SUPPLEMENTARY MATERIAL

Reinforcement alone does not explain increased reproductive isolation in sympatry Daniel R. Matute and Brandon S. Cooper

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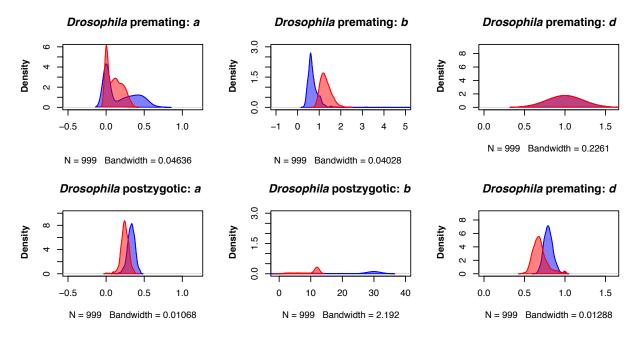
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34 TABLE S8. Datasets that have compiled data on the magnitude of reproductive		
	35	isolation in multiple species pairs

36 **FIGURE S1. 4PL regression parameters for** *Drosophila***.** The density plots

37 correspond to *a* (left), *b* (middle), and *d* (right) for premating isolation (top) and

38 postzygotic isolation (bottom). Blue: sympatric; red: allopatric. The values of *c*, the

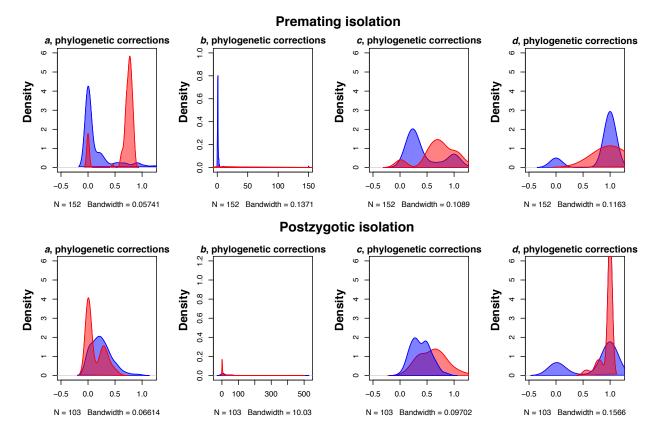
39 fourth parameter of the regression, are shown in Figure 1.



42 FIGURE S2. Drosophila 4PL regression parameters after phylogenetic

43 subsamplings at the species-group level. The density plots correspond to *a* (left), *b* 44 (middle left), *c* (middle right), and *d* (right) for premating isolation (top) and postzygotic

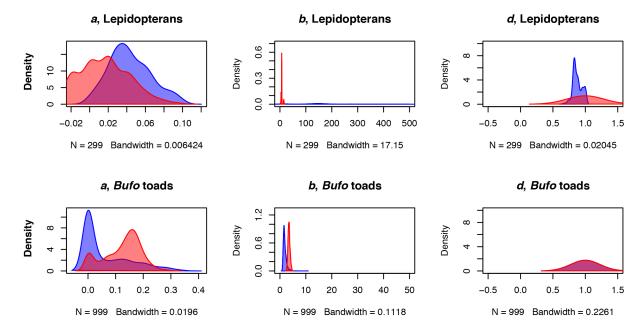
- 45 isolation (bottom). Blue: sympatric; red: allopatric.
- 46



