

The Accumulation of Genomic Barriers to Gene Flow in the Origin of Animal Species

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12 **Abstract**

13 The accumulation of genomic barriers to gene flow underpins speciation, thus its
14 trajectory fundamentally reflects speciation rate. Three trajectory models are: (1) the Beanbag
15 Effect Model predicts linear; (2) the Snowball Effect Model predicts exponential, whereas (3)
16 the Slowdown Effect Model predicts a logarithmic buildup of barriers to gene flow with the
17 genomic divergence between the parental lineages. Testing the three models in natural
18 populations ultimately explain speciation rate heterogeneity during the evolution of
19 reproductive isolation. Here, we established a global hybrid zone database, Hybrid Zone
20 Explorer, with 315 animal hybrid zones reported to date. This database empowered a rare
21 opportunity to directly infer in situ speciation with gene flow and test the three speciation rate
22 models. The Snowball Effect Model (2) is best supported with the full dataset. We further
23 refined the three models to consider independent and interactive effects of mitochondrial
24 (mtDNA) and nuclear genetic (nDNA) divergence on the selection against hybrids. The
25 Snowball Effect Model (2) was again best supported by the data, revealing positive roles of
26 independent mitonuclear genetic divergence. However, the interaction between mtDNA and
27 nDNA divergence negatively contributed to barriers to gene flow, revealing introgressive
28 mitonuclear discordance. Collectively, this data synthesis of animal speciation in situ reveals
29 an accelerated growth of barriers to gene flow with mitonuclear genetic divergence. This
30 speciation momentum profoundly illuminates the genomic principle shaping speciation rate
31 heterogeneity underlying animal biodiversity on our planet.

32

33 **Keywords:** Speciation rate, Hybrid zone, Snowball Effect, Genetic Incompatibility, Speciation,
34 Hybridization, Cline.

35

36 **Introduction**

37 The evolution of reproductive isolation is underpinned by genetic incompatibilities, which
38 accrue as the diverging lineages accumulate lineage-specific substitutions (Dobzhansky 1934;
39 Mayr 1959 & 1963; Orr 1995). The increased genetic incompatibilities contribute to the
40 selection against hybrids (Palopoli and Wu 1994; Orr 1995; Welch 2004), which attenuate
41 gene flow between diverging lineages, allowing speciation to unfold (Gavrilets 2004; Nosil
42 2008; Westram et al. 2022). The rate at which lineage-specific substitutions drive the selection
43 against hybrids ultimately prescribes the speciation rate (Coyne and Orr 1989).

44 The debates over this relationship have inspired decades of investigation of genomic
45 architecture and mechanisms of speciation (Mayr 1959 & 1963; Feder et al. 2014; Nosil and
46 Schluter 2011; Seehausen et al. 2014;). If divergent substitutions independently contribute to
47 speciation in the face of gene flow as a 'Beanbag' genetic process (Fisher, 1941; Haldane
48 1964), the genetic distance between the diverging lineages should predict linear growth of
49 genetic barriers to gene flow. However, this 'Beanbag Genetic Model' was coined and
50 criticized by Mayr (1959) as being oversimplified for omitting epistatic effects among co-
51 adaptive genes. If two-loci epistatic incompatibilities, or Dobzhansky & Muller Incompatibilities
52 (DMIs) (Dobzhansky 1934; Muller 1942; Mayr 1963), predominate barriers to gene flow, the
53 selection against hybrids would increase exponentially as genetic distance increases,
54 reflecting a 'Snowball Effect' (Orr 1995). A greater acceleration in speciation rate would be
55 observed if the genetic distance results in abundant higher-order incompatibilities (Orr 1995).
56 In contrast, an early 'slowdown' might be observed if premating isolation is established prior to
57 substantial genetic incompatibilities (Gourbiere and Mallet 2010).

58 Several taxon-specific empirical studies have supported the Snowball Effect hypothesis,
59 where the increase of genetic distance between parental lineages exponentially suppresses
60 hybrid fitness (Presgraves 2002; Matute et al. 2010; Moyle and Nakazato 2010). However,
61 others reported a more gradual, linear accumulation of selection against hybrids in response to
62 genetic divergence (Lijtmaer et al. 2003; Gourbiere and Mallet 2010; Stelkens et al. 2010). A
63 logarithmic relationship was also observed, reflecting a Slowdown Effect (Gourbiere and Mallet
64 2010). Collectively, the taxon-specific patterns concur with the heterogeneous speciation rates
65 across the tree of life that await large-scale comparative studies to identify evolutionary
66 principles underlying the regularities about speciation rates.

67 The heterogeneous patterns of speciation rate are likely attributed to the variability of
68 locus-specific effect sizes and the probability of incompatibility in different parts of the genome.
69 For instance, the smaller effective population size in the mitochondrial genome than the
70 nuclear genome predicted greater drift, counteracting selection (Slatkin and Maruyama 1975).
71 In addition, mitochondrial deleterious mutations reportedly exhibit a greater average effect size
72 than nuclear mutations (Popadin et al. 2013). Moreover, the probability of incompatibility can
73 differ between intergenomic and intragenomic discordance.

74 More broadly, the heterogeneous patterns of selection against hybrids could also be
75 explained by the stages of speciation along the speciation continuum (Roux et al. 2016). In the
76 early stages of divergence, large-effect single-locus genetic barriers are more effective than
77 DMIs to overcome extensive gene flow (Felsenstein 1981; Wu 2001; Nosil and Schluter 2011;
78 Wang et al. 2020; de Zwaan et al. 2022). If the effect sizes of single-locus barriers were
79 constant through time, the selection against hybrids should linearly increase as lineage-

80 specific substitutions accumulate over time (Fig. 1, Beanbag Effect). If the effect sizes were
81 larger earlier (Fisher 1930) in speciation, when the lineage-specific substitutions were scarce
82 and exhibiting diminishing returns in response to further increments of genetic divergence, a
83 Slowdown Effect in the form of a logarithmic function is expected (Gourbiere and Mallet 2010)
84 (Fig. 1, Slowdown Effect). As the gene flow between diverging lineages attenuates, allowing
85 DMIs to build up, a Snowball Effect can be observed (Orr 1995) (Fig. 1, Snowball Effect),
86 where the selection against hybrids grows exponentially as lineage-specific substitutions
87 accrue. The heterogeneous tension between genetic incompatibility and gene flow across the
88 speciation continuum is naturally encapsulated in decades of hybrid zone studies (Fig. 2).

