

## 1 **Supporting Information**

### 2 **Habitat variables and diet categories**

3 The following represent the specific habitat variables and diet categories that were scored for species pairs in order to calculate ecological  
 4 divergence indices. Habitat variables and diet categories are not presented for the Lepidoptera and *Drosophila* datasets because, as stated in  
 5 the main text, the host plant represents both habitat and diet for these insects and degree of host plant sharing was thus used to calculate  
 6 associated indices. **Angiosperms:** Habitat variables: altitude, flower color, flower scent, general habitat characteristics, mesic vs. xeric,  
 7 pollination mechanism, selfing vs. outcrossing, soil type, temperature, time of flower opening. **Fishes:** Habitat variables: freshwater vs.  
 8 marine, lentic vs. lotic, pelagic vs. benthic, substrate type, surrounding habitat, temperate vs. tropical, turbid vs. clear water, vegetated  
 9 habitat?, water depth, water pH, water speed, water temperature; Diet categories: algae, detritus, fish, fish eggs, invertebrates, plankton,  
 10 plants, tetrapod vertebrates. **Darters** Habitat variables: altitude, pools vs. streams, nature of riffles, runs present?, springs present?,  
 11 substrate type, vegetation present?, water body size, water clarity, water depth, water speed, water temperature. **Frogs** Habitat variables:  
 12 breeding time, egg-laying habits, habitat surrounding water body, means of predator avoidance, miscellaneous habitat characteristics,  
 13 restricted to wetlands?, salinity, source of cover, temporary vs. permanent water bodies, terrestrial vs. arboreal, vegetation, water body type,  
 14 water depth. **Birds:** Habitat variables: aquatic vs. terrestrial, elevation, forest type, freshwater vs. saline habitats, latitude/climate, lives in  
 15 coastal areas?, miscellaneous habitat characteristics, open vs. closed habitats, rocky habitats?, terrestrial habitat type, type of aquatic  
 16 habitat, xeric vs. mesic; Diet categories: flowers, fruits, fungi, invertebrates, miscellaneous plant tissues (leaves, roots, buds, etc.), nectar,  
 17 seeds/grains/nuts, vertebrates. **Doves/pigeons:** Habitat variables: associated with water?, elevation, mesic vs. xeric, rocky habitats?,  
 18 vegetation type; Diet categories: flowers, fruits, miscellaneous plant tissues (leaves, roots, buds, etc.), seeds/grains/nuts.

19

### 20 **Evaluating non-linearity and incorporation of nonlinear regression analysis**

21 We evaluated the possible presence of non-linearity in our data, identified an appropriate nonlinear regression model where required, and  
 22 then incorporated a combination of linear and nonlinear regression as appropriate in our individual analyses. Here, we describe a five-step  
 23 process for doing so. First, we used standard diagnostic techniques to test our assumption of linearity between genetic distance and each of  
 24 the two focal variables of our study: ecological divergence and reproductive isolation. Second, if the assumption of linearity was violated,  
 25 we tested whether a non-linear quadratic model fit the data significantly better than did the linear model. If so, the third step was to use the  
 26 diagnostic test from step one to verify that the quadratic regression could be used to accurately calculate residuals. Fourth, for these cases  
 27 of nonlinearity we estimated residuals using quadratic, rather than linear, regression. Fifth, we conducted our t-test analyses using  
 28 associations from the constituent individual analyses, each of which was conducted using the regression model (linear or quadratic) found

29 to be most appropriate for each. Each step is described in detail below. (We took this approach in evaluating both the unadjusted and the  
30 phylogenetically adjusted data, but only describe the results of our non-linearity tests for the unadjusted data (Table 5).)