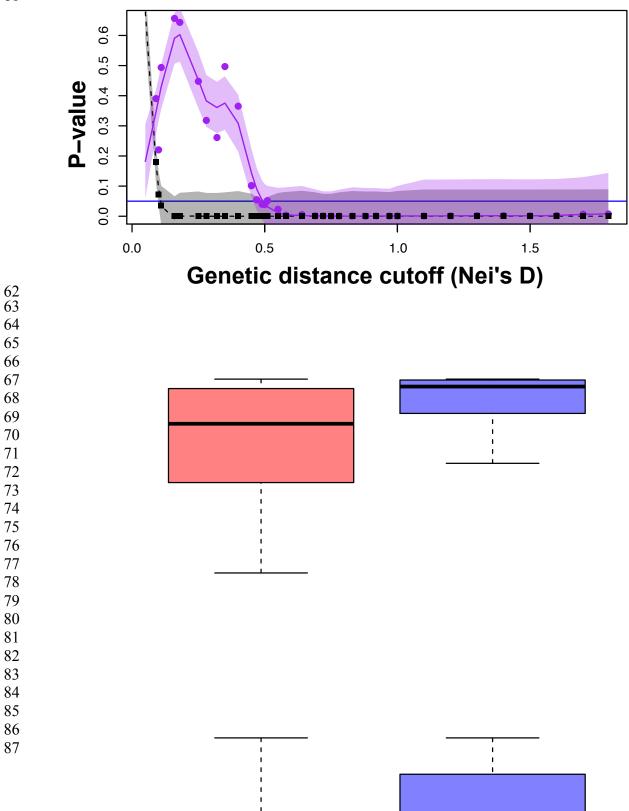
49 FIGURE S3. 4PL regression parameters for Lepidopterans and Bufo frogs. The

50 density plots correspond to *a* (left), *b* (middle), and *d* (right). Blue: sympatric; red:

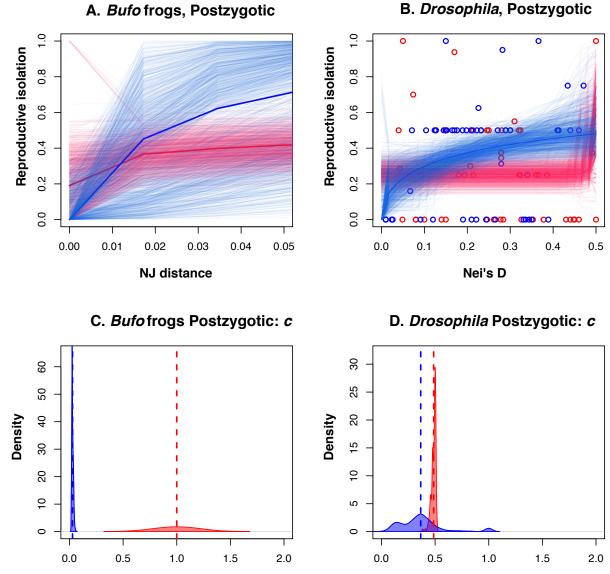
allopatric. The values of *c*, the fourth parameter of the regression, are shown in Figure2.



56 FIGURE S4. Comparisons of RI depend greatly on divergence time. Wilcoxon tests 57 comparing the strength of premating (black) and postzygotic (purple) RI in sympatry and 58 allopatry when using different thresholds to define young species. The solid horizontal 59 line indicates the threshold of significance defined by Coyne and Orr (1, 2). Shaded 60 intervals represent 95% confidence intervals.



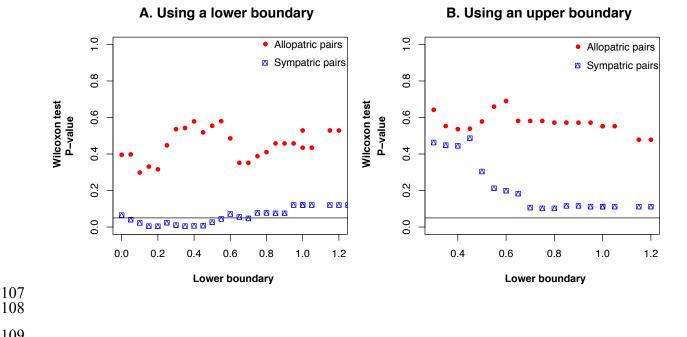
88 FIGURE S5. Postzygotic isolation evolves faster in sympatric species than in 89 allopatric species when the data set is restricted to 'young' species. For these analyses we defined young as the species with the third lowest divergence time (i.e., 90 91 Nei's *D* < 0.5 for *Drosophila* and NJ distance < 0.05 for *Bufo*). 4PL regressions in the youngest Drosophila (Nei's D < 0.51) and youngest Bufo species (Nei's D < 0.51) show 92 similar results to the full range of divergence. In both cases (young species and full 93 94 divergence), sympatric species pairs show an earlier inflection point. Note that we did 95 not do the same analyses for Lepidopterans as the 4PL regressions did not converge in datasets that did not include all the data. 96 97