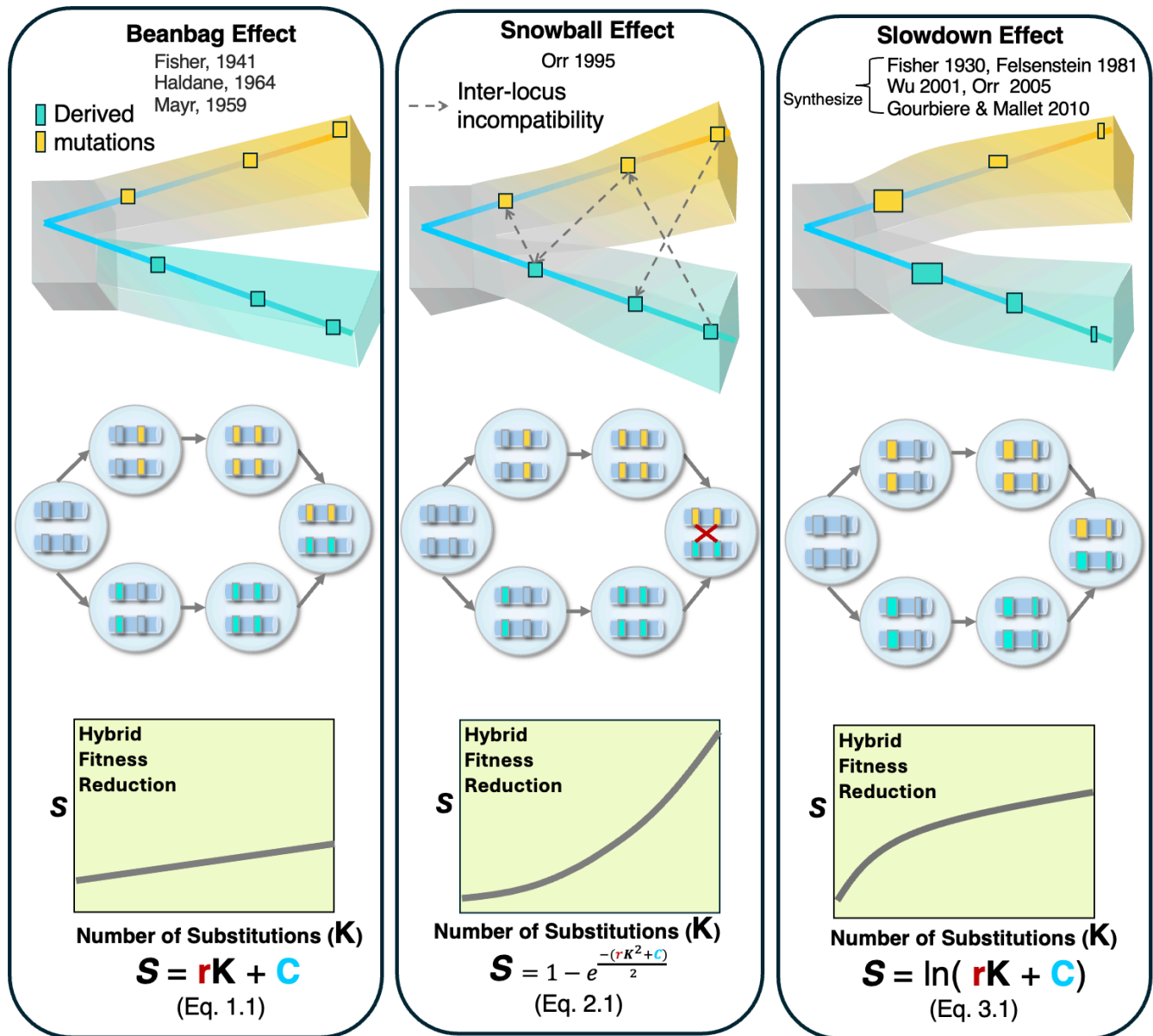
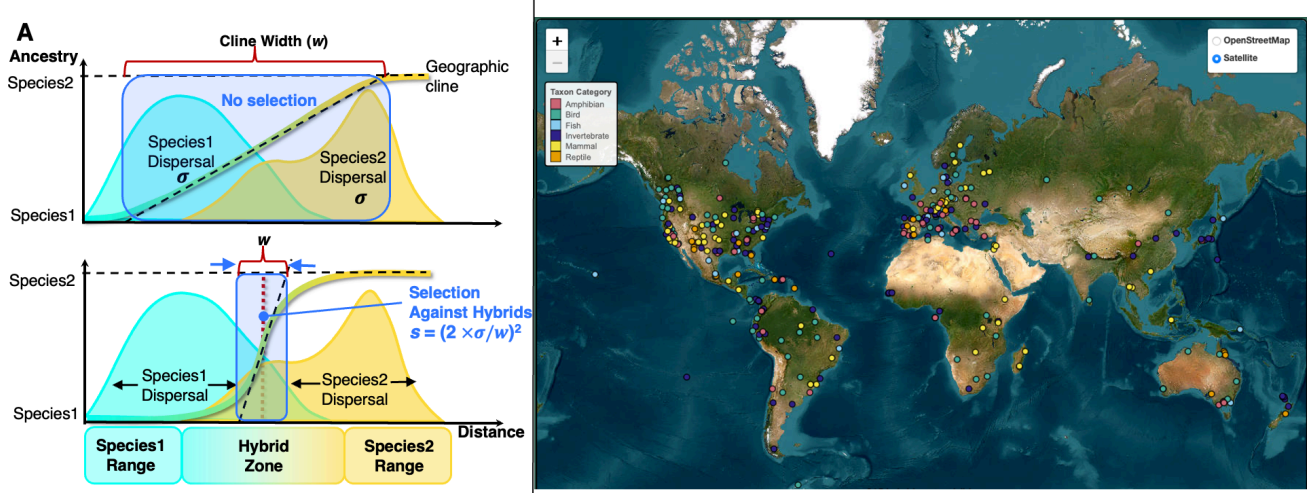


Fig. 1 Three speciation models: (1) Beanbag Effect, (2) Snowball Effect, and (3) Slowdown Effect.

Hybrid zones are geographic areas where diverging lineages hybridize (Barton 1979).

They provide unique opportunities to observe the progression of speciation as the tension between genetic incompatibility and gene flow is resolved (Barton and Hewitt 1985). This tension is effectively theorized as the tension zone model, where the width of the cline (w) is a balance of selection (s) and dispersal (σ) (Fig. 2A) (Barton 1979). The selection (s) is a

98 composite expression of genetic incompatibility and divergent selection, or reproductive
 99 isolation in the face of gene flow. The rate at which s increases with the extension of genetic
 100 distance between diverging lineages ultimately translates to the speciation rate (Fig. 2B).



101
 102 **Fig.2 Hybrid zones are natural laboratories for understanding speciation. A**, The cline
 103 width (w) of a hybrid zone is a balance of gene flow (σ) and selection (s) against hybrids. In the
 104 absence of s , σ will widen w over time as species 1 hybridizes with species 2 (top). Bottom:
 105 strong selection against hybrids counteracts with σ and shrinks w . **B**, World distribution of
 106 animal hybrid zones represented in the Hybrid Zone Explorer database. Over 315 animal
 107 hybrid zones have been reported. Color categories correspond to six different organismal
 108 groups: mammals in yellow (59), birds in green (69), reptiles in orange (25), amphibians in
 109 maroon (45), invertebrates in purple (94), and fish in blue (23).

110
 111
 112 Here, we conduct a data synthesis to examine the buildup rate of selection against
 113 hybrids (s) in animal hybrid zones (Fig. 2). We tested three alternative models that predict the
 114 selection against hybrids with the genetic distance between parental lineages. Model 1
 115 predicts a linear increase of s as genetic distance increases (Fig. 1, Beanbag Effect); Model 2
 116 predicts an exponential growth of s (Fig. 1, Snowball Effect); while Model 3 predicts a
 117 logarithmic relationship (Fig. 1, Slowdown Effect).