31  
32 Step 1: Testing for nonlinearity: Linear regression techniques assume linear relationships between variables. A standard diagnostic test of  
33 this assumption is to plot the standardized residuals from a regression of the dependent variable against standardized estimates (i.e.,  
34 predicted values) of this variable (Cohen and Cohen 1983). Under linearity, this plot should show a random pattern. If nonlinearity is  
35 present, a curvilinear pattern should be observed. Whether the relationship is indeed curvilinear can be tested by statistically evaluating  
36 whether a quadratic regression of the residual values against the predicted values is significant.

37 We tested whether ecological divergence (ED hereafter) and reproductive isolation (RI hereafter) were non-linearly associated with  
38 genetic distance (GD hereafter) using these standard diagnostic techniques. For each form of RI for each study taxon we plotted the  
39 residuals from a regression of RI on GD against the predicted values for RI and tested for curvilinearity in this association using quadratic  
40 regression. The same analyses were repeated for each form of ED. The results of these analyses are shown in Table 5.

41  
42 Step 2: Testing whether a (nonlinear) quadratic model provides a better fit: In 8 of 30 cases, significant curvilinearity was detected (Table  
43 5). For these 8 cases, we used partial F-tests to analyze whether adding a quadratic term significantly improved the regression model. That  
44 is, we assessed the significance of increases in  $r^2$  when a quadratic term was added to the linear regression model (Norusis 1993). Quadratic  
45 regression is the simplest and most general type of nonlinear regression, and is the usual starting point when departures from linearity are  
46 detected (Cohen and Cohen 1983; see Step 3 for explicit tests of whether the quadratic model is justified for calculation of residuals). In all  
47 8 cases, the quadratic regression did significantly improve the fit of the model (Table 6). For the sake of completeness, we also conducted  
48 these tests for cases where the assumption of linearity was confirmed in Step 1. These tests further confirm that a linear model provides a  
49 sufficient fit to the data.

50  
51 Step 3: Testing whether residuals can be accurately calculated using quadratic regression: By repeating the diagnostic tests from Step 1, we  
52 similarly evaluated whether residuals obtained from quadratic regression displayed the expected random pattern. Nonsignificant results  
53 supported this inference in 7/8 cases (and in all 8 if a Bonferroni correction is applied; Rice 1989) (Table 5). This demonstrated that the  
54 quadratic model fits the data well and that residuals can be accurately calculated from a quadratic regression.

55  
56 Step 4: Calculation of residuals from models which fit the data properly: For the 8 cases where quadratic regression was most appropriate,  
57 we estimated associations between RI and ED using the residuals from a quadratic regression of RI on GD and ED on GD (rather than

58 residuals calculated using linear regression). For the other cases, we continued to use residuals derived from the earlier justified linear  
59 regressions.

60

61 Step 5: Individual and cross-taxon t-test analyses: Residuals derived from appropriate linear and/or quadratic regressions were used in the  
62 individual analyses (Table 1) to provide the ecology-isolation association values for use in the cross-taxon t-tests (Table 2). Our results  
63 demonstrate that when nonlinearity is explicitly and statistically accounted for, there is a significantly positive association between ED and  
64 RI across the eight datasets for each of our four methods of analysis (Table 2). We additionally conducted all of our analyses using only  
65 linear regression (analyses and results not discussed here). These strictly linear analyses generally yielded somewhat lower p-values than  
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441 216.  
 442 **Table 3. Summary of individual analyses of the strength of the association between indices of ecological divergence and components of**  
 443 **reproductive isolation for the phylogenetically adjusted data.**

