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Review

The strength and genetic basis of reproductive isolating barriers in flowering plants

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Speciation is characterized by the evolution of reproductive isolation between two groups of organisms. Understanding the process of speciation requires the quantification of barriers to reproductive isolation, dissection of the genetic mechanisms that contribute to those barriers and determination of the forces driving the evolution of those barriers. Through a comprehensive analysis involving 19 pairs of plant taxa, we assessed the strength and patterns of asymmetry of multiple prezygotic and postzygotic reproductive isolating barriers. We then reviewed contemporary knowledge of the genetic architecture of reproductive isolation and the relative role of chromosomal and genic factors in intrinsic postzygotic isolation. On average, we found that prezygotic isolation is approximately twice as strong as postzygotic isolation, and that postmating barriers are approximately three times more asymmetrical in their action than premating barriers. Barriers involve a variable number of loci, and chromosomal rearrangements may have a limited direct role in reproductive isolation in plants. Future research should aim to understand the relationship between particular genetic loci and the magnitude of their effect on reproductive isolation in nature, the geographical scale at which plant speciation occurs, and the role of different evolutionary forces in the speciation process.

Keywords: speciation; asymmetry; Bateson–Dobzhansky–Muller incompatibilities; chromosomal rearrangements; quantitative trait loci

1. INTRODUCTION

A formidable challenge in evolutionary biology is to understand how and why reproductive isolating barriers arise during speciation (Dobzhansky 1937; Mayr 1942; Stebbins 1950; Clausen 1951; Grant 1981; Coyne & Orr 2004). To address these issues, a comprehensive study of closely related species should attempt to identify all reproductive barriers limiting hybridization and introgressive gene flow. Such studies should also quantify the individual effects of each barrier on reproductive isolation and genomic patterns of introgression (Coyne & Orr 1989; Ramsey *et al.* 2003; Nosil *et al.* 2005). In addition, studies should aim both to determine the precise molecular genetic basis for each barrier (Ting *et al.* 1998; Presgraves *et al.* 2003; Brideau *et al.* 2006; Masly *et al.* 2006; Bomblies *et al.* 2007) and to obtain a mechanistic understanding of the evolutionary forces that caused each barrier to evolve (Kelly & Noor 1996; Schluter 2001; Rundle & Nosil 2005; Orr *et al.* 2007). While such ambitious goals have yet to be achieved for any single pair of species, substantial progress has been made during the last decade towards addressing the ecological and genetic basis of reproductive isolation.

A major goal of speciation biology is to determine the relative importance of different types of reproductive isolating barriers (Ramsey *et al.* 2003; Coyne & Orr 2004; Nosil *et al.* 2005; Martin & Willis 2007; Cozzolino & Scopece 2008). Plant evolutionary biologists have recently made major progress in quantifying the individual strengths of a suite of reproductive isolating barriers in a number of different plant systems (Chari & Wilson 2001; Ramsey *et al.* 2003; Husband & Sabara 2004; Kay 2006; Martin & Willis 2007; Lowry *et al.* in press). For the most part, these studies support the assertion that prezygotic barriers make a greater overall contribution than postzygotic barriers to total reproductive isolation. This argument is based on the observation that prezygotic barriers are often individually stronger than postzygotic barriers and that prezygotic barriers act earlier over the life history of an organism than postzygotic barriers (Ramsey *et al.* 2003; Coyne & Orr 2004; Nosil *et al.* 2005; Kay 2006). However, there are still only a handful of studies that have quantified the