118 We further developed three refined models to dissect mitochondrial and nuclear genetic
 119 effects on speciation. Since the mitochondrial and nuclear genomes have distinct substitution

rates (Ballard and Whitlock 2003) and coevolve to maintain critical eukaryotic energetics (Eddo et al. 2025), ‘Endosymbiotic Snowball Effect’ was articulated (Wang et al. 2025). To test ‘Endosymbiotic Snowball Effect’, we further partitioned the genetic distance into mtDNA and nDNA distance to understand their independent and interactive effects on speciation. Together, this study will inform the mode of speciation in the origin of animal biodiversity.

Method

Hybrid zones

We studied 315 animal hybrid zones reported to date (McEntee et al. 2020; Nieto Feliner et al. 2023) and categorized them into six taxonomic groups: amphibians, birds, fishes, invertebrates, reptiles, and mammals (Fig. 2B). We filtered the data with the following criteria: (1) there has been sufficient spatial sampling to fit the genetic and/or phenotypic clines across the hybrid zone; (2) there have been direct estimations of cline widths with genetic markers; (3) there is sufficient information on dispersal distance in the unit of Kilometer/generation or Kilometer/generation^{0.5}; (4) for the dispersal distance reported in Kilometer/generation, there is information on generation time to calculate the Kilometer/generation^{0.5}. These criteria resulted in 152 animal hybrid zones for model testing (see below).

Mitonuclear genetic distance

To infer the genetic distance between hybridizing species, we calculated the genetic distance of the most sequenced mitochondrial and nuclear genes in animals. We surveyed a broad panel of markers, including mitochondrial genes (Cytb, ND2, COI, ND3, ND4, Msh1), nuclear protein-coding genes (RAG1, RAG2, MC1R, BDNF, ODC1, MHCII, ASIP, ACTB, IRBP, ENC1, TYR, EF1A, GAPDH, Wnt1, H3, H4, FGB), cytoribosomal genes (5S, 18S, 28S, ITS1, ITS2) and mitoribosomal genes (12S, 16S). We fetched DNA sequences of the target

144 genes for each parental species from GenBank and only kept the genes if both species had at
145 least three sequences per species. To ensure even sampling depths among the species pairs
146 in our meta-analysis, for species with more than 15 sequence records of a gene, we randomly
147 sampled 15 sequences among all the records. We then aligned all the sequences of each
148 gene in each species pair with the msa function in the msa package (Bodenhofer et al. 2015)
149 in R (R Core Team 2023). Specifically, we used MUSCLE (Edgar 2004) with gapOpening = 20
150 and gapExtension = 10. With the alignment, we calculated the mean pairwise distance
151 between parental species with dist.dna function with the TN93 substitution rate model in the
152 ape package (Paradis and Schliep 2019). Then, we averaged the between-species distance of
153 all the mitochondrial genes and nuclear genes separately to result in the mean pairwise
154 mtDNA distance ($\overline{K_m}$) and the mean pairwise nDNA distance ($\overline{K_n}$) between each pair of
155 parental species. Finally, we calculated the mean pairwise genetic distance ($\overline{K}$) between each
156 pair of parental species by averaging the nDNA and mtDNA distances. To account for the
157 uncertainty in $\overline{K}$ estimations, we calculated the standard deviation of $\overline{K_m}$, $\overline{K_n}$, and $\overline{K}$. Among the
158 152 hybrid zones, 99 hybrid zones had sufficient genetic information to calculate the genetic
159 distance between parental species. To evaluate the reliability of our genic estimation of
160 parental species divergence, we computed the ratio of between-species distance over the sum
161 of between- and within-species distance and conducted correlation tests of F_{ST} estimates for
162 several pairs of species reported in the literature (see supplementary data).

163 *Selection against hybrids*

164 We extracted the dispersal, center, and width of hybrid zones from McEntee et al.
165 (2020), Nieto Feliner et al. (2023), and additional literature (supplementary data). We unified

the unit of dispersal rate as kilometers (KM) $/\sqrt{generation}$ with the average generation time information of each species pair. To account for sex-specific dispersal, we conducted another round of literature search for female vs male dispersal rate in each species pair (supplementary information). We calculated the average cline width (w) and center for mtDNA and nDNA data, as well as phenotypic data separately. We calculated selection (s) against heterozygotes as a function of cline width (w) and dispersal (σ): $s = (2 \times \frac{\sigma}{w})^2$ (Fig. 1) (Barton and Gale 1993). To infer the cumulative selection against hybrids, we substituted w with averaged cline width ($\bar{w}$) among mtDNA and nDNA clines (Fig. 3B). To account for uncertainty of selection estimates due to heterogeneous dispersal rates and cline width estimates, we monitored sex-specific dispersal, and the variance of cline width estimates for each hybrid zone. For hybrid zones with known sex-specific dispersal rates, we calculated selection on mtDNA and nDNA, respectively, as $s_{mt} = (2 \times \frac{\sigma(female)}{w})^2$ and $s_{nt} = (2 \times \frac{\sigma(average)}{w})^2$. We derived the standard deviation of s , $SD(s)$, as $(8\sigma^2 / w^3) SD(w)$.

Speciation models

To investigate the relationship between genetic distance and strength of selection against hybrids (s) in the hybrid zones, we test three conventional hybrid incompatibility models: (1) Linear Effect (Eq. 1.1), (2) Snowball Effect (Eq. 2.1; Fig. S1), and (3) Slowdown Effect (Eq. 3.1). In each model, selection (s) is a function of genetic distance (K) with a rate parameter r and a constant parameter C as divergent plasticity, epigenetic divergence, or genetic assimilation (Pigliucci and Murren 2003; Ehrenreich and Pfennig 2016; Mueller et al. 2025). The rate parameter corresponds to the average effect size of each pair of incompatibilities.

$$s = rK + C \quad (\text{Eq. 1.1})$$

$$s = 1 - e^{-(rK^2 + C)} \quad (\text{Eq. 2.1})$$

$$s = \ln(rK + C) \quad (\text{Eq. 3.1})$$

To account for the effects of mtDNA and nuclear genetic distance between the parental species (Fig. 3), we further refined the conventional expressions (Eq. 1.1, 2.1, and 3.1) into mitonuclear models (Eq. 1.2, 2.2, and 3.2) to partition K into K_m (mtDNA distance) and K_n (nuclear DNA distance) that correspond to rate parameters r_m and r_n , respectively, besides mitonuclear interaction r_{mn} , and a epigenomic constant of C .

