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Review

Does Asymmetric Reproductive Isolation Predict the Direction of Introgression in Plants?

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Abstract: Background/Objectives: The evolution of reproductive isolation (RI) results in the reduction of interspecific hybridization and the maintenance of species boundaries. Asymmetries in RI, where one species more frequently serves as the maternal or paternal parent in initial F_1 hybrid formation, are commonly observed in plants. Asymmetric introgression, the predominantly unidirectional transfer of genetic material through hybridization and backcrossing, has also been frequently documented in hybridizing plant taxa as well. This study investigates whether asymmetries in total RI measured between species can predict the direction of introgression in naturally hybridizing plant taxa. **Methods**: A meta-analysis was conducted on 19 plant species pairs with published data on both asymmetric total RI, and asymmetric introgression. Species pairs that met these criteria were identified through a comprehensive literature review. A two-tailed binomial test was performed to evaluate whether asymmetric RI was associated with asymmetries in introgression. **Results**: No significant relationship was found between asymmetries in total RI and the direction of introgression ($p = 0.3593$). **Conclusions**: Asymmetric RI largely does not predict the direction of introgression. Rather, introgression patterns may be better understood by examining F_1 and later-generation hybrids in natural settings, focusing on their fitness, mating behaviors, and the ecological and demographic factors that shape hybrid zones.

Keywords: asymmetric reproductive isolation; asymmetric introgression; natural hybridization; gene flow; hybrid zones; plant speciation

1. Introduction

Reproductive Isolation and Introgression: Speciation is a fundamental evolutionary process characterized by the development of reproductive isolation, which limits gene flow between genetically diverging taxa. The total RI observed between taxa typically encompasses a diverse suite of reproductive barriers that act collectively to limit gene flow and maintain species boundaries [1–3]. These barriers are broadly categorized based on the timing at which they occur during the life cycle of the organisms. Prezygotic barriers, which act prior to fertilization, reduce the likelihood of F_1 hybrid formation (e.g., temporal and ecological isolating barriers) [4], whereas postzygotic barriers act after fertilization, manifesting as reduced hybrid viability and/or fertility [3,5]. According to the biological species concept, speciation is complete when RI prevents the production of fertile hybrids, thereby halting gene flow entirely [2].

Complete reproductive isolation rarely evolves instantaneously [but see [6–9]], and the total RI observed between diverging taxa is often incomplete (i.e. RI ≠ 1.0) allowing for occasional F_1 hybrid formation. A number of methods have been developed to quantify RI for individual reproductive barriers and their relative contributions to the total RI observed between species. These methods generally seek to quantify the degree to which F_1 hybrid formation is reduced relative to that of pure-species formation [5,10–14]. Because initial F_1 hybrid formation can occur bi-directionally (i.e. either

species may serve as the maternal or paternal parent), measures of RI are often calculated reciprocally [12,13,15,16]. A key finding across a broad suite of plants is that total RI is frequently asymmetric, with one species more likely to serve as the maternal or paternal parent during initial F_1 hybrid formation [15,16]. Such asymmetric isolation has led researchers to suggest that this could influence patterns of introgression–the transfer of genetic material between species via hybridization and subsequent backcrossing [17,18]. Like RI, introgression is also often observed to be asymmetric, with gene flow predominantly occurring from one species into the other [17,19–22]. However, it remains an open question whether asymmetries in RI are predictive of the direction of introgression.

Asymmetries in RI have been widely documented in plants and may result from a combination of sequentially acting prezygotic and postzygotic barriers that may ultimately favor one parent species over the other during F_1 hybrid formation [13,15,16]. Similarly, asymmetric introgression is also a frequently observed phenomenon in plants [17,19–22]. Some studies have posited that the directionality in RI might be indicative of the directionality of introgression, often assuming that the favored paternal parent in F_1 hybrid formation will also serve as the primary genetic donor in subsequent gene flow [17,18].

The direction of introgression is influenced by multiple factors, the interactions of which can be complex [17]. The initial proximity of F_1 hybrids to one or the other species may play a crucial role, particularly in plants, where pollen and seed dispersal mechanisms can bias backcrossing toward either the most abundant or geographically closest species [22–24]. After viable and fertile F_1 hybrids are formed, selection pressures on later-generation backcross hybrids, whether ecological or intrinsic, can influence the direction of introgression, as selectively advantageous alleles can be incorporated into heterospecific genomic backgrounds [17,25–28]. Importantly, the predominant direction of introgression may not necessarily be determined by the direction of initial F_1 hybrid formation, but rather the fitness and mating patterns of those F_1 and later-generation backcross hybrids. This highlights the need for empirical studies that consider not only RI asymmetries, but also the other factors that may predict the direction of introgression once F_1 hybrids are formed in natural populations.