Taxon	Ecolo- gical trait	RI	N	Traditional analyses		Analyses of <i>a priori</i> residuals	
				(1)	(2)	(3)	(4)
				Parametric	Nonpara- metric	Parametric	Nonpara- metric
				r	rho	r	rho
Angiosperms	habitat	pre-	16	-0.04	-0.05	-0.04	-0.00
Angiosperms	habitat	post-	26	0.46	0.34	0.45	0.31
Angiosperms	habitat	total	14	0.11	0.12	0.11	0.15
Lepidoptera	diet/hab	post-	46	0.17	0.01	0.17	0.01
Lepidoptera	diet/hab	total	33	-0.01	0.04	-0.01	-0.01
<i>Drosophila</i>	diet/hab	pre-	14	0.03	-0.04	-0.06	-0.18
<i>Drosophila</i>	diet/hab	post-	19	-0.20	-0.13	-0.22	-0.12
<i>Drosophila</i>	diet/hab	total	13	0.42	0.16	0.37	0.12
Fishes	habitat	post-	37	0.12	0.03	0.12	0.01
Fishes	diet	post-	28	-0.16	-0.18	-0.15	-0.12
Fishes	size	post-	36	-0.01	-0.03	0.00	0.00
Darters	habitat	pre-	10	-0.08	-0.17	-0.08	-0.22
Darters	habitat	post-	8	-0.25	-0.20	-0.25	-0.12
Darters	habitat	total	7	0.19	0.17	0.20	0.11
Darters	size	pre-	10	0.57	0.52	0.62	0.37
Darters	size	post-	8	-0.20	0.17	-0.22	0.05
Darters	size	total	7	0.16	0.23	0.17	0.18



Frogs	habitat	post-	32	0.20	0.17	0.21	0.17
Birds	habitat	post-	10	0.02	0.01	0.03	0.15
Birds	diet	post-	10	0.09	0.30	0.09	0.21
Birds	size	post-	9	0.89	0.88	0.87	0.83
Doves	habitat	post-	4	0.83	0.95	0.88	1.00
Doves	diet	post-	4	0.35	0.26	0.96	0.20
Doves	size	post-	4	-0.69	-0.40	-0.88	-0.20

444 Note: these analyses of the phylogenetically adjusted data are specifically presented as a further test of the robustness of our results; analyses of the  
445 unadjusted data provide a sufficient test of the focal hypothesis of this study. This table presents results from regression analyses for each data set. N =  
446 number of species comparisons analyzed. r and rho values indicate the strength of the association between ecological divergence and reproductive  
447 isolation after time (genetic distance) has been statistically removed. These association values provide the data for the cross-taxon analyses (Table 4).  
448 Abbreviations: diet/hab = diet/habitat, pre- = prezygotic reproductive isolation, post- = postzygotic reproductive isolation, total = total reproductive  
449 isolation. Numbers in parentheses above each column of association values refer to the particular method of regression analysis used to derive these  
450 values (see text for details). For Lepidoptera, “postzygotic isolation” data were in the form of hybrid inviability and “total isolation” represented a  
451 combined index of hybrid inviability and hybrid sterility (data for which were not provided in the original paper).  
452

453 Table 4. One-sample cross-taxon t-tests of the association between ecological divergence and reproductive isolation across datasets using  
 454 the phylogenetically adjusted data

Comparison	N	Traditional analyses				Analyses of <i>a priori</i> residuals				
		(1)		(2)		(3)		(4)		
		Parametric		Nonparametric		Parametric		Nonparametric		
		Mean r	P	Mean rho	P	Mean r	P	Mean rho	P	
Overall	8	0.14354	0.005*	0.13	0.021*	0.15	0.007*	0.13	0.037*	
By ecological trait										
Habitat	8	0.18	0.505	0.16	0.113	0.19	0.059	0.17	0.084~	
Diet	5	0.09	0.168	0.08	0.212	2.01	0.180	0.05	0.267	
Size	4	0.09	0.398	0.19	0.268	0.05	0.454	0.21	0.210	
By component of reproductive isolation										
Prezygotic	3	0.23	0.227	0.03	0.370	0.06	0.320	-0.04	0.339	
Postzygotic	8	0.11	0.012*	0.12	0.063~	0.13	0.101	0.13	0.056~	
Total	3	0.24	0.066~	0.16	0.012*	0.22	0.054~	0.14	0.003*	