$$s = r_m K_m + r_n K_n + r_{mn} K_m K_n + C \quad (\text{Eq. 1.2})$$

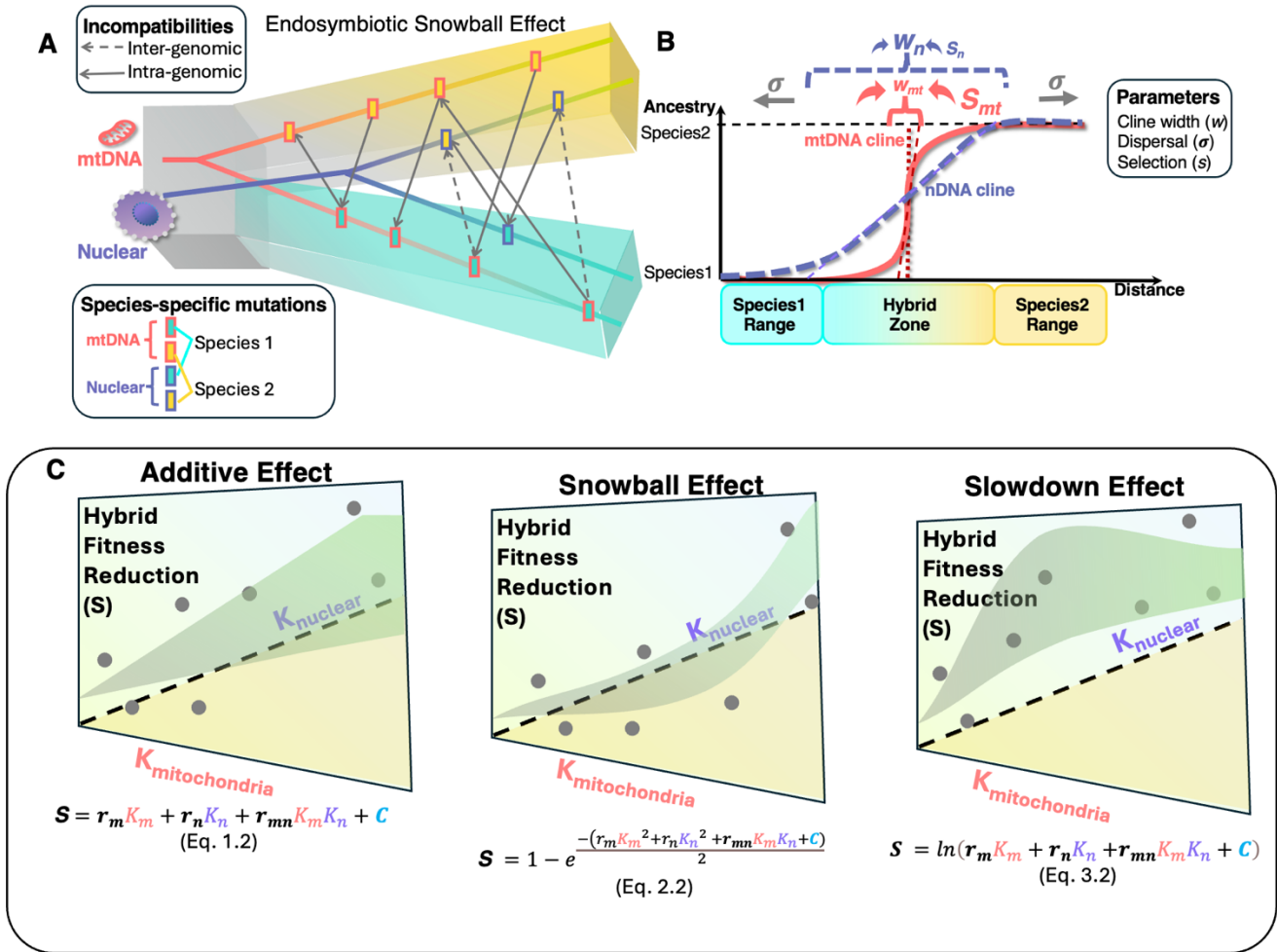
$$s = 1 - e^{-(r_m K_m^2 + r_n K_n^2 + r_{mn} K_m K_n + C)} \quad (\text{Eq. 2.2})$$

$$s = \ln(r_m K_m + r_n K_n + r_{mn} K_m K_n + C) \quad (\text{Eq. 3.2})$$

We conducted model fitting of the above three models with the nls2 function of nls2 package (Grothendieck, 2007) in R (R Core Team 2023). With the default algorithm and starting value of $r = 0.01$, $C = 0.1$, model fitting was conducted. The AIC of each fitted model is obtained with the AIC function. In each conventional model fitting (Eq. 1.1, 2.1, 3.1), we estimated the rate parameter r and constant C with selection against hybrid incompatibility (s) in response to mean pairwise genetic distance ($\bar{K}$) between the parental species. To evaluate independent effects of mtDNA and nDNA distance, we tested Eq. 1.1, 2.1, and 3.1 with K being $\bar{K}_m$ and $\bar{K}_n$.

To evaluate taxon-specific patterns, we conducted conventional model fitting (1.1, 2.1, 3.1) within each taxonomic group (Amphibians, Birds, Invertebrates, Mammals, and Reptiles), except for Fishes, due to data insufficiency. Furthermore, we estimated r_m , r_n , and C in the

213 refined model fitting (1.2, 2.2, 3.2). Only 38 hybrid zones had sufficient mtDNA and nDNA
 214 sequence data to participate in refined model fitting, thus further taxon-specific modeling fitting
 215 was omitted.