Rationale and scope of review: This study seeks to clarify the relationship between asymmetric total RI and asymmetric introgression in plants. While numerous studies have documented asymmetric RI in plant taxa [5,15,16], and others have observed asymmetric introgression [17,19–22], no comprehensive effort has yet been made to synthesize these findings and determine whether asymmetries in RI are predictive of the directionality of introgression. Given the prevalence of hybridization and the recognized evolutionary significance of introgression in plants, understanding whether asymmetries in RI are predictive of the direction of introgression could enhance the understanding of speciation in the face of gene flow and improve the ability to anticipate patterns of gene flow and species integrity in hybridizing taxa.

This review focuses exclusively on plants due to the extensive documentation of hybridization and introgression across a diversity of taxa, as well an extensive body of literature providing quantitative measures of prezygotic, postzygotic, and total RI [5,15,16]. Plant systems are particularly suitable for studying these dynamics because many species readily hybridize, and reproductive barriers in plants are often characterized by a combination of ecological and genetic factors [29]. Additionally, the relatively large sample size of available plant studies allows for robust meta-analyses and the identification of general patterns across taxa. By examining plant species with documented asymmetries in both RI and introgression, this review aims to test whether the direction of RI is predictive of the directionality of introgression.

Objectives: A meta-analysis approach was utilized to address two main objectives: (1) to identify plant-species pairs where *both* total RI and asymmetries in introgression have been documented (either within the same publication or across separate studies) and (2) to test whether asymmetric total RI is predictive of the direction of introgression. These findings ultimately suggest that asymmetrical RI is not predictive of introgression directionality, highlighting the need for empirical

studies on introgression patterns in natural populations to avoid oversimplified assumptions based solely on RI asymmetry.

2. Materials and Methods

Naturally hybridizing plant species (i.e. not "ecotypes" or other sub-species designations) appropriate for this meta-analysis were identified in a two-step process. First, studies of species pairs must have included at least one measure of prezygotic isolation, at least one measure of postzygotic isolation, and bi-directional calculations of total isolation all reported within a single manuscript. Second, these same species pairs also needed documented evidence of asymmetric introgression, either within the same manuscript described above or in other publications.

Christie et al. (2022) [16] compiled a comprehensive dataset of studies conducted before January 15, 2021 that satisfied the first criterion. Total RI calculations derived from those studies are presented in Table 1 (See Table S1 for calculations of Total RI based on methods by Sobel and Chen (2014) [13]). Additional studies meeting the first criterion and published from January 2021 to 30 May, 2024 were also identified by using the *Google Scholar* "cited by" link to the Christie et al. (2022) [16] review, as well as the Lowry et al. (2008) [15] and Baack et al. (2015) [5] reviews. The *Google Scholar* database from 2021 onward (up until 30 May, 2024) was additionally searched using combinations of the phrases "reproductive isolation," "plants," "prezygotic barriers," "postzygotic barriers," "total isolation," "prezygotic isolation," and "postzygotic isolation." These are also presented in Table 1.

Table 1. Total reproductive isolation measured using methods by Sobel and Chen (2014) [13]. $RI_{species1}$ indicates total RI calculated with species 1 – the species with the highest measure of total RI - as the seed parent, and $RI_{species1}$ indicates total RI calculated with species 2 as the seed parent. The predominant direction of asymmetric introgression is indicated in the last column, as well as citations for total RI and introgression measures.