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strengths of individual reproductive isolating barriers in plants. Many other previous studies have involved field and laboratory experiments that contain data that are yet to be quantified and compiled for a comprehensive analysis of the evolution of reproductive isolation in plants (but see Nosil *et al.* 2005 for a comparable review that includes plants and animals). These data have the potential to determine the relative importance of prezygotic and postzygotic barriers. Such data could also address a number of additional questions regarding the strength of reproductive isolating barriers, such as how often are single barriers sufficient to completely prevent gene flow, what is the average strength of reproductive isolation when the strength of all barriers is combined and is the strength of reproductive isolating barriers dependent on the direction in which hybridization occurs? In particular, asymmetries in the strength of barriers that depend on the direction of gene exchange can provide an insight into the forces and mechanisms that drive reproductive isolation (Arnold *et al.* 1996; Tiffin *et al.* 2001; Takami *et al.* 2007). For example, asymmetries in intrinsic postzygotic isolation are probably caused by incompatibilities involving cytonuclear or gametophyte–sporophyte interactions, as well as patterns of maternal or paternal silencing that differ between reciprocal crosses (Tiffin *et al.* 2001; Turelli & Moyle 2007). On the contrary, asymmetries in intrinsic postzygotic barriers are not likely to be caused by chromosomal rearrangements or deleterious epistatic incompatibilities of nuclear genes, owing to the nature of action of these genetic mechanisms of reproductive isolation. While previous studies have found that individual postmating barriers are frequently asymmetric in their action (Tiffin *et al.* 2001; Turelli & Moyle 2007), it is unclear whether such asymmetries also occur for premating barriers.

Recent advances in molecular techniques are beginning to enable researchers to identify the particular genomic regions and genes responsible for reproductive isolating barriers in plants (Josefsson *et al.* 2006; Sweigart *et al.* 2006; Bomblies *et al.* 2007; Rieseberg & Willis 2007; Case & Willis 2008; see also Lexer & Widmer 2008). This knowledge of the genetic mechanisms of reproductive isolation promises to provide an insight into how reproductive isolating barriers may evolve. Importantly, the genetic architecture of reproductive isolation can control the genome-wide pattern of introgression among hybridizing species, since genomic regions tightly linked to reproductive isolation genes will not introgress across species boundaries at the same rate as other regions of the genome (Wu 2001; Wu & Ting 2004; Turner *et al.* 2005). Therefore, a more complex genetic architecture may reduce the rate of gene flow across the entire genome to a greater extent than would a barrier governed by only a few major loci. Currently, it is unclear whether the evolution of reproductive isolating barriers is under simple genetic control or due to more complex additive and/or epistatic control, involves loci of major or minor effect, and whether genetic architecture is dependent on the type of reproductive isolating barrier. In addition, the relative role of genic changes versus the chromosomal rearrangements in the evolution of intrinsic postzygotic reproductive isolation (Stebbins 1950, 1958; Coyne & Orr 2004; Gottlieb 2004) now appears to be on the verge of resolution with the implementation of modern molecular techniques.

Here, we conduct a comprehensive literature review to address two broad questions regarding the strength and genetic basis of reproductive isolating barriers in plants. First, are prezygotic barriers stronger than postzygotic barriers, and do they differ in their level of asymmetry? Second, is the genetic architecture of reproductive isolating barriers generally simple or complex, and what is the relative role of chromosomal rearrangements versus genic changes in the formation of these barriers? Our goal with this review is to evaluate the state of current progress in regard to the quantification of the strength of reproductive isolation, as well as the genetic and genomic basis of isolating barriers and to propose future avenues of research.

2. ANALYSIS OF THE STRENGTH AND GENETIC BASIS OF REPRODUCTIVE ISOLATION

(a) *Strength of reproductive isolation literature survey*

We conducted a literature survey to broadly characterize the individual contribution of different reproductive isolating barriers in plants, and to test whether postmating barriers act more asymmetrically than premating barriers. We used a few key criteria in order to select study systems for inclusion in our analysis. Primarily, we required that reproductive isolating barriers be described in a manner that allowed for direct quantification. Some study systems were discarded because only a qualitative description of barrier strength was given. For example, we did not include studies where authors made statements such as 'sister species shared pollinators' or 'species had overlapping flowering times'; the actual quantification of floral visitation and phenology was required for inclusion. Each included system also had to have been evaluated for at least one prezygotic and one postzygotic barrier, so that we could compare the magnitude of prezygotic and postzygotic barriers. We excluded studies of crosses among distantly related species, such as those across genera, since these barriers may have evolved after speciation occurred. We also excluded studies involving changes in the level of ploidy or hybrid origin (e.g. Husband & Sabara 2004; Lowe & Abbott 2004), even though these studies otherwise met our criteria. After identifying suitable study systems, we conducted additional searches for papers that quantified the strengths of other reproductive isolating barriers within those systems. In total, we identified 19 study systems that met these criteria (table 1).

