SUPPORTING INFORMATION

The strength of reproductive isolating barriers in seed plants: insights from studies quantifying premating and postmating reproductive barriers over the past 15 years. Christie, K., L.S. Fraser, and D.B. Lowry. *Evolution* (2022).

**Description of Isolating Barriers**

*Ecogeographic isolation:* Ecogeographic isolation refers to allopatry or spatial segregation enforced by adaptation (Sobel et al. 2010; Schemske 2010). For example, if two lineages are adapted to different soil types and precipitation regimes, they may rarely co-occur because neither can survive in the other’s habitat. We included estimates of ecogeographic isolation at a broad scale (e.g., predicted range overlap as determined from climatic niche models) as well as habitat segregation at a fine scale (e.g., estimates of microhabitat isolation within areas of sympatry). We provide summary statistics for both sub-categories, as well as overall summary statistics for Ecogeographic Isolation in Table 1. Many authors used herbarium records or other databases of spatial occurrence data to determine the frequency of sympatric and allopatric occurrences. It is debatable whether simple occurrence data accurately reflect ecogeographic isolation or other forms of allopatry arising due to chance (Rincón-Barrado et al. 2021); yet, for the purposes of this study we included all such estimates of spatial segregation here. We used Equations RI4C (Sobel and Chen 2014) based on shared and unshared occurrences or regions of spatial overlap to calculate barrier strengths associated with ecogeographic isolation.

*Immigrant inviability:* Individuals migrating into foreign habitats they are not adapted to may suffer reduced survival or reduced reproductive output compared to local residents (Nosil et al. 2005). Reduced survival of migrants in foreign habitats may strengthen spatial segregation among maladapted migrants and locally adapted residents, reducing potential gene exchange. Here, we utilize a “local” vs. “foreign” definition of local adaptation (Kawecki and Ebert 2004) to quantify the strength of immigrant inviability as a reproductive barrier (Richards and Ortiz-Barrientos 2016). Specifically, we use Equation RI4A (Sobel and Chen 2014), substituting the heterospecific (H) and conspecific (C) parameters for F and L, respectively, where F represents the fitness of a foreign migrant, and L represents the fitness of a local resident. In contrast to ecogeographic isolation, in which authors typically quantified RI using observational or correlative methods, here authors quantified RI associated with immigrant inviability using reciprocal transplant experiments.
**Phenology**: Phenology refers to flowering time differences, specifically the temporal extent to which two taxa may be able to exchange genes through pollen transfer. Authors quantified RI arising from phenological differences both in the field and in common gardens, often providing a count of shared and unshared flowering days, or phenological distribution curves for both taxa. For many studies we extracted the shared and unshared portions of these phenological distributions using *ImageJ*. We used Equations RI\textsubscript{IC} (Sobel and Chen 2014) based on shared and unshared flowering times to calculate barrier strengths associated with phenological isolation.

**Mating system differences**: Self-fertilization can act as a reproductive barrier by shielding selfing taxa from heterospecific pollen (Brys et al. 2014). To calculate RI associated with mating system, we use Equation RI\textsubscript{IA}, where the heterospecific (H) parameter represents outcrossed offspring, and the conspecific (C) parameter represents selfed offspring. In most cases, authors obtained RI data by genotyping or phenotyping offspring; in a few cases, RI was inferred through differences in pollen transfer rates. Alternatively, authors sometimes quantified *Differential Pollen Production* as a proxy for mating system differences. When authors quantified multiple facets of mating system RI (e.g., observed selfing/outcrossing rates, as well as differences in pollen production between putative selfers and outcrossers), we took the average of these different components to represent overall RI associated with mating system.

**Floral isolation**: Floral isolation refers to compositional, behavioral, or mechanical mechanisms imposed by pollinators that reduce pollen transfer between plant taxa (Grant 1994; Mitchell 1994). Pollinator differences, differences in the preference or constancy of shared pollinators, and mechanical isolation can all contribute to floral isolation. Within this broad category, researchers used a variety of methods to gather RI data, including documenting differences in the richness or abundances of pollinator communities, differences in visitation rates or pollinator constancy, and by directly quantifying patterns of conspecific and heterospecific pollen transfer. Depending upon the data provided by primary authors, we used both Equations RI\textsubscript{IC} and RI\textsubscript{IA} (Sobel and Chen 2014) to calculate barrier strengths associated with floral isolation. Specifically, when primary authors provided data based on the number of floral visits from shared and unshared pollinators, we used Equation RI\textsubscript{IC} to calculate barrier strengths; when authors provided data for the number of heterospecific and conspecific pollinator transitions, or measures of conspecific and heterospecific pollen depositions, we used we used Equation RI\textsubscript{IA} to calculate barrier strengths associated with floral isolation.
Pollen-pistil interactions: Our estimate of RI associated with pollen-pistil interactions represents a variety of postmating prezygotic barriers. A competitive situation arises when a flower is simultaneously pollinated with both hetero- and conspecific pollen. Conspecific pollen precedence refers to instances in which conspecific pollen outcompetes heterospecific pollen and thus, successfully fertilizes ovules at a relatively higher rate (Broz and Bedinger 2021). Such gametic selection was typically assessed using mixed pollen crosses. Some authors also assessed postmating prezygotic RI in non-competitive (i.e., single donor crosses) by comparing pollen dynamics in conspecific compared to heterospecific crosses and quantifying pollen adhesion, pollen germination, pollen tube growth rates, or the likelihood of pollen tubes reaching the ovule. In all cases we used Equation RI_{4A} (Sobel and Chen 2014) based on conspecific and heterospecific fitness to calculate barrier strengths associated with pollen-pistil interactions.