Species 1	Species 2	$RI_{species1}$	$RI_{species2}$	Introgression Direction
Iris douglasiana	*Iris innominata*	1.0	0.72975	Species 2 [37,50]*
Ipomopsis tenuituba	*Ipomopsis aggregata*	0.87208889	0.4684096	Species 2 [33,34]*
Penstemon centranthifolius	*Penstemon spectabilis*	0.97913942	0.47501345	Species 2 [40,41]*
Mimulus cardinalis	*Mimulus lewisii*	0.99842332	0.98956871	Species 2 [11,38]*
Costus pulverulentus	*Costus scaber*	1.0	0.99754474	Species 2 [44,51]*
Pinus yunnanensis	*Pinus densata*	0.556039	0.464428	Species 2 [52,53]*
Pinus tabuliformis	*Pinus densata*	0.739177	0.612348	Species 2 [52,53]*
Primula beesiana	*Primula bulleyana*	1.0	0.61760791	Species 2 [30]*
Primula secundiflora	*Primula poissonii*	0.961773	0.62171704	Species 2 [45]*
Quercus mogolica	*Quercus liaotungensis*	0.3808	0.123289	Species 2 [35,36]*
Ipomoea lacunosa	*Ipomoea cordatotriloba*	0.607344	0.490876	Species 2 [42,43]
Mimulus glaucescens	*Mimulus guttatus*	0.632	0.39	Species 2 [39]
Mimulus guttatus	*Mimulus nasutus*	0.98973968	0.16531978	Species 1 [12]*
Helianthus petiolaris	*Helianthus annuus*	0.99989231	0.99979006	Species 1 [54]*
Centaurium erythraea	*Centaurium littorale*	0.98969618	0.98601173	Species 1 [46]*
Ophrys incubacea	*Ophrys garganica*	1.0	0.86108599	Species 1 [55,56]*
Primula vulgaris	*Primula elatior*	0.93715411	0.88941822	Species 1 [18,49]*
Silene yunnanensis	*Silene ascelepiadae*	0.7954445	0.685	Species 1 [48]*
Primula vulgaris	*Primula veris*	0.91873543	0.654731	Species 1 [49,57]*

* Total isolation derived from RI measures reported by Christie et al. (2022) [16] – See Table S1.

Once species pairs meeting criterion 1 were identified, a comprehensive search of *Google Scholar* was again conducted in order to determine whether additional studies were published that examined introgression between the species pairs identified above and, if so, to ascertain any asymmetries with respect to such gene flow. For each species pair, relevant literature was identified by performing a three-word search combining the genus and both specific epithets. This approach was necessary as some authors did not use the full species names for both taxa and may have abbreviated the genus name for one or the other resulting in their studies not appearing in a *Google Scholar* search that

utilized the unabbreviated names of both species. The resulting papers were examined to determine whether any asymmetry in gene flow existed. For some species pairs, the initial search produced an unwieldy number of results. For these taxa, the search was further narrowed by incorporating additional combinations of the keywords "introgression," "gene flow," and "asymmetric."

3. Data Analysis

To assess whether asymmetries in total RI were predictive of asymmetries in introgression, a two-tailed binomial test was performed using the *binom.test* function in Program R. For each species pair with identified asymmetries in both total RI and introgression, whether or not those asymmetries were in the same or opposite directions was recorded. For this analysis, a 'success' was recorded when RI and introgression occurred in the same direction, and a 'failure' was recorded when the direction was in opposite directions. The null hypothesis was that there was no relationship between asymmetries in reproductive isolation and asymmetries in introgression (e.g., the directionality of RI was not predictive of the directionality of introgression; probability of success = 0.5).

4. Results and Discussion

This study investigated whether asymmetries in total reproductive isolation (RI) were predictive of the direction of introgression in hybridizing plant taxa. A total of 19 species pairs were identified where published information existed for both total RI and asymmetric introgression (Table 1). The binomial test (N successes = 12, N trials = 19, p = 0.3593) showed no significant relationship between directionality of asymmetries in total RI and introgression, suggesting that introgression patterns are instead more often shaped by a combination of system-specific ecological, genetic, evolutionary and/or demographic factors.

In many of the systems identified here (N = 12/19), asymmetry in total RI corresponds with that of introgression, where the species serving primarily as the maternal parent also tends to receive more introgressed genetic material from the species that serves as the pollen parent (Table 1). In a majority of these cases (8 of 12), the direction of introgression appears to be driven primarily by demographic factors such as relative species abundances, range expansions of one species into the habitat of another, or spatial shifts in hybrid zones. For instance, in *Primula*, directional introgression from *P. beesiana* into P. *bulleyana* was attributed primarily to a greater abundance of *P. bulleyana* in hybridizing populations, which facilitated increased amounts of pollinator-mediated backcrossing towards *P. bulleyana* [30]. In an *Ipomopsis* hybrid zone, asymmetric introgression from *I. tenuituba* into *I. aggregata* was attributed to *I. aggregata* advancing into *I. tenuituba* habitats facilitated by pollinator behavior and habitat selection on hybrids [31–34]. Similarly, in *Quercus*, alleles from *Q. mongolica* were found to have introgressed into *Q. liaotungensis*, likely resulting from northward migration of *Q. liaotungensis* into already-colonized *Q. mongolica* habitats during warmer climatic periods [35]. Asymmetric introgression in two Pinus hybrid zones was attributed to unidirectional pollen flow and historical range shifts influenced by geological and historical climatic changes [36]. Similar patterns are also observed in *Iris*, where introgression of chloroplast DNA from *I. innominata* into *I. douglasiana* was attributed to hybrid zone movement [37]. In *Mimulus*, asymmetric introgression from *M. cardinalis* into *M. lewisii* has been reported, though it is unclear if such introgression is due to range expansion or the spread of adaptive alleles via natural selection [38]. Similarly in *M. glaucescens* and *M. guttatus* hybridizing populations, gene flow from *M. glaucescens* into *M. guttatus* was attributed to increased migration rates of the former, though selective costs of introgressed *M. guttatus* alleles into *M. glaucescens* backgrounds were not ruled out [39].