Of the 19 study systems, only four (Ramsey *et al.* 2003; Kay 2006; Martin & Willis 2007; Lowry *et al.* in press) quantified the strength of reproductive isolating barriers. Unfortunately, the formulae used to calculate the components of reproductive isolation often differed among these studies. To make estimates of reproductive isolation in those four systems comparable with each other as well as with estimates from the remaining 15 systems, we recalculated the components of reproductive isolation (tables S1–S3, electronic supplementary material). Using consistent

Table 1. Strength of reproductive isolating barriers in flowering plant systems, in which multiple barriers have been measured. (Type identifies the pair of species, varieties or ecotypes between which barriers were measured. Long dash indicates an absence of data available for a given barrier. A designation of 'low' is given for those barriers in which the qualitative data suggest that the barrier is not strong. References and notes on the calculations are given in table S2 of the electronic supplementary material.)

system	type	geography	immigrant inviability	phenology	pollinator	mating system	pollen competition	F_1 seed formation	F_1 seed germination	F_1 viability	F_1 male fertility	F_1 seed set	extrinsic postzygotic	total prezygotic	total postzygotic
Artemisia tridentata	basin	—	0.727	—	—	—	—	—	—	—	—	—	0.909	0.850	0.773
	mountain	—	0.973	—	—	—	—	—	—	—	—	—	0.636		
Chamaecrista desvauxii	var. *graminea*	—	—	0.467	low	—	—	1.000	—	—	—	—	—	0.301	1.000
	var. *latistipula*	—	—	0.135	low	—	—	1.000	—	—	—	—	—		
Costus	*scaber*	0.478	—	0.166	0.769	—	—	0.801	0.297	—	−0.016	—	—	0.946	0.881
	pulverulentus	0.348	—	0.230	1.000	—	—	0.976	−0.433	—	0.021	—	—		
Gelsemium	*sempervirens*	—	—	0.963	low	—	—	0.613	—	—	—	—	—	0.964	0.802
	rankinii	—	—	0.964	low	—	—	0.990	—	—	—	—	—		
Gilia capitata	coast	—	0.870	—	—	—	—	0.750	—	—	—	—	—	0.749	0.875
	inland	—	0.628	—	—	—	—	1.000	—	—	—	—	—		
Helianthus exilis	serpentine	—	0.953	low	—	—	—	0	—	—	—	—	—	0.971	0
	riparian	—	0.989	low	—	—	—	0	—	—	—	—	—		
Helianthus	*annuus*	—	—	0.410	—	—	0.970	−0.022	—	—	0.949	0.991	—	0.989	1.000
	petiolaris	—	—	0.291	—	—	0.996	−0.021	—	—	0.949	0.991	—		
Ipomopsis	*aggregata*	—	1.000	low	0.732	—	0.487	—	—	—	—	—	0.247	0.988	0.278
	tenuituba	—	0.923	low	0.711	—	−0.674	—	—	—	—	—	0.308		
Iris (1)	*douglasiana*	—	1.000	0.160	—	—	—	−0.702	—	—	—	—	—	0.942	−0.564
	innominata	—	0.864	0.125	—	—	—	−0.425	—	—	—	—	—		
Iris (2)	*chrysophylla*	—	—	0.339	—	—	—	−0.200	—	—	—	—	—	0.177	0.315
	innominata	—	—	0.014	—	—	—	0.829	—	—	—	—	—		
Iris (3)	*fulva*	—	0.061	0.322	0.634	—	1.000	1.000	0.044	−0.124	—	−0.150	−0.030	0.961	0.432
	hexagona	—	0.083	0.499	0.634	—	0.611	0.196	0.026	−0.331	—	−0.137	−0.056		
Iris (4)	*fulva*	—	−0.119	0.934	0.534	—	0.696	—	—	—	—	−1.112	−0.045	0.752	−2.063
	brevicaulis	—	−0.052	0.782	−1.000	—	−1.306	—	—	—	—	−2.500	−0.138		
Mimulus (1)	*lewisii*	0.587	0.994	—	0.950	—	0.958	0.405	0.203	−1.392	0.662	0.409	—	1.000	0.878
	cardinalis	0.587	0.874	—	1.000	—	0.281	0.489	0.047	0.056	0.628	0.737	—		
Mimulus (2)	*guttatus*	—	—	0.415	—	—	0.892	—	0.275	0.190	0.705	0.686	—	1.000	0.634
	nasutus	—	—	0.584	—	0.999	0.000	—	−0.018	−0.042	0.167	−0.294	—		
Mimulus (3)	*guttatus*	—	—	low	0.939	—	—	0.958	—	—	—	—	—	0.947	0.958
	nudatus	—	—	low	0.955	—	—	0.958	—	—	—	—	—		
Mimulus guttatus	coast	—	0.874	1.000	—	—	—	—	—	0.002	—	—	−1.800	1.000	−0.780
	inland	—	0.999	1.000	—	—	—	—	—	0.002	—	—	0.233		
Penstemon	*centranthifolius*	—	—	—	0.615	—	—	0.963	—	—	0.826	—	—	0.530	0.872
	spectabilis	—	—	—	0.444	—	—	0.524	—	−0.031	0.202	—	—		
Pedicularis	*rhinanthoides*	—	—	0.598	0.947	—	—	−0.076	—	—	—	—	—	0.975	0.462
	longiflora	—	—	0.634	0.921	—	—	1.000	—	—	—	—	—		
Phlox	*cuspidata*	—	0.222	low	—	—	−0.222	−0.433	—	—	0.908	1.000	—	0.888	0.974
	drummondii	—	0.158	low	0.826	—	0.630	0.851	—	—	0.664	0.694	—		