455 Note: these analyses of the phylogenetically adjusted data are specifically presented as a further test of the robustness of our results;  
 456 analyses of the unadjusted data provide a sufficient test of the focal hypothesis of this study. N = the number of datasets available for use  
 457 in an analysis. Tests compare the mean observed associations (r, rho values) between ecological divergence and residual reproductive  
 458 isolation from the individual analyses (Table 3) with an absence of association (i.e., r, rho = 0). A significant result indicates a positive  
 459 association that is independent of time. Numbers in parentheses above each column of association values refer to the particular method of  
 460 regression analysis used to derive these values (see text for details). ~P < 0.10; \*P < 0.05.  
 461

462 **Table 5. Tests for non-linearity of the data.**

Taxon	RI or ED	Step 1: quadratic regression of predicted versus residual values from linear regression			Step 2: Fit of raw data to linear versus quadratic regression models				Step 3: quadratic regression of predicted versus residual values from quadratic regression		
		r <sup>2</sup>	F	p	r (linear)	r (quad.)	F-change	p	r <sup>2</sup>	F	p
Unadjusted data											
Angios.	pre-	0.210	10.47	0.000	0.526	0.654	20.95	0.000	0.012	0.48	0.622
Angios	post-	0.021	1.06	0.349	0.339	0.365	2.13	0.148	-	-	-
Angios	total	0.509	26.40	0.000	0.808	0.911	52.80	0.000	0.100	2.83	0.070
Angios	hab	0.038	3.07	0.049	0.081	0.211	6.14	0.014	0.023	1.81	0.167
Lepid.	post-	0.369	18.97	0.000	0.735	0.843	37.95	0.000	0.038	1.30	0.279
Lepid.	total	0.003	0.08	0.927	0.648	0.649	0.15	0.699	-	-	-
Lepid.	d/h	0.020	0.65	0.525	0.027	0.145	1.03	0.258	-	-	-
<i>Dros.</i>	pre-	0.041	1.12	0.333	0.394	0.436	2.24	0.140	-	-	-
Dros.	post-	0.275	10.79	0.000	0.649	0.762	21.58	0.000	0.039	1.17	0.318
Dros.	total	0.189	7.34	0.001	0.527	0.643	14.69	0.000	0.113	4.03	0.023
Dros.	d/h	0.036	1.39	0.255	0.427	0.461	2.78	0.100	-	-	-
Fishes	post-	0.129	2.51	0.096	0.779	0.811	5.03	0.032	-	-	-
Fishes	hab.	0.000	0.00	0.998	0.061	0.062	0.00	0.950	-	-	-
Fishes	diet	0.001	0.01	0.990	0.083	0.087	0.02	0.891	-	-	-
Fishes	size	0.000	0.01	0.994	0.304	0.305	0.01	0.916	-	-	-
Darters	pre-	0.157	0.93	0.427	0.772	0.812	1.87	0.203	-	-	-
Darters	post-	0.006	0.02	0.981	0.123	0.147	0.04	0.850	-	-	-