216
 217 **Fig. 3 Mitonuclear genetic divergence underlying the selection against hybrids. A.**
 218 Endosymbiotic Snowball Effect (Wang et al. 2025): Lineage-specific substitutions in the
 219 mitochondrial genome (red) and nuclear genome (purple) can lead to intragenomic and
 220 intergenomic Dobzhansky-Muller Incompatibilities, which emergently contribute to selection
 221 against hybrids. **B.** This selection counteracts gene flow (σ) across hybrid zones and reduces
 222 the cline width (w). Greater substitution rates in the mitochondrial genome than the nuclear
 223 genome can result in a greater rate of incompatibility and thus stronger selection on mtDNA
 224 clines than the nDNA clines. **C.** Refined speciation models with independent and interactive
 225 mitonuclear effects: (1) Linear (Eq. 1.2), Snowball (Eq. 2.2), Slowdown (Eq. 3.2).
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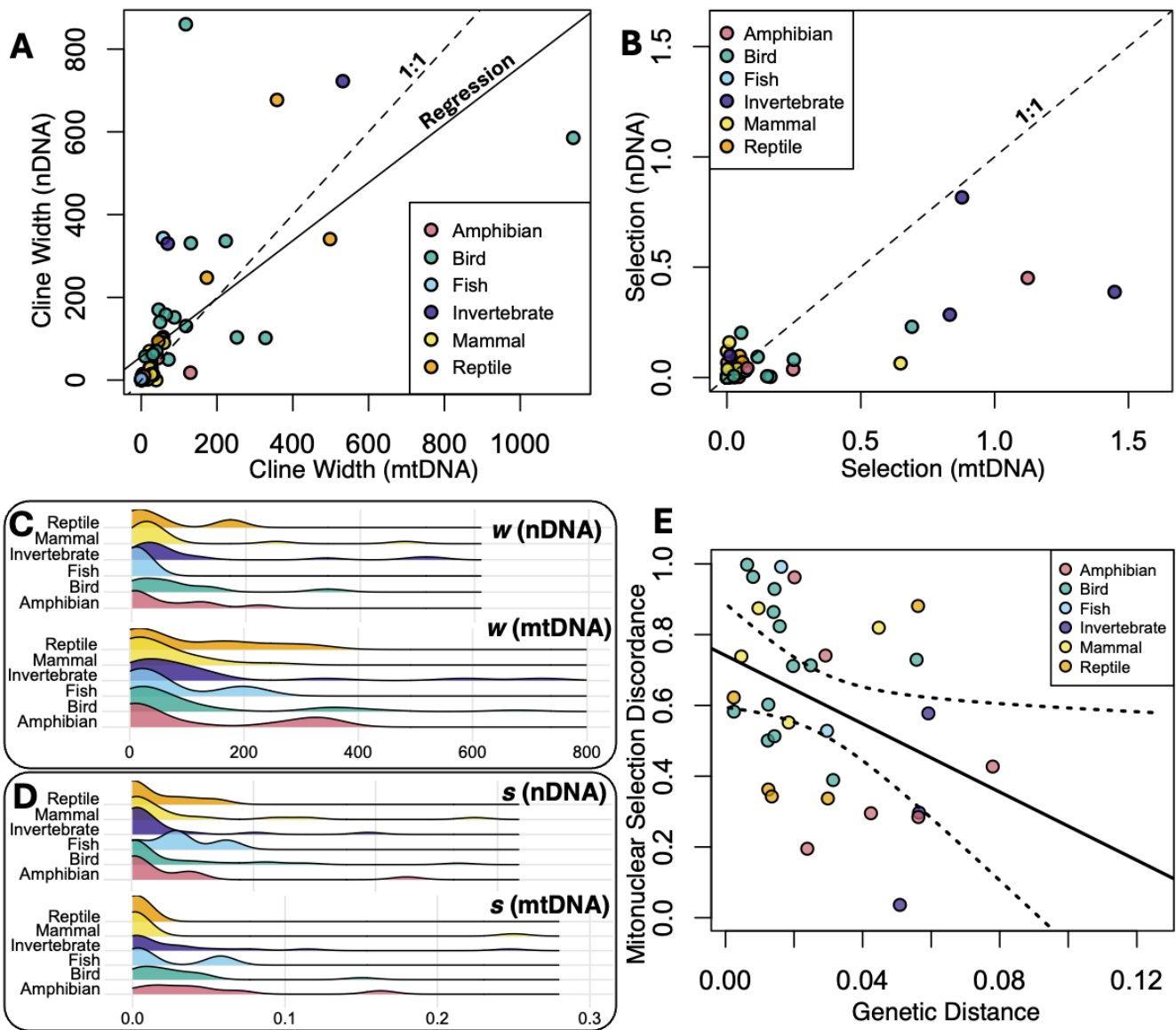
227 To test the significance of observed regression coefficients, we conducted 1,000
 228 bootstrap iterations to generate a bootstrap distribution of regression coefficients $\hat{r}$ and $\hat{C}$. In
 229 each bootstrap iteration, we randomly sampled $N = 152$ hybrid zones with replacement from
 230 the full dataset. For each bootstrap sample of $K^* = [K_1^*, K_2^*, \dots, K_i^*, \dots, K_{152}^*]$ and $s^* = [s_1^*, s_2^*, \dots,$
 231 $s_i^*, \dots, s_{152}^*]$, we simultaneously fitted the sample to (1) Linear, (2) Snowball, and (3) Slowdown
 232 Effect Models (Fig.1; Eq. 1.1, 2.1, & 3.1) to calculate $\hat{r}$ and $\hat{C}$. To account for the uncertainty in
 233 K and s , we randomly sampled K_i^* and s_i^* respectively from normal distributions: $N(K_i, sd(K_i))$
 234 and $N(s_i, sd(s_i))$. To generate a null distribution of $\hat{r}$ and $\hat{C}$, we randomly shuffled and
 235 mismatched K^* and s^* in each bootstrap sample and simultaneously fitted the three models
 236 (Eq. 1.1, 2.1, & 3.1) again. We used paired-T tests to compare the observed distribution of r
 237 and C , r_{Obs} and C_{Obs} , to the corresponding null distributions, r_{H0} and C_{H0} .

238 A similar bootstrapping test was applied to the refined models (Fig. 3C; Eq. 1.2, 2.2, &
 239 3.2), where (1) Linear, (2) Snowball, (3) Slowdown Models were compared in terms of AICs,
 240 and the observed bootstrap distribution of $\widehat{r_m}$, $\widehat{r_n}$, $\widehat{r_{mn}}$, and $\hat{C}$, r_{m_Obs} , r_{n_Obs} , r_{mn_Obs} and
 241 C_{Obs} , was respectively compared to r_{m_H0} , r_{n_H0} , r_{mn_H0} , and C_{H0} .

242 **Results**

243 *Discordant mitonuclear selection*

244 The mtDNA and nDNA cline widths are significantly correlated ($R^2 = 0.44$, $p < 10^{-7}$).
245 However, the selections against hybrids on mtDNA and nDNA are not significantly correlated
246 ($R^2 = 0.02$, $p > 0.05$). The discrepancy of selection on mtDNA and nDNA clines significantly
247 decreases with greater genetic distance between the parental species ($R^2 = 0.14$, $p = 0.03$).



248 **Fig. 4 Mitonuclear selection in animal hybrid zones.** A, the cline widths of mitochondrial
249 and nuclear DNA were significantly correlated. The two variables are significantly correlated (r
250

251 = 0.44, $p < 10^{-7}$). **B**, Stronger selection on mitochondrial clines than the nuclear DNA clines. **C**-
252 **D**, The distribution of nuclear (nDNA) and mitochondrial (mtDNA) genetic cline widths (**C**) and
253 selection on the clines (**D**) across animal taxa. **E**, the discordance between selections on
254 mtDNA and nDNA significantly decreases with genetic distance ($p < 0.05$).
255

256 *Speciation models*

257 The Snowball Effect Model (3.2) (Fig. 1, 5A) has the lowest AIC when fitted to the full
258 data, but it is only marginally superior with ΔAIC being 0.36. However, the Snowball Effect
259 Model showed stronger superiority within amphibians ($\Delta AIC = 1.15$), mammals ($\Delta AIC = 3.46$),
260 and reptiles ($\Delta AIC = 2$) than the alternative model with the second lowest AIC (Table 1).
261 However, the three models were about equal ($\Delta AIC = 0.05$) for birds with the Beanbag Effect
262 Model retaining the lowest AIC. When evaluating the effect of mitochondrial and nuclear
263 genomic divergence separately, the Snowball Effect was strongly supported ($\Delta AIC = 4.21$) for
264 the nuclear genomic divergence (Table S1), but not mitochondrial genomic divergence (Table
265 S2).

266 The bootstrap mean of r_{Obs} in the Snowball Effect model (Eq. 3.2) was 7.38 (Fig.
267 5B), which was significantly different from r_{H0} (mean difference: 4.79, 95% CI of the
268 difference: 0.69 to 10.58, $p < 10^{-15}$), indicating a significantly positive effect of genetic
269 divergence on selection against hybrids. However, the estimates of r were heterogeneous
270 among taxonomic groups (Table 1), with birds ($r = 15.39$) being the highest, then mammals (r
271 = 10.84), amphibians ($r = 8.89$) and invertebrates ($r = 8.69$) intermediate, and lower for reptiles
272 ($r = 0.74$) (Table 1).

Table 1 The effect of genetic divergence on the selection against hybrids among animal taxa. Standard errors (SE) of the regression coefficients for the rate parameter(r) and constant (C). The models (Fig. 1, Eq 1.1-3.1) with the lowest AICs are in bold. The Snowball model (equation 3.1) has the lowest AIC fitted to the full dataset.