formulae, the components of reproductive isolation were calculated from raw data extracted from the text, tables and figures of papers as well as raw data supplied as personal communications from investigators of both previously published and unpublished studies. The strength of reproductive isolating barriers was quantified reciprocally when possible, using the rationale and methods outlined in Martin & Willis (2007) and Lowry *et al.* (in press; see the electronic supplementary material for details on calculations (table S1), notes and raw data involved in calculations (table S2) and calculations of reproductive isolation due to phenological differences among species (table S3)). In general, the quantification of prezygotic reproductive isolating barriers followed the form

$$\mathrm{RI}_{\mathrm{prezygotic}} = 1 - \frac{\text{observed/expected heterospecific matings}}{\text{observed/expected conspecific matings}},$$

where RI is the strength of reproductive isolation. Quantifying prezygotic barriers in this form allows for a direct comparison with postzygotic barriers (Martin & Willis 2007), which were quantified as

$$\mathrm{RI}_{\mathrm{postzygotic}} = 1 - \frac{\mathrm{F}_1 \text{ hybrid fitness}}{\text{mean parental fitness}}.$$

We restricted our assessment of postzygotic barriers to the F_1 generation, as quantification of reproductive isolation in more advanced generations is difficult, as it is often unclear how many naturally produced hybrids will be backcross or F_2 progeny. Furthermore, trait segregation in advanced generation hybrids, especially transgressive segregation (Rieseberg *et al.* 2003), means that the effects of postzygotic isolation will vary greatly among different genotypes within any single advanced generation hybrid class. Therefore, for advanced generation hybrids, it is much more meaningful to quantify how reproductive isolation acts on particular traits or loci (Barton & Bengtsson 1986; Wu 2001; Mallet 2005; Lexer & Widmer 2008). For extrinsic postzygotic isolation, only studies that assessed the fitness of hybrids in parental habitats were included in our analysis.