N = 999 Bandwidth = 0.001654

N = 999 Bandwidth = 0.03386

- 99 FIGURE S6. The association between premating and postzygotic asymmetries is
- 100 contingent on the way that data are filtered in Drosophila. The y-axis shows the
- significance (P-value) of a Wilcoxon rank sum test with continuity correction. A. 101
- 102 Correlation between premating and postzygotic isolation when we used a lower
- threshold (i.e., including data from the lower threshold to the maximum of the range) for 103
- allopatric (red) and sympatric (blue) species. B. Correlation between premating and 104
- 105 postzygotic isolation when we used a upper threshold (i.e., including data from 0 to the
- 106 upper threshold) for allopatric (red) and sympatric (blue) species.



110 FIGURE S7. Mean premating and postzygotic RI are higher in sympatry in

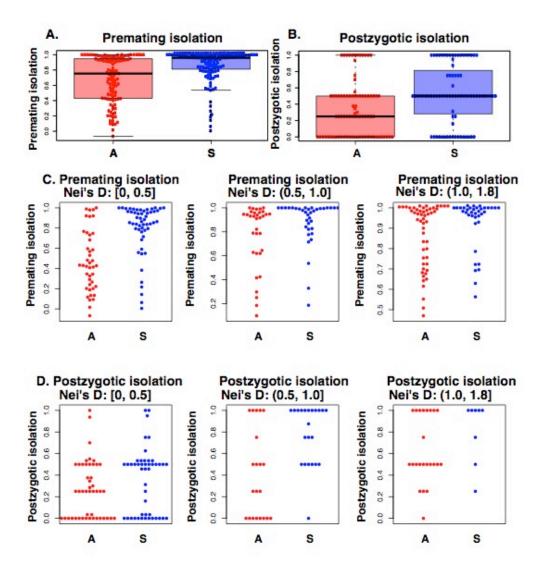
111 Drosophila but the ranges are overlapping. Premating (A) and postzygotic (B) RI in

sympatry (S) is a subset of the range in allopatry (A). This result for premating (C) and

113 postzygotic (**D**) RI is independent of the arbitrary threshold chosen to define young

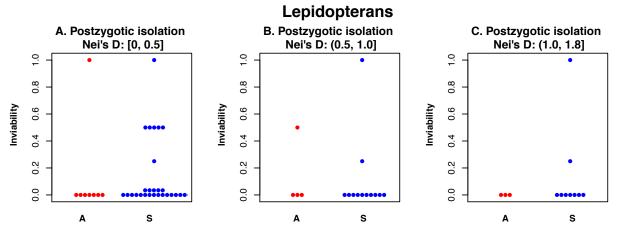
species. Similar plots for postzygotic RI in Lepidopterans and *Bufo* are shown in Figure

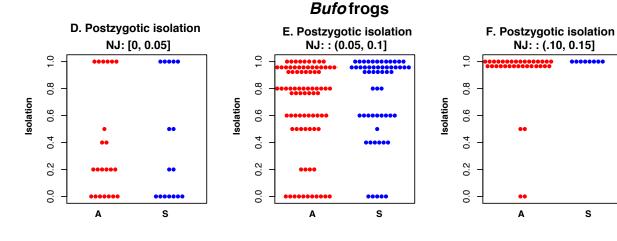
115 **S8**.



118 FIGURE S8. The range of postzygotic isolation in sympatry is similar to that in

- 119 allopatry in Lepidopterans and in *Bufo* toads. Regardless of the cutoff of genetic
- 120 distance, the range of postzygotic isolation usually overlaps with the range of RI in
- 121 allopatry (highly diverged Lepidopterans being an exception). Top panels:
- 122 Lepidopterans; lower panels: Bufo frogs.