The remaining studies where asymmetry in total RI corresponded with the direction of introgression (N = 4/12) suggested alternative explanations beyond demographic factors, including natural selection, differences in mating systems, or no clear mechanism for explaining the observed asymmetries. For example, in *Penstamon*, introgression from *P. centranthifolius* into *P. spectabilis* was been observed [40], with significant reductions in seed number and seed mass being observed in

backcrosses towards *P. centranthifolius* (but not towards *P. spectabilis*) posited as a possible driver of this asymmetry [41]. Selection on floral traits and mating system differences likely explain asymmetric introgression from the predominantly selfing *Ipomopsis lacunosa* into the outcrossing *I. cordatotriloba* [42,43]. In the hybridizing systems involving *Costus pulverulentus* and *C. scaber* [44] and *Primula poissonii* and *P. secundiflora* [45], the directionality of asymmetric total RI aligned with introgression patterns, though no clear mechanism for these asymmetries were proposed.

The remaining study systems examined showed contrasting patterns (N = 7/19), where asymmetries in total RI and introgression occurred in *opposite* directions. In these cases, the species serving primarily as the maternal parent received gene flow *less* frequently than in the reciprocal direction. For example, in *Centaurium*, total RI favored F_1 hybrid production with *C. littorale* as the maternal parent and *C. erythraea* as the paternal parent (Table 1, $RI_{C.erythraea}$ = 0.794, 0.989; $RI_{C.\ littorale}$ = 0.775, 0.986, Table 1). However, introgression occurred predominantly into *C. erythraea*, a pattern that was largely attributed to differences in mating systems, with *C. littoral* exhibiting higher rates of selfing, and F_1 and late-generation hybrids being more likely to mate with the outcrossing species *C. erythrea* [46,47]. Similarly, asymmetric introgression in *Mimulus* occurred predominantly from the selfing *M. nasutus* into the largely outcrossing *M. guttatus*, this despite total RI being complete when *M. guttatus* acted as the F_1 pollen parent ($RI_{M.guttatus}$ = 1.0, Table 1) [12,20]. In protandrous *Silene* species, flowering asynchrony was identified as a primary driver of asymmetric total RI ($RI_{S.asclepiadae}$ = 0.685, $RI_{S\ yunnanensis}$ = 0.795). *Silene asclepiadae* flowering precedes that of *S. yunnanensis*, and late-flowering *S. asclepiadae* are more likely to serve as seed parents during F1 hybrid formation. However, the flowering times of hybrids are most similar to those of *S. yunnanensis* allowing for more backcrossing and introgression towards this species [48]. In *Primula*, total RI was higher when *P. vulgaris* was the maternal parent compared to *P. elatior* ($RI_{P.vulgaris}$ = 0.937m $RI_{P.elatior}$ = 0.889), and similarly higher in comparisons between *P. vulgaris* and *P. veris*, RI was also higher with *P. vulgaris* as the maternal parent ($RI_{P.vulgaris}$ = 0.919, $RI_{P.veris}$ = 0.655) [18]. Subsequent genomic analysis revealed likely adaptive mechanisms favoring directional introgression from *P. elatior* and *P. veris* into *P. vulgaris* across multiple hybrid zones, including increased fertility and improved tolerance to iron-rich waterlogged soils [49].

Collectively, these findings highlight the critical roles of ecological, genetic, and demographic factors in shaping the mating patterns and fitness of F_1 and later generation hybrids, which ultimately influence patterns of introgression. Notably, the directionality of asymmetric RI does not reliably predict the direction of introgression. Although studies that measure reproductive isolation are important for identifying key barriers to initial hybridization, they offer limited insight into the direction of subsequent introgression.

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