The relative contribution of individual barriers to total reproductive isolation allows for the direct comparison of barriers based on both the strength and the chronology in which they occur (Ramsey *et al.* 2003; Coyne & Orr 2004). However, we did not calculate the relative contribution of individual barriers to total reproductive isolation, in part owing to the conceptual difficulties in calculating these quantities outlined by Martin & Willis (2007) and because we were mainly interested in understanding the rate at which barriers arise during speciation. Instead, we calculated the individual contribution of each barrier averaged over the study systems. For any given species pair, all barriers have had the same time to evolve since the start of the species' divergence. A comparison of the individual contribution of barriers for a particular system reveals those barriers that have increased the most during the period of divergence, and thus those barriers that have evolved the fastest.

Overall, we were able to quantify 80 barriers (40 prezygotic and 40 postzygotic) to gene flow, and calculate the degree of asymmetry for 71 of those barriers. For the nine remaining barriers, we were unable to calculate the degree of asymmetry because the barrier strength had only been measured for one direction of gene flow or only an average value was provided. We also recorded qualitative descriptions of barrier strengths for the 19 selected systems that were not quantified by authors (table 1). These qualitative values were not included in further analysis. For each study system, we calculated the total combined strength of prezygotic and postzygotic barriers, following the methods for determining total isolation presented in Ramsey *et al.* (2003). In order to determine whether there is a difference in the strength of prezygotic and postzygotic barriers, we conducted a paired *t*-test to compare the total strength of prezygotic and postzygotic isolation in the 19 study systems. We calculated the asymmetry of each barrier as the absolute value of the difference between the strengths of a given barrier for both crossing directions. A *t*-test, which included all the calculated values of asymmetries, was used to determine whether premating and postmating barriers differed in their degree of asymmetry. While there are issues associated with the assumption of independence, such as the use of multiple values from each study system, each value was treated as an independent sample (table S4, electronic supplementary material). In assuming independence, we were able to combine multiple means across taxa to compare trends in the levels of asymmetry across premating and postmating barriers.

(b) *Genetic basis of reproductive isolation literature survey*

We surveyed the current literature for studies that used rigorous crosses or modern molecular techniques to establish the genetic architecture or identify a gene or pathway causal to phenotypes with a plausible role in reproductive isolation. We did not include studies that investigated the genetic basis of reproductive isolation among polyploids and diploids (e.g. Josefsson *et al.* 2006, submitted), even though the genetic basis of reproductive isolation between tetraploids and diploids is clearly relevant to the evolution of plant species, given the preponderance of polyploidization in plants (Otto & Whitton 2000). Our logic is that the lethality of F_1 hybrids between diploids and polyploids, frequently referred to as the triploid block, is likely to be fundamentally different from that of incompatibilities in crosses among diploid species (Ramsey & Schemske 1998, 2002). Overall, we identified a total of 25 studies that fit our criteria across 10 genera. We restricted our evaluation to the genetic architecture of reproductive isolating barriers and the role of underdominant chromosomal rearrangements (e.g. Stebbins 1958). Such rearrangements are underdominant because individuals heterozygous for rearrangements produce aneuploid gametes during meiosis, which in turn leads to hybrid sterility (Stebbins 1950, 1958; Grant 1981; Fishman & Willis 2001). It should be noted that we did not evaluate how chromosomal rearrangements might indirectly facilitate the accumulation of reproductive isolating barriers (Noor *et al.* 2001; Rieseberg 2001; Kirkpatrick & Barton 2006) or how gene duplications or transpositions can cause incompatibilities (Werth & Windham