123 124

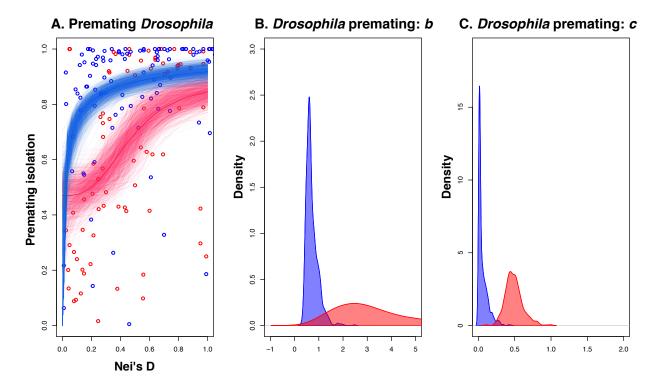
s

FIGURE S9. Premating isolation accumulates faster in sympatric than in allopatric

Drosophila species, even after including previously estimated metrics of

premating isolation in allopatric population pairs of the same species. These estimates are similar to the ones shown in Figures 1 and S1 but include metrics of

- isolation provided in (3-5).



SUPPLEMENTARY TABLES

- TABLE S1. 4PL models fit the data better than linear regressions. Akaike information criterion for each fitted model.

Taxon	Barrier	Linear	Logistic	4PL
Drosophila	Premating. Allopatric	3.111	7.167	-19.557
Drosophila	Premating. Sympatric	-44.350	-45.059	-53.562
Drosophila	Postzygotic. Allopatric	53.975	53.383	50.630
Drosophila	Postzygotic. Sympatric	36.043	138.178	30.949
Lepidopterans	Postzygotic. Allopatric	-13.427	-16.391	-19.366
Lepidopterans	Postzygotic. Sympatric	-11.07777	5.3061	-44.391

TABLE S2. Pairwise comparisons between coefficients of the four-parameter

- logistic model between sympatric and allopatric species. To assess the significance of the difference between coefficients we compared bootstrapped
- distributions using a Wilcoxon test.

	Coefficient	Coef _{sympatric}	Coef _{allopatric}	Wilcoxon test
Premating, Drosophila	a	0.184 (0.211)	0.115 (0.102)	W = 468,480; P = 0.015
Premating, Drosophila	b	0.740 (0.517)	1.316 (0.270)	W = 949,860; P < 0.0001
Premating, Drosophila	С	0.059 (0.083)	0.318 (0.085)	W = 978,080; P < 0.0001
Premating, Drosophila	d	1 (0)	1 (0)	W = 499,000; P = NA
Postzygotic, Drosophila	а	0.329 (0.049)	0.242 (0.050)	W = 93,010; P < 0.0001
Postzygotic, Drosophila	Ь	22.843 (9.801)	9.402 (3.532)	W = 164,870; P < 0.0001
Postzygotic, Drosophila	С	0.439 (0.044)	0.751 (0.114)	W = 993,050; P < 0.0001
Postzygotic, Drosophila	d	0.799(0.061)	0.682 (0.091)	W = 126,760; P < 0.0001
Postzygotic, Lepidopterans	а	0.015 (0.025)	0.043 (0.021)	W = 17155; P < 0.0001
Postzygotic, Lepidopterans	b	7.252 (2.955)	222.696 (426.678)	W = 1,189; P < 0.0001
Postzygotic, Lepidopterans			0.686 (0.012)	W = 89,401; P < 0.0001
Postzygotic, Lepidopterans	d	1(0)	0.872 (0.069)	W = 85,364; P < 0.0001
Postzygotic,	а	0.062 (0.087)	0.124 (0.065)	W = 739,185;

<i>Bufo</i> toads				<i>P</i> < 0.0001
Postzygotic, <i>Bufo</i> toads	b	1.854(0.699)	3.520 (0.414)	W = 971,916; P < 0.0001
Postzygotic, <i>Bufo</i> toads	С	0.028 (0.008)	0.038 (0.003)	W = 877,636; P < 0.0001
Postzygotic, <i>Bufo</i> toads	d	1 (0)	1 (0)	W = 499,000, P = NA

TABLE S3. Coefficients for a phylogenetically informed linear regression for premating and postzygotic isolation between *Drosophila* species pairs. The confidence intervals are based in 5 different chains.

