

1 Supplementary Material

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3 For: Freeman BG, J Rolland, G Montgomery & D Schluter. Faster evolution of a pre-mating
4 reproductive barrier is not associated with faster speciation rates in New World passerine birds.

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12 For Datasets A and B (Dataset A = summary data necessary to conduct analysis; Dataset B = raw
13 data on playback experiments), please see Dryad: <https://doi.org/10.5061/dryad.fbg79cnw1>

14 *Methods for phylogenetic analyses*

15 Our dataset consists of phylogenetically independent comparisons. However, closely related
16 taxon pairs in our dataset may have similar speciation rates because they share many branches in
17 the tree. We therefore tested the association between evolutionary rates of song discrimination
18 and speciation rates with two additional analyses that incorporated phylogenetic information.

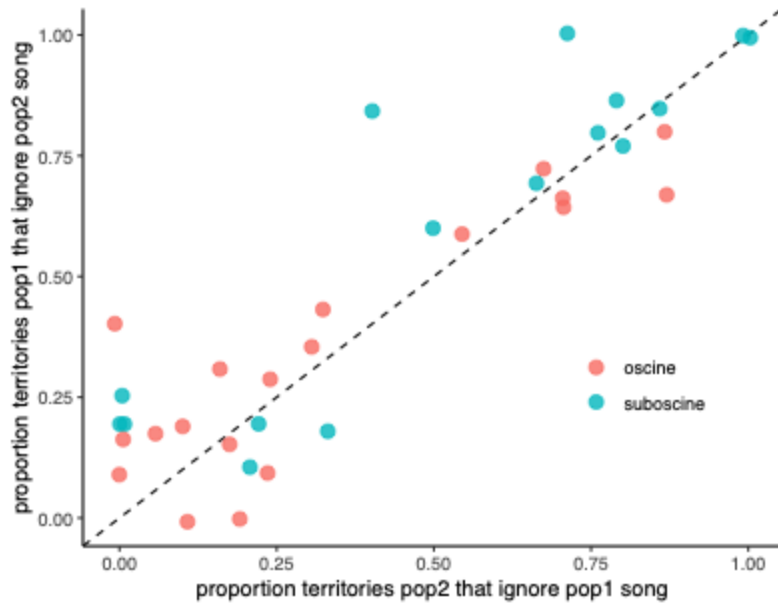
19 First, we tested whether the rate of evolution of song discrimination was associated with the
20 recent speciation rate using ES-sim (Harvey and Rabosky 2018,
21 <https://github.com/mgharvey/ES-sim>). We estimated speciation rate using the DR statistic using
22 species-level phylogenies as described in the main text. We then built a tree for the ES-sim
23 analysis using separate trees for oscines and suboscines: for oscines, we used a MCC tree from
24 trees downloaded from Jetz et al. 2012 Nature (available at birdtree.org), and for suboscines we
25 used the recently published Harvey tree (Harvey et al. 2020 Nature). We pasted these two trees
26 together using the “bind.tree” function from the “ape” package, and rescaled the basal branches
27 to map the dating for the origin of the oscine/suboscine split at 64.6 million years ago (this date
28 was obtained from the visualization of the Jetz et al. 2012 tree available at
29 http://www.onezoom.org/OZtree/static/OZLegacy/EDGE_birds.htm). Our dataset contained 175
30 taxon pairs. However, there are several cases where multiple taxon pairs referred to the same tip
31 (e.g., in widely distributed species such as *Henicorhina leucophrys*), and we therefore used a
32 dataset of 158 taxon pairs for which each taxon pair could be represented as a single unique tip
33 on the phylogenetic tree. We then ran ES-sim simulations on the pruned ultrametric tree of 158
34 species, as this analysis requires an ultrametric tree. We used ES-sim was then used to assess the
35 significance of the association between speciation rate and the ratio between song discrimination
36 and genetic distance, and used 1000 simulations to build the null distribution. We found no

37 significant effect of the rate of evolution of song discrimination on recent speciation rate ($\rho =$
38 $0.040, p = 0.61$). This indicates that our results are robust to the inclusion of phylogenetic
39 information in our models.