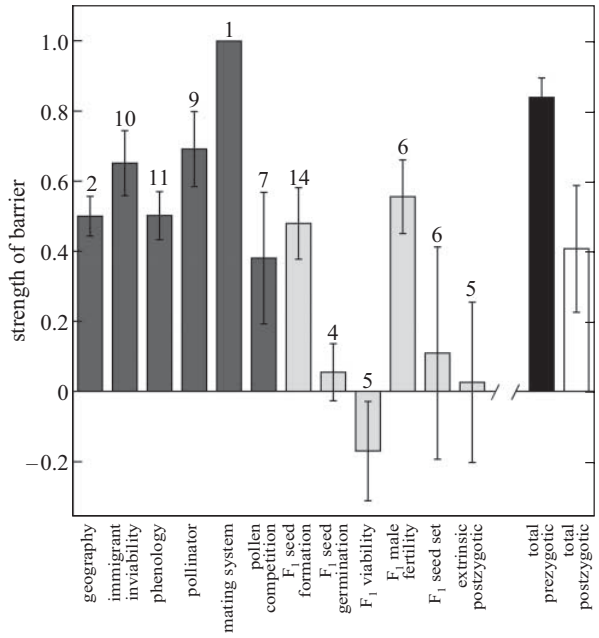

Figure 1. The individual strengths of prezygotic (dark grey) and postzygotic (light grey) reproductive isolating mechanisms compiled from 19 flowering plant systems in which at least two barriers were assayed. Values range from 0.0 for no isolation to 1.0 for complete isolation. Each bar indicates the mean ± s.e. and numbers above each bar indicate the sample size for the corresponding barrier.

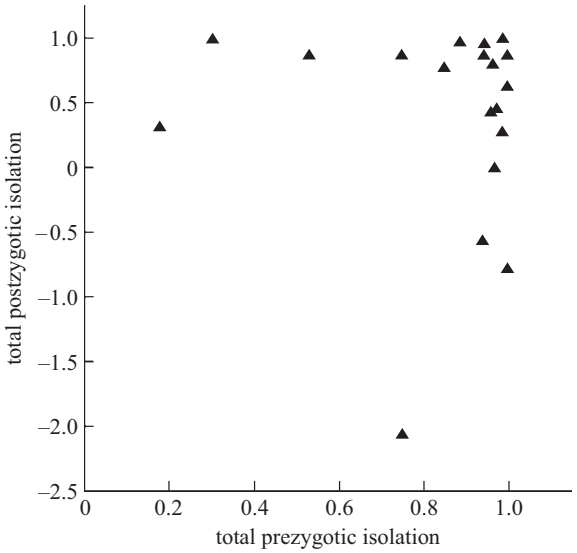

Figure 2. Comparison of total prezygotic isolation versus postzygotic isolation for 19 pairs of taxa. Note that the scale of the two axes is not identical. Negative values for total postzygotic isolation reflect hybrid performance that is greater than that of the parental taxa.

1991; Lynch & Force 2000; Masly *et al.* 2006), as there are few empirical studies that have thus far identified these phenomena in plants. For a complementary review of genetic differentiation among plant species in five model plant genera, see Lexer & Widmer (2008).

3. RESULTS OF ANALYSES

(a) *Strength of reproductive isolating barriers*

Through our analysis, we found that individual reproductive isolating barriers are rarely sufficient on their own to cause complete reproductive isolation. In fact, only 2 out of the 80 quantified barriers were equal to 1.000, while only 7 out of the 78 remaining barriers were greater than 0.950. Out of the 19 study systems, nine systems had an individual barrier greater than 0.950. However, the combination of reproductive isolating barriers led to a reproductive isolation greater than 0.950 in 15 out of the 19 taxon pairs examined. Total isolation in the remaining four systems was 0.240 (*Iris* 4), 0.435 (*Iris* 2), 0.909 (*Iris* 1) and 0.940 (*Penstemon*). Overall, these results suggest that very strong reproductive isolation can result from strong individual isolating barriers, but may often require a combination of barriers.

When the strengths of individual reproductive isolating barriers were averaged over all systems, we found that multiple prezygotic barriers, including immigrant inviability, flowering time isolation and pollinator isolation were, on average, strong barriers to gene flow (greater than 0.500; figure 1; table 1). This contrasts with the strength of postzygotic barriers, where only F_1 male fertility had an average strength greater than 0.500 (figure 1).