Isolation	Coefficient	Isolation	95% CI	Effective	рМСМС
		mean		sample size	
Premating (Intercept)		0.417	[0.253,	888.760	<0.001
			0.616]		
Premating	Genetic Distance	0.297	[0.179,	11.710	<0.001
			0.414]		
Premating	Sympatry	0.364	[0.245,	575.470	<0.001
	(Sympatric-		0.464]		
	Allopatric)				
Premating	Genetic Distance ×	-0.184	[-0.323, -	1,000.00	0.014
	Sympatry		0.058]		
(Sympatric-					
	Allopatric)				
Postzygotic	Intercept	0.214	[-0.018.	910.500	0.084
			0.446]		
Postzygotic	Genetic Distance	0.306	[0.087,	704.400	0.004
			0.530]		
Postzygotic	Sympatry	0.034	[-0.121,	1,000.00	0.660
	(Sympatric-		0.198]		
	Allopatric)				
Postzygotic	Genetic Distance ×	0.388	[0.138,	984.50	0.002
	Sympatry		0.699]		
	(Sympatric-				
	Allopatric)				

TABLE S4. Regression coefficients from a phylogenetically informed regression for postzygotic isolation between Lepidopteran and *Bufo* species pairs. The confidence intervals are based in 5 different chains.

Group		Postzygotic mean	95% CI	Effective sample size	рМСМС
Lepidopterans	(Intercept)	-0.100	[-0.392, 0. 273]	1,000.0	0.488
Lepidopterans	Genetic Distance	0.906	[0.670, 1.125]	892.2	<0.001
Lepidopterans	Sympatry (Sympatric- Allopatric)	0.123	[-0.018, 0.257]	1,000.0	0.076
Lepidopterans	Genetic Distance × Sympatry (Sympatric- Allopatric)	-0.382	[-0.674, -0.093]	1,000.0	0.014
Bufo frogs	(Intercept)	0.502	[0.294, 0.691]	1,000.0	<0.001
Bufo frogs	Genetic Distance	4.849	[3.668, 5.950]	1,000.0	<0.001
<i>Bufo</i> frogs	Sympatry (Sympatric- Allopatric)	-0.161	[-0.272, -0.032]	1,000.0	0.006
<i>Bufo</i> frogs	Genetic Distance × Sympatry (Sympatric- Allopatric)	1.938	[0.486, 3.637]	1,000.0	0.012

156 **TABLE S5. Recently diverged species show faster accumulation of postzygotic**

157 isolation when they are sympatric than when they are allopatric in *Bufo* frogs.

158 Wilcoxon tests comparing the strength of postzygotic isolation in sympatry and allopatry

159 when using different thresholds to define young species in lepidopterans and *Bufo*

160 frogs. NC: regression did not converged and the analysis was not possible. Figure 3

161 shows similar results for premating and postzygotic isolation in *Drosophila*.

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Group	Cutoff	Csymp	Callop	W	<i>P</i> -value
Lepidopterans	0.5	NC	NC	—	—
Lepidopterans	1	NC	NC	—	—
Bufo frogs	0.05	0.028	>0.05	995,004	<0.0001
Bufo frogs	0.06	0.028	>0.06	946,053	<0.0001
Bufo frogs	0.07	0.028	0.037	816,440	<0.0001
Bufo frogs	0.08	0.029	>0.08	971,028	<0.0001
Bufo frogs	0.09	0.028	0.037	839,586	<0.0001
Bufo frogs	0.10	0.029	0.038	868,986	<0.0001
Bufo frogs	0.11	0.029	0.038	869,901	<0.0001
Bufo frogs	0.12	0.028	0.038	871,175	<0.0001

TABLE S6. Linear models show that the overlap of geographic range significantly affects the magnitude of premating and postzygotic isolation between *Drosophila*

species pairs.