Taxa	Models	r	SE (r)	C	SE (C)	AIC	Δ AIC
Altogether	Beanbag	6.452	1.995	-0.006	0.062	40.063	0.36
	Snowball	11.376	2.021	0.051	0.054	39.706	
	Slowdown	7.699	2.585	0.985	0.068	40.330	
Amphibian	Beanbag	5.497	2.998	-0.114	0.140	0.655	1.16
	Snowball	8.890	2.317	-0.047	0.098	-0.500	
	Slowdown	5.636	3.485	0.895	0.145	1.036	
Bird	Beanbag	10.002	4.828	-0.024	0.149	27.597	0.05
	Snowball	15.394	4.828	0.058	0.134	27.649	
	Slowdown	12.972	6.935	0.953	0.167	27.705	
Invertebrate	Beanbag	5.271	2.112	-0.024	0.097	3.660	0.40
	Snowball	8.685	2.066	0.022	0.087	3.265	
	Slowdown	6.343	2.892	0.970	0.103	3.915	
Mammal	Beanbag	6.060	2.224	-0.113	0.078	-11.164	3.46
	Snowball	10.842	1.667	-0.062	0.050	-14.625	
	Slowdown	5.957	2.462	0.898	0.080	-10.417	
Reptile	Beanbag	0.047	0.028	0.001	0.001	-60.764	2.01
	Snowball	0.739	0.158	0.001	0.001	-62.765	
	Slowdown	0.047	0.028	1.001	0.001	-60.760	

When considering mitonuclear genetic divergence independently and interactively (Fig. 3), the Snowball Effect model (Eq. 2.2) has the lowest AIC (Table 2; Fig. 5 C-D). Remarkably, the Δ AIC between the Snowball Effect model and the next model was 8.8. We observed a significantly positive effect of nuclear DNA distance and mDNA distance independently, but a negative interactive effect of mitonuclear genetic distance (Fig. 5C) on the selection against hybrid DNA. Specifically, the nuclear genetic divergence effect (r_n) was large (Table 2). In the Snowball Effect Model, bootstrap mean of r_n was significantly different from r_{n_H0} (mean difference: 667.01, 95%CI of the difference: 620.15 to 713.87 $p < 10^{-15}$) (Fig. 5D). In

286 comparison, the estimated mitochondrial genetic divergence effect (r_m) was much smaller
 287 (Table 2) but was significantly different than r_{m_H0} (mean difference: 77.12, 95%CI of the
 288 difference: 71.75 to 82.49; $p < 10^{-15}$). The regression coefficient of mitonuclear interactive
 289 effect (r_{mn}) was significantly negative, with the bootstrap mean significantly more negative than
 290 r_{mn_H0} (mean difference: -635.573, 95%CI of the difference: -671.84 to -599.31; $p < 10^{-15}$).

291

292 **Table 2** Regression coefficients and standard errors (SE) of the r_m , r_n , and C of the refined
 293 models. The Snowball Effect model has the lowest AIC.

Models	r_m	SE (r_m)	r_n	SE (r_n)	r_{mn}	SE (r_{mn})	C	SE (C)	AIC
Beanbag	6.10	2.15	19.65	4.718	-400.28	107.44	-0.09	0.08	8.18
Snowball	189.59	73.72	1214.69	610.01	-1096.37	439.58	0.14	0.107	-0.63
Slowdown	6.38	2.73	21.01	7.819	-417.79	139.83	0.92	0.10	11.82

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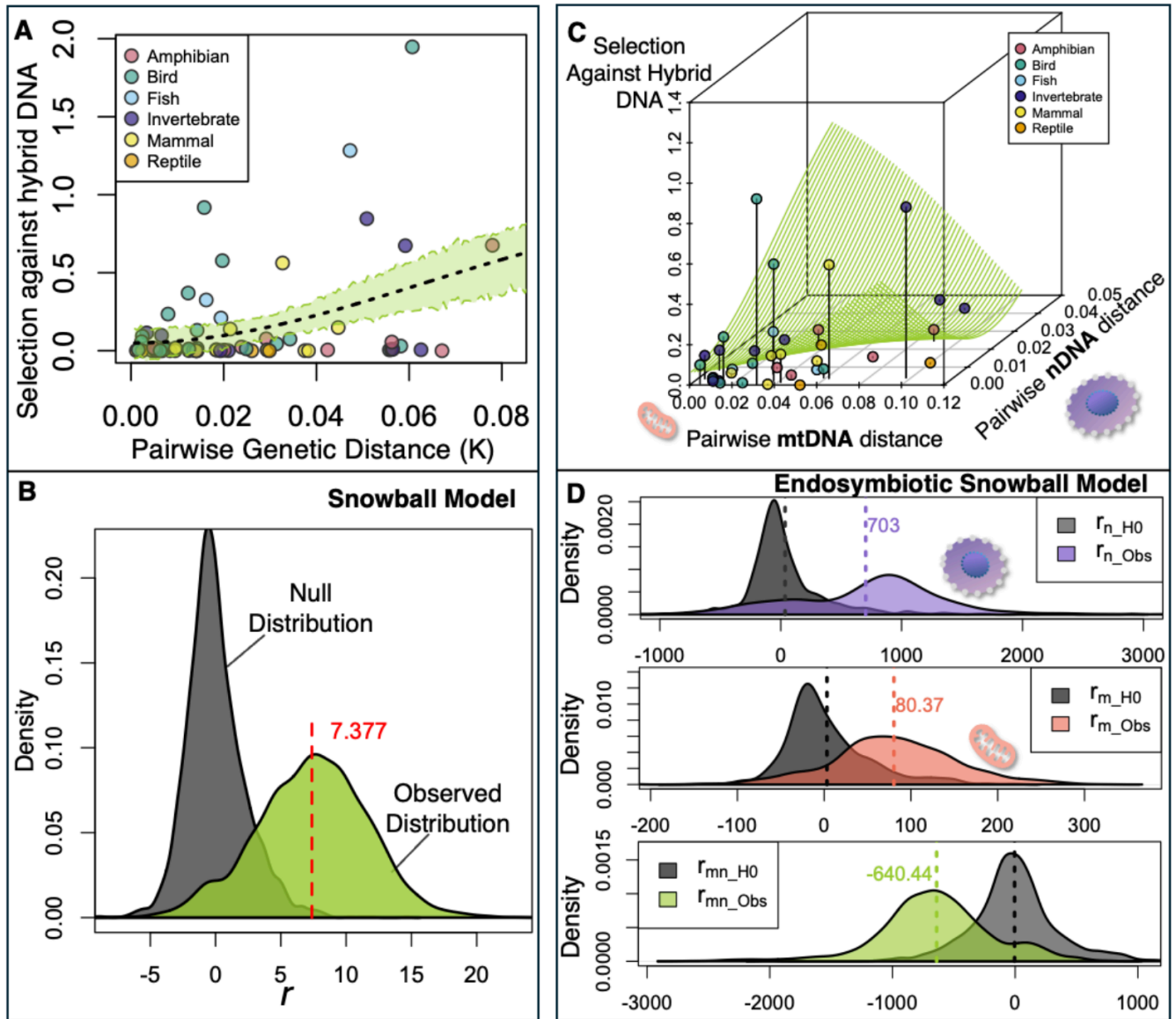


Fig. 5 Coefficients of estimates in the best-fitted models for conventional versus refined models of speciation. **A**, Conventional models (equations 1.1, 2.1, and 3.1) considered the total genetic distance as the predictor for selection against hybrids. The Snowball Effect model (dotted line) has the lowest AIC. **B**, Bootstrap distributions of the rate parameter r (equation 1.3) estimations based observed relationship between K and s across animal hybrid zones (blue) versus r estimates based on randomly mismatched K and s (grey). Estimates of r are based on the best-fitted conventional models. **C**, Refined models considered the effect of mtDNA and nDNA divergence and their interactions separately. The Snowball Effect model again has the lowest AIC. **D**, The bootstrap distributions of rate parameter estimations for r_m , r_n , and r_{mn} which correspond to the independent effects of mtDNA and nDNA distance as well as their interaction. Estimates of r_m , r_n , and r_{mn} are from the best-fitted refined model. **C-D**, vertical dotted lines represent bootstrap means.