40 Second, we fit a generalized nonlinear regression model using the “gnls” function in R. This
41 model incorporates phylogenetic branch length information in the weights and the correlation
42 structure in the model. To fit this model required that each taxon-pair in our dataset be
43 represented as a tip on a phylogenetic tree. Roughly half of our taxon pairs consisted of
44 comparisons between sister species on this tree; the other half were comparisons between
45 populations of the same species. For each interspecific taxon pair, we edited the phylogenetic
46 tree so that the tip was located at the most recent common ancestor of the sister pair rather than
47 at the present (i.e., the “sister node” became the tip). Consequently, the resulting tree was no
48 longer ultrametric. After constructing this tree, we then fit full and reduced Michaelis-Menten
49 models as described in the main text (with genetic distance and clade as predictor variables in the
50 reduced model and genetic distance, clade, and recent speciation rate as predictor variables in the
51 full model). We modeled variances as by the diagonal of the variance-covariance matrix using
52 the “weights” option in the model formula, and specified a Brownian correlation structure using
53 the “correlation” option in the model formula. The results of this model were almost identical to
54 the results we previously reported. The full model was not a better fit to the data than the reduced
55 model ($p = 0.36$) and, in the full model, recent speciation rate was unrelated to song
56 discrimination (parameter estimate of recent speciation rate = $0.0038 \pm 0.0053, t = 0.72, p =$
57 0.47). This provides further confidence that the results we report in the main text are robust to the
58 inclusion of phylogenetic information in our models.

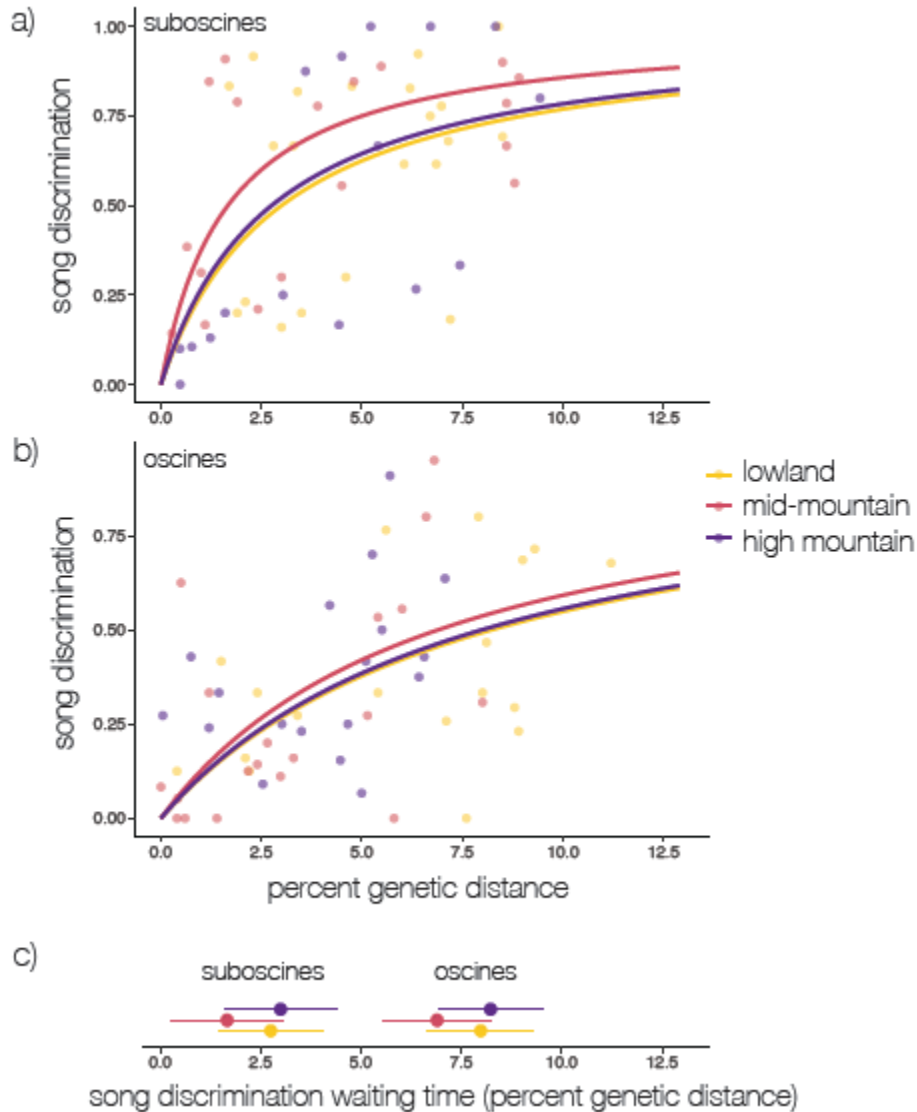
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- 60 Harvey M. G. & Rabosky, D. L. 2018. Continuous traits and speciation rates: Alternatives to
61 state-dependent diversification models. *Methods in Ecology and Evolution* **9**, 984-993.
- 62 Harvey, M. G. et al. 2020 The evolution of a tropical biodiversity hotspot. *Science* **370**, 1343–
63 1348.
- 64 Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K. & Mooers, A. O. 2012 The global diversity of
65 birds in space and time. *Nature* **491**, 444–448.