Response (type of barrier)			F-value	P-value
Premating	mating Factorial Genetic dista (Nei's D)		57.187	7.171 × 10 ⁻¹³
Premating	Factorial	% Sympatry	50.809	1.044 × 10 ⁻¹¹
Premating	Factorial	Genetic distance × % Sympatry	3.982	0.047
Premating	No-interaction	Genetic distance (Nei's D)	56.528	9.338 × 10 ⁻¹³
Premating	No-interaction	% Sympatry	50.224	1.329 × 10 ⁻¹¹
Postzygotic Factorial		Genetic distance (Nei's D)	28.259	6.097 × 10 ⁻⁷
Postzygotic	Factorial	% Sympatry	14.283	2.625 × 10 ⁻⁴
Postzygotic Factorial		Genetic distance × % Sympatry	2.380	0.126
Postzygotic	No-interaction	Genetic distance (Nei's D)	27.892	6.98 × 10 ⁻⁷
Postzygotic	No-interaction	% Sympatry	14.098	2.85 × 10 ⁻⁴

171 **TABLE S7.** The effect of geographic overlap on the effect of the magnitude of

172 premating and postzygotic RI is mostly driven by species with disjoint

173 geographic ranges. For each overlap threshold, we fit a linear model in which the

174 strength of isolation, either premating or postzygotic, depends on genetic distance and

175 the percentage of overlap (% sympatry).

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		Ne	i's D	% sy	mpatry
RI type	Lower threshold (not included)	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value
Premating	All data	56.528	9.338 × 10 ⁻¹³	50.22	1.329 × 10 ⁻¹¹
Premating	0.00	10.873	1.268 × 10 ⁻³	7.022	9.082 × 10 ⁻³
Premating	0.01	10.873	1.268 × 10 ⁻³	7.022	9.082 × 10 ⁻³
Premating	0.02	10.450	1.569 × 10 ⁻³	7.487	7.121 × 10 ⁻³
Premating	0.03	8.946	3.36 × 10 ⁻³	6.704	0.011
Premating	0.04	9.158	3.030 × 10 ⁻³	8.601	4.026 × 10 ⁻³
Premating	0.05	9.443	2.637 × 10 ⁻³	3.242	0.074
Premating	0.10	9.920	2.101 × 10 ⁻³	0.095	0.758
Postzygotic	All data	27.892	6.98 × 10 ⁻⁷	14.098	2.85 × 10 ⁻³
Postzygotic	0.00	16.626	1.632 × 10 ⁻⁴	1.520	0.223
Postzygotic	0.01	16.626	1.632 × 10 ⁻⁴	1.520	0.223
Postzygotic	0.02	16.626	1.632 × 10 ⁻⁴	1.520	0.223
Postzygotic	0.03	14.006	4.788 × 10 ⁻⁴	1.459	0.233
Postzygotic	0.04	14.274	4.443 × 10 ⁻⁴	2.355	0.132
Postzygotic	0.05	14.263	4.549 × 10 ⁻⁴	2.841	0.090
Postzygotic	0.10	12.629	9.371 × 10 ⁻⁴	3.641	0.063

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TABLE S8. Datasets that have compiled data on the magnitude of reproductive isolation in multiple species pairs of animals.

	N	Premating	Postzygotic	Distance	Geographic	Reference
		isolation	isolation		status	
Drosophila	630	Х	Х	Х	Х	(6)
Toads	669		Х	Х	Х	(7, 8)
Lepidoptera	212		Х	Х	Х	(9)
Heliconius	12	Х	Х	Х		(10)
Birds	407		Х	Х		(11)
Sticklebacks	5	Х	Х	Х		(12)
Centrarchid	130		Х	Х		(13)
fish						