Recent studies of the components of reproductive isolation in single systems have suggested that prezygotic barriers are stronger than postzygotic barriers (Ramsey *et al.* 2003; Nosil *et al.* 2005; Kay 2006; Martin & Willis 2007; Lowry *et al.* in press). Our comparison of total prezygotic with total postzygotic isolation across study systems supports this hypothesis. Overall, the mean (±s.e.) strength of total prezygotic isolation (0.838 ± 0.056) was, on average, twice as strong as the total strength of postzygotic isolation (0.407 ± 0.181, paired *t*-test, d.f. = 18, $p = 0.0375$). Although this pattern is striking there are exceptions, as postzygotic barriers sometimes are stronger than prezygotic barriers (e.g. Costa *et al.* 2007; figure 2). Interestingly, the strengths of total prezygotic or postzygotic isolation, but not necessarily both, were very strong (greater than 0.750; figure 2) for all but one system we examined (*Iris* 2; Young 1996). While our results show that prezygotic isolation tends to be greater than postzygotic isolation in plant species, our results also highlight the great diversity of barriers that reproductively isolate plant species, as has been noted in the classic plant evolutionary literature (Clausen 1951; Stebbins 1958; Grant 1981).

(b) *Asymmetries in reproductive isolating barriers*

In our analysis, the most asymmetrically acting barriers were pollen competition, F_1 viability and F_1 seed set, which had a degree of asymmetry greater than 0.500 (figure 3; table S4, electronic supplementary material). Phenological isolation and immigrant inviability were nearly symmetric in their action, with values of asymmetry less than 0.150. Overall, the results of our analysis were consistent with previous studies (Tiffin *et al.* 2001; Turelli & Moyle 2007), in that postmating barriers are highly asymmetric and

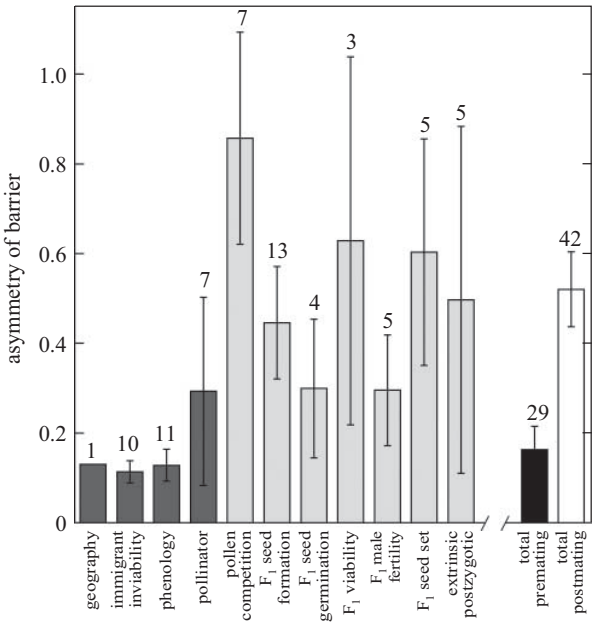

Figure 3. Asymmetry of reproductive isolating barriers, calculated for individual barriers as the absolute value of the difference in strength between the direction of potential matings or crosses, for premating (dark grey) and postmating (light grey) barriers. Values of zero indicate that the barrier acts symmetrically regardless of the direction of the cross or potential mating, and the upper bound is unlimited. Bars indicate mean $\pm$ s.e., and numbers above each bar indicate the sample size for each barrier.

significantly greater than zero (0.520 ± 0.08, *t*-test, $p < 0.0001$). Interestingly, while asymmetries of premating barriers were also significantly greater than zero (0.163 ± 0.05, *t*-test, $p = 0.0040$), they were, on average, three times less asymmetric than postmating barriers (*t*-test, d.f. $= 69$, $p = 0.0017$).