308 Discussion

309 The accumulation of selection against hybrids in the hybrid zones between diverging
310 lineages is key to unraveling speciation rates *in situ*. Here, we leveraged decades of hybrid
311 zone studies to understand the evolution of reproductive isolation in the face of gene flow.
312 Overall, the selection against hybrids increased with genetic divergence between the parental
313 species, despite the heterogeneous speciation rates across taxa (Table 1, 2; Fig. 5).

314 The Snowball Effect Models (Equation 2.1 & 2.2) best fitted the full data (Fig. 5, Table 1-
315 2) among the conventional models (Equation 1.1-3.1, Fig. 1) as well as the refined models
316 (equation 1.2-3.2, Fig. 3). The taxon-specific results are largely concordant with the full data
317 supporting the Snowball Effect. Altogether, our results suggest that Dobzhansky & Muller
318 Incompatibilities (DMIs) (Dobzhansky 1934; Muller 1942; Mayr 1963) predominately contribute
319 to barriers to gene flow. This conclusion is consistent with previous empirical studies on fruit
320 flies and wild tomato (Presgraves 2002; Matute et al. 2010; Moyle and Nakazato 2010).
321 Despite the challenge to maintain epistatic co-adaptive variants during speciation with gene
322 flow (Felsenstein, 1981), epistasis can snowball barriers to gene flow across the genomes,
323 allowing speciation to rapidly proceed (Orr, 1995).

324 Specifically, estimate of rate parameter r was the highest in birds (Table 1) which
325 reflects strong role of genetic divergence in speciation with gene flow. High basal metabolic
326 rates in endotherms could require stronger selection on genetic incompatibility, especially
327 between the mitochondrial and nuclear genomes (Hill 2019). As endotherms exhibit faster
328 climatic niche shifts than ectotherms (Rolland et al. 2018), the underlying genetic divergence
329 between endothermic sister populations could disproportionately contribute to divergent

adaptation. Both the proximate and ultimate differences in endotherms vs ectotherms could lead to greater effect sizes of genetic divergence on the selection against hybrids.

The three speciation models (Fig. 1) are not necessarily mutually exclusive and could be parallel, sequential, or hierarchical in the genome evolution underlying speciation. In the early stage of speciation, the growth of reproductive isolation via a single or few large-effect substitutions (Nosil and Schluter 2011; Servedio et al. 2011), genetic assimilation (Waddington 1961), and/or cultural evolution (Lachlan and Servedio 2004) prior to extensive genetic divergence renders a Slowdown Effect (Gourbiere and Mallet 2010). Independent genetic/epigenetic effects could rapidly and synergistically couple with other locally adaptive loci to form DMIs (Dobzhansky 1934; Muller 1942; Mayr 1963) and/or genome-wide barriers to gene flow (Flaxman et al. 2013).

The initial independent reproductive isolation might experience organismal constraints and thus are heterogeneous among taxonomic groups. For example, vocal learning in songbirds might uniquely increase the likelihood of cultural evolution as premating isolation (Slabbekoorn and Smith 2002; Lachlan and Servedio 2004), though it can also disrupt reproductive isolation. Future combinatorial examination of the three models in different parts of the nuclear genome could further unveil the evolutionary genomic mechanism of speciation.

When we consider the mtDNA and nDNA divergence separately (Eq. 1.2, 2.2, 3.2), Snowball Effect model (Eq. 2.2) fits the data best (Fig. 1). In this model, nuclear and mitochondrial divergence showed large positive effects, while the mitonuclear interaction exhibited negative effects (Fig. 5C, Table 2). This result is concordant with the conventional model (Fig. 5A) in terms of the overall positive role of mtDNA or nDNA divergence on barriers

352 to gene flow. The interaction of mtDNA and nDNA distance showed a negative role for barriers
353 to gene flow (Fig. 5C). This pattern may seem inconsistent with the growing evidence that
354 mitonuclear incompatibility being important for early-stage speciation (Hill 2017; Wang et al.
355 2021; Burton 2022; Moran et al. 2024). Greater mutation rates of mtDNA than nDNA in animal
356 cells (Ballard and Whitlock 2003; Lynch et al. 2006) were expected to result in mitonuclear
357 ancestry discordance. While some of the mitonuclear ancestry discordance may contribute to
358 interspecific incompatibility (Orr 1995), some could be beneficial (Havird et al. 2019; Garde et
359 al. 2025).

360 We observed much smaller effect sizes of independent mitochondrial substitutions on
361 hybrid fitness (Fig. 5, r_m), which could be a result of strong mitochondrial purifying selection
362 and nuclear compensatory evolution. The roughly 5-fold greater effect sizes of mitochondrial
363 deleterious mutations than nuclear mutations (Popadin et al. 2013) incurs greater purifying
364 selection (Popadin et al. 2013) in the mitochondrial genome. Further compensatory evolution
365 of the nuclear genome (Princepe and De Aguiar 2024) could mask the initial independent
366 effects of mitochondrial substitutions. Indeed, we observed the narrower mtDNA cline than the
367 nDNA cline (Fig. 4) despite the smaller effective population size of mtDNA than nDNA
368 expected greater drift and a wider cline under neutrality (Slatkin and Maruyama 1975).

369 *Caveats*

370 The available 315 animal hybrid zone studies have been concentrated in the global
371 northwest (Fig. 2B, S2), with an underrepresentation of global biodiverse hotspots. Our
372 observation could be predominantly representing animal speciation patterns in western
373 temperate ecoregions (Weir & Schluter, 2007), which may not apply for tropical ecoregions as

374 eco-evolutionary dynamics can be intricate and complex in highly biodiverse ecosystems
375 (Burney & Brumfield, 2009). There are far fewer aquatic hybrid zones than terrestrial hybrid
376 zones (Fig. 2B), which may constrain our conclusion within the terrestrial domain. Life history
377 information, such as independently measured generation time and dispersal rate, was
378 unavailable for a large proportion of the hybrid zones, with only 152 of the 315 cases
379 containing sufficient information to calculate selection against hybrids. For the 152 hybrid
380 zones for which we could calculate selection, there were insufficient sequence data in
381 GenBank for commonly sequenced genes in the nuclear genomes of the species pairs. This
382 sequencing gap limits the inference from the refined models to a small sample size. Despite
383 the limited dataset, the significant mitonuclear effect on the selection against hybrid DNAs
384 supported the Endosymbiotic Snowball Effect (Wang et al. 2025). Future studies that broaden
385 the sequencing scope of hybrid zone studies could provide valuable modeling resolution to
386 illuminate speciation heterogeneity across the genomes.