Darters	total	0.257	0.69	0.551	0.630	0.743	1.39	0.304	-	-	-
Darters	hab	0.028	0.17	0.843	0.214	0.269	0.35	0.567	-	-	-
Darters	size	0.051	0.32	0.731	0.302	0.371	0.64	0.438	-	-	-
Frogs	post-	0.010	0.47	0.625	0.358	0.370	0.95	0.333	-	-	-
Frogs	hab	0.014	0.74	0.482	0.153	0.193	1.47	0.228	-	-	-
Birds	post-	0.089	6.17	0.003	0.637	0.677	12.33	0.001	0.003	0.18	0.833
Birds	hab.	0.200	1.21	0.303	0.250	0.284	2.41	0.123	-	-	-
Birds	diet	0.011	0.63	0.535	0.284	0.302	1.26	0.264	-	-	-
Birds	size	0.007	0.42	0.656	0.392	0.399	0.85	0.360	-	-	-
Doves	post-	0.006	0.06	0.947	0.592	0.596	0.11	0.745	-	-	-
Doves	hab.	0.292	3.71	0.045	0.322	0.604	7.41	0.014	0.034	0.32	0.730
Doves	diet	0.134	1.39	0.274	0.209	0.414	2.78	0.113	-	-	-
Doves	size	0.016	0.13	0.882	0.029	0.128	0.25	0.622	-	-	-
Phylogenetically adjusted data											
Angios.	pre-	0.454	5.41	0.020	0.668	0.835	10.82	0.006	0.021	0.14	0.837
Angios.	post-	0.006	0.07	0.931	-	-	-	-	-	-	-
Angios.	total	0.270	2.04	0.176	-	-	-	-	-	-	-
Angios.	hab	0.048	0.778	0.468	-	-	-	-	-	-	-
Lepid.	post-	0.407	14.75	0.000	0.791	0.882	24.50	0.000	0.047	1.07	0.353
Lepid.	total	0.001	0.020	0.980	-	-	-	-	-	-	-
Lepid.	d/h	0.041	0.91	0.410	-	-	-	-	-	-	-
<i>Dros.</i>	pre-	0.032	0.43	0.654	-	-	-	-	-	-	-
<i>Dros.</i>	post-	0.190	3.40	0.047	0.663	0.739	6.81	0.014	0.019	0.29	0.753
<i>Dros.</i>	total	0.107	1.20	0.321	-	-	-	-	-	-	-

<i>Dros.</i>	d/h (pre)*	0.011	0.14	0.870	-	-	-	-	-	-	-
<i>Dros.</i>	d/h (post)*	0.021	0.32	0.731	-	-	-	-	-	-	-
<i>Dros.</i>	d/h (tot.)*	0.004	0.041	0.960	-	-	-	-	-	-	-
Fishes	post-	0.034	0.25	0.784	-	-	-	-	-	-	-
Fishes	hab.	0.156	1.30	0.305	-	-	-	-	-	-	-
Fishes	diet	0.057	0.30	0.745	-	-	-	-	-	-	-
Fishes	size	0.039	0.28	0.758	-	-	-	-	-	-	-
Darters	pre-	0.352	1.90	0.219	-	-	-	-	-	-	-
Darters	post-	0.042	0.11	0.899	-	-	-	-	-	-	-
Darters	total	0.322	0.95	0.459	-	-	-	-	-	-	-
Darters	hab	0.027	0.11	0.898	-	-	-	-	-	-	-
Darters	size	0.091	0.40	0.683	-	-	-	-	-	-	-
Frogs	post-	0.144	2.44	0.105	-	-	-	-	-	-	-
Frogs	hab	0.011	0.16	0.856	-	-	-	-	-	-	-
Birds	post-	0.127	0.51	0.622	-	-	-	-	-	-	-
Birds	hab.	0.008	0.03	0.971	-	-	-	-	-	-	-
Birds	diet	0.051	0.19	0.832	-	-	-	-	-	-	-
Birds	size	0.000	0.00	0.999	-	-	-	-	-	-	-
Doves	post-	0.884	3.80	0.341	-	-	-	-	-	-	-
Doves	hab.	0.446	0.40	0.744	-	-	-	-	-	-	-
Doves	diet	0.996	128.48	0.062	-	-	-	-	-	-	-
Doves	size	0.981	26.01	0.137	-	-	-	-	-	-	-

463 These analyses evaluate whether the fit of ecological divergence (ED) and reproductive isolation (RI) data to genetic distance data is better  
464 described by linear versus nonlinear regression. Each step in these analyses is outlined in the Supporting Information text. Few departures  
465 from linearity were detected. In these cases, a quadratic model fit the data significantly better and thus generated unbiased residuals. \* The  
466 linearity of diet/habitat data in *Drosophila* had to be examined separately for each component of reproductive isolation for the adjusted  
467 datas. This is because different components of reproductive isolation were available for different subsets of species pairs, necessitating the  
468 creation of separate adjusted datasets.

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