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67 Figure S1. Song discrimination was reciprocal within taxon pairs for both oscines and
 68 suboscines. The dashed line shows the 1:1 line where song discrimination was perfectly
 69 correlated within taxon pairs; the correlation is $r = 0.90$ ($p \ll 0.0001$). Song discrimination in
 70 our analysis is calculated as the proportion of territories that ignore allopatric song. Data comes
 71 from 36 taxon pairs for which we conducted five or more experiments for both populations.



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74 Figure S2. Evolutionary rates of song discrimination are similar between elevational zones

75 within the tropics. A full model that included elevational zone was not a better fit compared to a

76 reduced model that did not include elevational zone (F test; $df = 1$, $F = 1.29$, $p = 0.28$).

77 Predictions from the full Michaelis-Menten model are plotted as trendlines for suboscines and

78 oscines (panel a and b); raw data is shown as points. Song discrimination waiting times were

79 similar for lowland, mid-mountain and high mountain taxon pairs (panel c).

80 Table S1. Details for 16 taxon pairs included in this study that have not been studied in their parapatric contact zone. We use our data
 81 measuring song discrimination in allopatry to predict whether populations regularly interbreed with each other or not in this contact
 82 zone. Locations where playback experiments took place are given in parentheses. For further details on these comparisons please see
 83 Dataset A.

Population 1	Population 2	Contact zone	Prediction
Xiphorhynchus erythrogygius aequatorialis (Costa Rica)	Xiphorhynchus erythrogygius punctigula (Ecuador)	western Colombia	do not regularly interbreed
Formicarius analis hoffmanni (Costa Rica)	Formicarius analis monoliger	Honduras	do not regularly interbreed
Hafferia zeledoni zeledoni (Costa Rica)	Hafferia immaculata	north-central Colombia	do not regularly interbreed
Myrmeciza exsul occidentalis (Costa Rica)	Myrmeciza exsul maculifer (Ecuador)	western Colombia	regularly interbreed
Leptopogon superciliaris transandinus (Ecuador)	Leptopogon superciliaris superciliaris (Ecuador)	central Colombia	do not regularly interbreed
Hylophilus decurtatus decurtatus (Costa Rica)	Hylophilus decurtatus minor (Ecuador)	western Colombia	regularly interbreed
Vireo bellii arizonae (SE Arizona)	Vireo bellii bellii	southwest USA	regularly interbreed
Vireo gilvus swainsoni group (Pacific NW)	Vireo gilvus gilvus group	central USA/Canada	regularly interbreed
Toxostoma curvirostre oberholseri (Texas)	Toxostoma curvirostre palmeri (SE Arizona)	northern Mexico	regularly interbreed
Cantorchilus modestus (Costa Rica)	Cantorchilus elutus	Pacific slope Costa Rica	regularly interbreed
Microcerculus marginatus luscinioides (Costa Rica)	Microcerculus marginatus occidentalis (Ecuador)	western Colombia	do not regularly interbreed
Cardellina pusilla chryseola (Pacific NW)	Cardellina pusilla pusilla	central Canada	regularly interbreed
Geothlypis trichas occidentalis group (Pacific NW)	Geothlypis trichas trichas	central North America	regularly interbreed
Melospiza aberti (SE Arizona)	Melospiza crissalis	southwest USA	regularly interbreed
Arremon aurantirostris rufidorsalis (Caribbean slope Costa Rica)	Arremon aurantirostris aurantirostris (Pacific slope Costa Rica)	central Panama	do not regularly interbreed
Saltator maximus maximus (Ecuador)	Saltator maximus intermedius	western Colombia	regularly interbreed

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