387 The our metrics of selection against hybrids leveraged tension zone model (Barton and
388 Gale 1993) assuming the balance of selection and dispersal, which may not be generally
389 applicable. However, even without reaching selection and dispersal balance, the ratio of cline
390 width and dispersal rate still reflects the extent of barriers to gene flow across the nascent
391 species boundaries of naturally hybridizing taxa and thus remains the most direct population
392 metrics of speciation in situ. Since the understanding of organismal gene flow is crucial for
393 biodiversity science and conservation in the increasingly fragmented biosphere (Wang and
394 Yoder 2025), further developing direct and standardized metrics to monitor gene flow across
395 the species boundaries can be productive for speciation and conservation science.

396 *Conclusion*

397 We investigated the evolutionary mode of barriers to gene flow in the early stage of
398 speciation, empowered by decades of animal hybrid zone studies. We exponential growth of
399 selection against hybrid DNA with increased mitonuclear genetic divergence, consistent with
400 the Endosymbiotic Snowball Effect (Wang et al. 2025). Future broadening of taxonomic breadth
401 and dissection of the regularity and specificity of barriers to gene flow would be indispensable
402 for refining our understanding of speciation rates across the tree of life.

403
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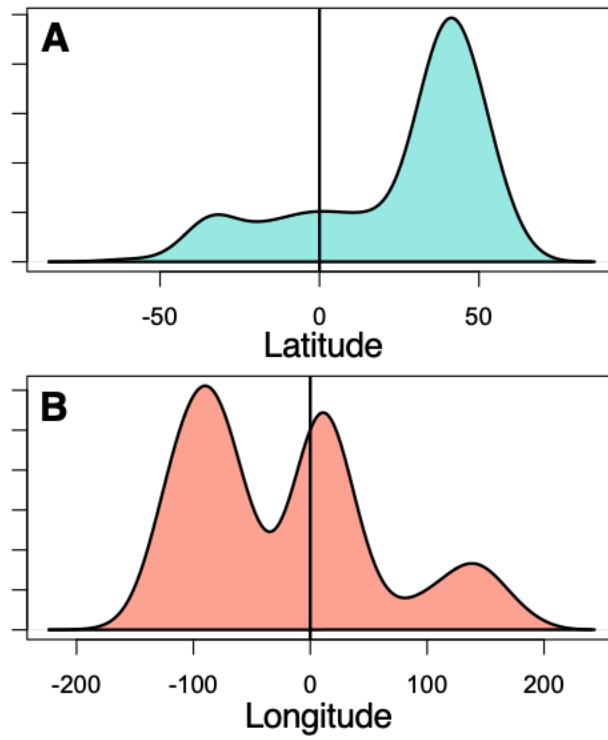
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547 **Data Accessibility**

548 All the data involved in the analysis is available in Hybrid Zone Explorer database.

549 <https://hzexplorer-production.up.railway.app/> (to be publicly available upon publication).

551
552 **Fig. S1. Modified Snowball Effect.** Orr (1995) derived the cumulative effect of K substitutions
553 with average effect size r on reduction of hybrid fitness, or the “strength of reproductive
554 isolation” as $1 - e^{-rK^2/2}$ (see Eq. 8 in Orr 1995). To incorporate epigenetic effects and be
555 directly comparable to Linear and Slowdown effects (Eq. 1.1 and 1.3), we modified the original
556 form and examined its form with different r values at moderate C .



557
558 **Fig. S2. Density distribution of the latitude and longitude of known hybrid zones.** The
559 known hybrid zones have been concentrated on the northern (A) and western (B) hemisphere.

560 **Table S1 The effect of nuclear genetic divergence on the selection against hybrids**
561 **among animal taxa.** Standard errors (SE) of the regression coefficients for the rate
562 parameter(r) and constant (C). The models (Fig. 1, Eq 1.1-3.1) with the lowest AICs are in
563 bold. The Snowball Effect Model (equation 3.1) has the lowest AIC fitted to the full dataset with
564 strong superiority ($\Delta AIC > 4$) than the second model. ΔAIC s (with >1 values bolded) represent
565 the absolute difference in AIC between the models with the lowest and second lowest AIC for
566 each taxonomic category.
567

Taxa	Models	r	SE (r)	C	SE (C)	AIC	ΔAIC
Altogether	Linear	5.568	3.050	0.085	0.057	16.771	
	Snowball	12.754	3.867	0.109	0.053	12.562	4.21
	Slowdown	5.497	4.154	1.100	0.066	17.521	
Amphibian	Linear	2.484	1.486	-0.004	0.025	-18.579	
	Snowball	10.104	2.281	0.005	0.016	-20.164	1.59
	Slowdown	2.533	1.538	0.996	0.026	-18.526	
Bird	Linear	-26.636	28.905	0.270	0.130	8.983	
	Snowball	NA	NA	NA	NA	NA	0.01
	Slowdown	-30.789	31.530	1.307	0.165	8.975	
Invertebrate	Linear	-4.519	5.268	0.179	0.118	5.750	
	Snowball	NA	NA	NA	NA	NA	0.02
	Slowdown	-4.777	5.512	1.192	0.137	5.768	
Mammal	Linear	-10.701	16.766	0.191	0.144	3.279	
	Snowball	NA	NA	NA	NA	NA	0.03
	Slowdown	-12.426	18.293	1.212	0.170	3.250	
Reptile	Linear	0.009	0.369	0.002	0.005	-19.659	
	Snowball	NA	NA	NA	NA	NA	0.00
	Slowdown	0.009	0.369	1.002	0.005	-19.659	

569 **Table S2 The effect of mitochondrial genetic divergence on the selection against**
570 **hybrids among animal taxa.** Standard errors (SE) of the regression coefficients for the rate
571 parameter(*r*) and constant (*C*). The models (Fig. 1, Eq 1.1-3.1) with the lowest AICs are in
572 bold. The Slowdown Effect Model (equation 3.3) has the lowest AIC fitted to the full dataset.
573 Δ AICs (with >1 values bolded) represent the absolute difference in AIC between the models
574 with the lowest and second lowest AIC for each taxonomic category.
575

Taxa	Models	<i>r</i>	SE (<i>r</i>)	<i>C</i>	SE (<i>C</i>)	AIC	Δ AIC
Altogether	Linear	3.565	1.606	0.040	0.065	45.306	
	Snowball	7.012	1.881	0.090	0.058	46.164	0.10
	Slowdown	4.395	2.017	1.031	0.072	45.202	
Amphibian	Linear	3.102	3.133	-0.055	0.184	3.001	
	Snowball	6.175	2.956	-0.012	0.140	2.830	0.17
	Slowdown	3.294	3.389	0.944	0.187	3.040	
Bird	Linear	10.002	4.828	-0.024	0.149	27.597	
	Snowball	15.394	4.828	0.058	0.134	27.649	0.05
	Slowdown	12.972	6.935	0.953	0.167	27.705	
Invertebrate	Linear	5.271	2.112	-0.024	0.097	3.660	
	Snowball	8.685	2.066	0.022	0.087	3.265	0.40
	Slowdown	6.343	2.892	0.970	0.103	3.915	
Mammal	Linear	6.060	2.224	-0.113	0.078	-11.164	
	Snowball	10.842	1.667	-0.062	0.050	-14.625	3.46
	Slowdown	5.957	2.462	0.898	0.080	-10.417	
Reptile	Linear	0.047	0.028	0.001	0.001	-60.764	
	Snowball	0.739	0.158	0.001	0.001	-62.765	2.00
	Slowdown	0.047	0.028	1.001	0.001	-60.760	