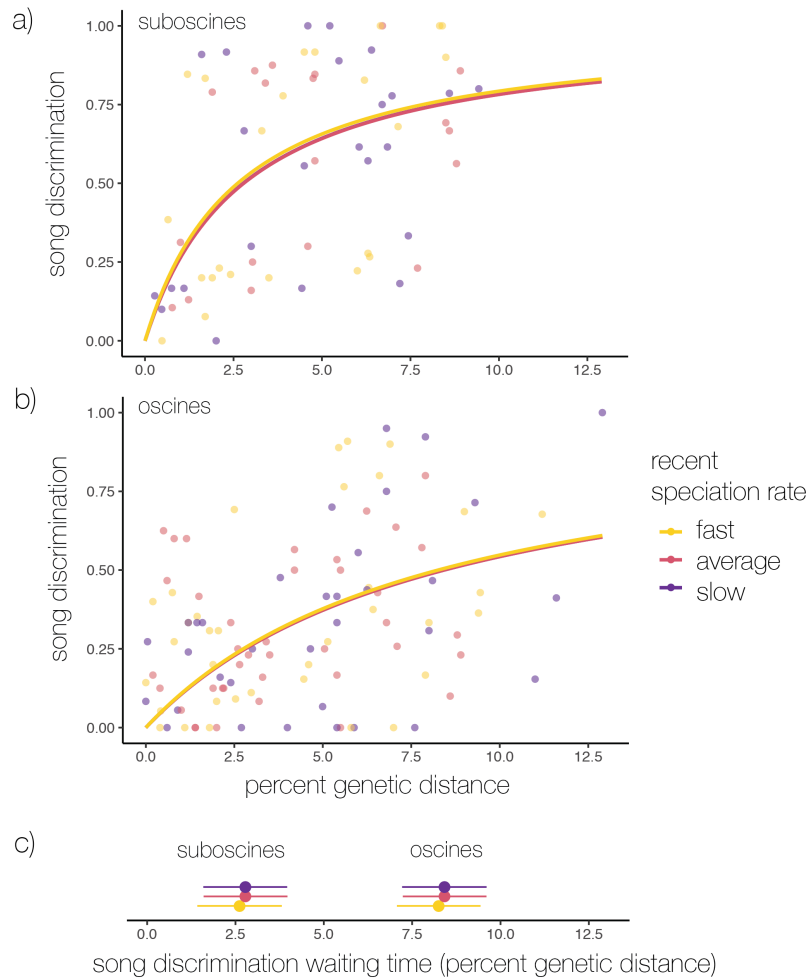
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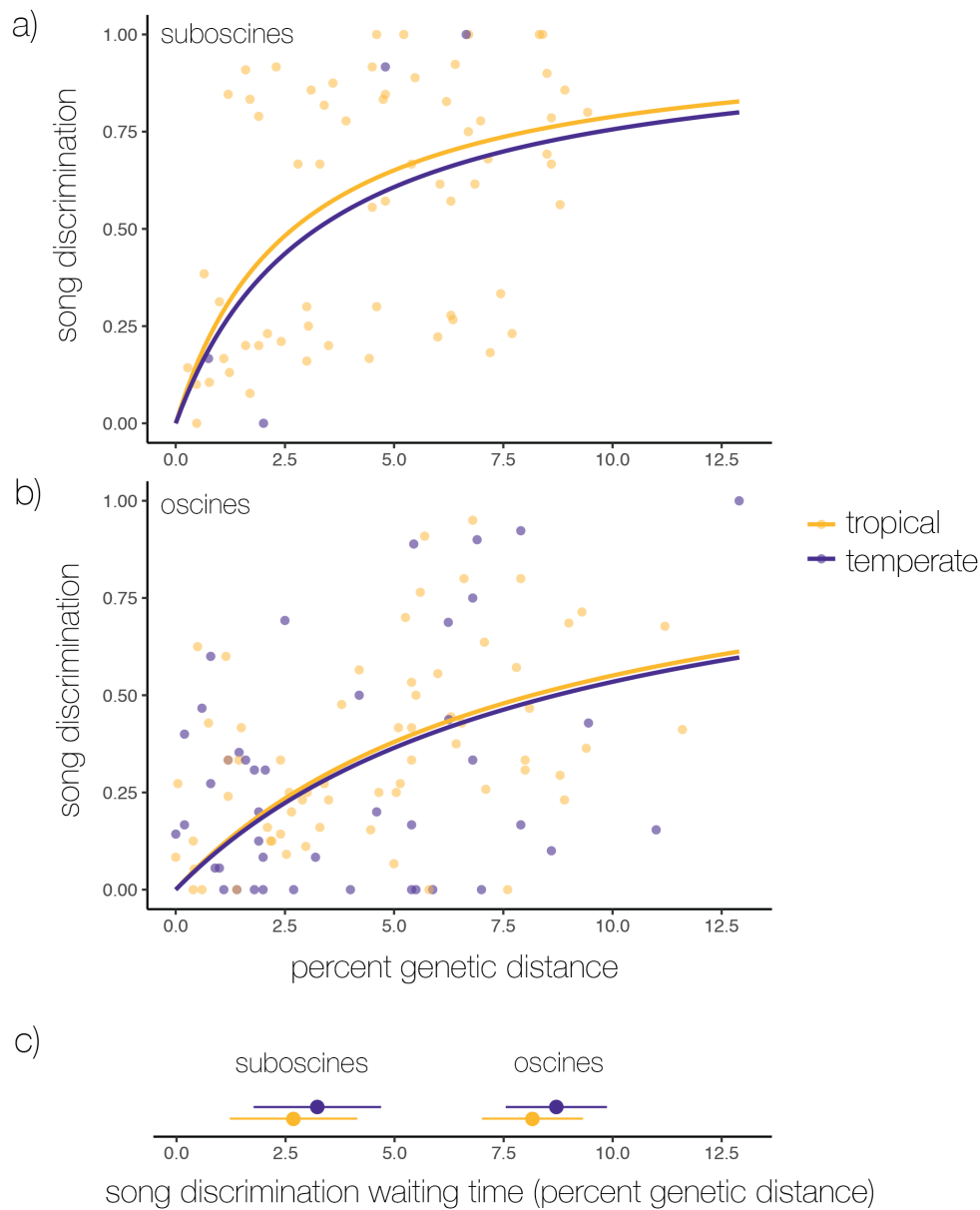
471 Figure 2. The evolution of song discrimination is faster in taxon pairs with innate song
 472 (suboscines) than taxon pairs with learned songs (oscines). Predictions from the full Michaelis-
 473 Menten that included clade (oscine versus suboscine) are plotted as trendlines in panel a; raw
 474 data is shown as points. This full model was a much better fit compared to a reduced model that
 475 did not include clade information (F test; $df = 1$, $F = 34.41$, $p < 0.0000001$). The song
 476 discrimination waiting time is 3.1 times smaller for suboscines (2.7 ± 0.40) than for oscines (8.4
 477 ± 1.1 ; panel b); this measures the genetic distance at which half of territories ignore allopatric
 478 song.

479



480

481 Figure 3. Evolutionary rates of song discrimination are not correlated with recent speciation
 482 rates. A full model that included log-transformed recent speciation rate as a continuous variable
 483 was not a better fit compared to a reduced model that did not include recent speciation rate (F -
 484 test; $df = 1$, $F = 0.57$, $p = 0.45$). Relationships are visualized using a model where recent
 485 speciation rate was coded as a categorical variable with three levels (slow, average and fast; each
 486 level contained one-third of data), with predictions plotted as trendlines and raw data are shown
 487 as points. The song discrimination waiting time is similar between taxon pairs with slow and fast
 488 recent speciation rates (panel c); this measures the genetic distance at which half of territories
 489 ignore allopatric song.



490

491 Figure 4. Evolutionary rates of song discrimination are similar between the tropics and temperate

492 zone; for suboscines (a) and oscines (b). A full model that included latitudinal zone was not a

493 better fit compared to a reduced model that did not include latitudinal zone (F test; $df = 1$, $F =$

494 0.015 , $p = 0.70$). Predictions from the full Michaelis-Menten model are plotted as trendlines; raw

495 data is shown as points. Song discrimination waiting times were similar for tropical and

496 temperate taxon pairs for both suboscines and oscines (panel c).