Fruit production: Unsuccessful fruit set following heterospecific pollen transfer might reflect pollen-pistil interactions (i.e., unsuccessful fertilization), or early seed and fruit abortion resulting from developmental problems in hybrid crosses (i.e., intrinsic postzygotic isolation). Authors typically scored fruit production or fruit development in experimental crosses. We used Equation RI_{4A} (Sobel and Chen 2014) based on conspecific and heterospecific fitness to calculate barrier strengths associated with fruit production.

Seed production: Authors commonly quantified the probability of successful seed production (i.e., seed set), seed number, and seed weight resulting from experimental crosses. These metrics reflect intrinsic postzygotic RI associated with seed development in hybrid crosses. Sometimes authors only scored the production of viable seeds here. We used Equation RI_{4A} (Sobel and Chen 2014) based on conspecific and heterospecific fitness to calculate barrier strengths associated with seed production.

F1 germination: To assess the viability of F1 seeds, authors germinated seeds from F1s and intraspecific crosses and calculated the relative successful germination fraction of the F1s. We used Equation RI_{4A} (Sobel and Chen 2014) based on parental and hybrid fitness to calculate barrier strengths associated with F1 germination.

F1 viability: To assess the viability of F1s compared to intraspecific progeny, authors grew F1s in laboratory or greenhouse conditions and quantified survival, survival to flowering, growth rates or biomass production, and flower number. We used Equation RI_{4A} (Sobel and Chen 2014) based on parental and hybrid fitness to calculate barrier strengths associated with F1 viability.
**F1 sterility:** F1 sterility included measures of both male sterility (*F1 Pollen Sterility*) and female fertility (*F1 Ovule Fertility*). Pollen viability/sterility was ubiquitously assessed via staining methodologies (Kearns and Inouye 1993) aimed at differentiating viable from non-viable pollen. Authors largely assessed female fertility in F1s through backcrosses in which the F1 received parental pollen. When measures of both male and female sterility were provided, we took the average to calculate overall RI associated with F1 sterility. We used Equation RI_{AA} (Sobel and Chen 2014) based on parental and hybrid fitness to calculate barrier strengths for F1 sterility.

**Extrinsic postzygotic isolation:** Authors quantified the magnitude of extrinsic postzygotic isolation (i.e., environmentally dependent selection against hybrids) by comparing the fitness (survival, reproductive output, or multiplicative fitness metric) of parental species growing in their native habitats in relation to hybrids occurring in these same habitats (e.g., Melo et al. 2014). F1 hybrids could occupy parental habitats either through heterospecific pollen reaching resident maternal plants and subsequent localized seed dispersal, resulting in hybrids derived from resident ovule mothers and migrant pollen fathers. Alternatively, although seemingly less likely, hybrids could also occur in parental habitats as a result of seed dispersal, in which a hybrid seed produced by a foreign mother and local pollen was transported into back into the native habitat of the pollen donor. In cases when authors assessed the fitness of hybrids resulting from both directions of the cross, we took the average of both values. We calculated barrier strengths associated with extrinsic postzygotic RI using a formulation of Equation RI_{AA} (Sobel and Chen 2014), where the conspecific (C) parameter represented conspecific or parental fitness at its home site, and the heterospecific (H) parameter represented F1 hybrid fitness at the same site. In the few cases in which authors also assessed this barrier in F2 hybrids, we calculated an average barrier strength associated with both F1s and F2s.
**Supplementary figures**

Figure S1: Pairwise comparison of the strengths of 12 reproductive isolating barriers. Black points indicate mean barrier strengths (estimated marginal means), gray bars represent 95% confidence intervals for barrier strength, and blue arrows represent comparisons among barriers. Barriers without overlapping blue arrows are significantly different ($p < 0.05$; Tukey’s HSD correction for multiple comparisons). Barrier strengths reflect the absolute strength of each barrier independent of the sequential action of multiple barriers or potential correlations or non-independence among barriers.
Figure S2: Pairwise p-values for 12 reproductive barriers indicating which individual isolating barriers are significantly different from one another. P-value thresholds are arranged along the x-axis by decreasing statistical support; isolating barriers are ordered along the y-axis by mean barrier strength. Pairs of isolating barriers are connected by blue vertical lines; pairwise comparisons with p-values < 0.05 have significantly different barrier strengths after correcting for multiple comparisons (Tukey’s HSD).
Figure S3: Density plots of barrier strengths for 12 reproductive isolating barriers as assessed in 89 flowering plant taxa. Barrier strengths are estimated using Sobel and Chen RI metrics and range from negative 1 (complete disassortative mating or heterosis) to 1 (complete RI). For each isolating barrier, an individual panel summarizes barrier strength by taxa type; cytotypes, ecotypes, and species depicted in different colors. Subspecies (n = 2 pairs) are grouped with ecotypes as both represent groupings of populations within species.
**Description of supplementary tables**

Table S1: *TableS1_raw_RI_data_FINAL.xlsx*. Metadata for the 89 taxa pairs from 70 publications used in this analysis. Numerical values for isolating barriers provide the raw data used in calculations of RI barrier strengths. Raw data for shared and unshared reproductive opportunities, and conspecific and heterospecific mating attempts, are provided for premating barriers. Conspecific and heterospecific fitness or crossing success are provided for postmating barriers. Values highlighted in green reflect Sobel and Chen RI values, as calculated and presented by primary authors. The “Notes” column provides details for how data were extracted, and if necessary, how RI values were calculated.

Table S2: *TableS2_SC_RI_data_FINAL.xlsx*. RI data for the 89 taxa pairs from 70 publications used in this analysis. Numerical values reflect the strength of individual isolating barriers, as calculated using Sobel and Chen (2014) RI metrics. These data were used for all analyses in the manuscript.
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