(c) *The genetic architecture of reproductive isolation*

The number of loci involved in prezygotic and postzygotic reproductive isolating barriers varied both among systems and among traits (range 1–17 loci; table 2). We found no clear differences in the genetic architecture of prezygotic and postzygotic barriers (table 2). Loci of large effect (more than 20% of the phenotypic variance or parental divergence) were found in a vast majority of these studies (table 2), and many barriers involve loci that explain over 50% of the parental divergence in traits thought to play a role in reproductive isolation (Bradshaw *et al.* 1998; Bouck *et al.* 2007).

Of all the prezygotic barriers to gene flow, the genetic basis of pollinator isolation is the most represented in our survey (table 2). This is probably the product of a historical emphasis by evolutionary biologists on the importance of pollinator isolation (Prazmo 1965; Grant 1994; Schemske & Bradshaw 1999), and the fact that flower colour derived from anthocyanins involves a simple and well-characterized genetic pathway (Dooner *et al.* 1991; Holton & Cornish 1995; Koes *et al.* 2005; Grotewold 2006). Major genes involved in flower colour evolution have been identified in *Antirrhinum* (Schwinn *et al.* 2006) and *Petunia* (Hoballah *et al.* 2007). It is not surprising then that several studies suggest that pollinator isolation can have a very simple genetic basis (table 2). For example, the introgression of a single flower colour locus (*YUP*) among bee-pollinated *Mimulus lewisii* and hummingbird-pollinated *Mimulus cardinalis* through reciprocal backcrosses was sufficient to cause major shifts in pollinator visitation in the field (Bradshaw & Schemske 2003). A separate quantitative trait locus (QTL) accounted for 41% of the parental divergence of nectar volume between these species in the greenhouse, and led to a twofold change in hummingbird visitation in an experimental F_2 population placed into the field (Schemske & Bradshaw 1999).

While pollinator isolation is clearly an important component of reproductive isolation, phenological isolation, immigrant inviability and mating system isolation can be just as important prezygotic barriers to gene flow (figure 1). However, only a handful of studies have been able to progress towards understanding the genetic basis of these prezygotic barriers. Recent work, using coast and inland ecotypes of *Mimulus guttatus*, has determined that flowering time and morphological divergence involve two pleiotropic loci of large effect, coupled with many loci of small effect (Hall *et al.* 2006). These genetic changes in flowering time contribute not only to strong phenological isolation among coast and inland populations, but also to habitat-mediated selection against immigrants moving from the coastal habitat into the inland habitat (table 1; Hall & Willis 2006; Lowry *et al.* in press).

Recent studies have also made major progress in understanding the genetic basis of postzygotic isolation in plants. Researchers working with *Mimulus* and *Arabidopsis* have fine-mapped (Sweigart *et al.* 2006) and even cloned (Bomblies *et al.* 2007; Case & Willis 2008) genic incompatibilities. In crosses between *M. guttatus* and *Mimulus nasutus*, two major interacting nuclear loci as well as other minor factors control hybrid male sterility (Sweigart *et al.* 2006). In the same cross, pollen fertility is also affected by a simple two-locus cytonuclear interaction (Fishman & Willis 2006; Case & Willis 2008), indicating that two-locus systems can act independently in parallel to produce hybrid sterility. Furthermore, a recent review identified 35 independent examples of two-locus genic interactions that contribute to hybrid inviability or lethality in plants (Bomblies & Weigel 2007). These studies suggest that two-locus incompatability systems may be very common in plants, although a recent study in *Arabidopsis* found more complex interactions involved in postzygotic isolation (Josefsson *et al.* submitted).

(d) *The role of chromosomal rearrangements in intrinsic postzygotic isolation*

The relative role of genic factors and chromosomal rearrangements in reproductive isolation has been long debated among plant biologists (Stebbins 1950, 1958; Lewis & Roberts 1956; Grant 1981; Rieseberg 2001). Today, researchers believe that underdominant chromosomal rearrangements, which cause aneuploidy during meiosis in heterozygous hybrids, may have little

Table 2. Number, effect and type of loci affecting different reproductive isolating barriers between pairs of taxa, populations of the same species or ecotypes of the same species.

type of barrier	taxa	number of loci per trait	major effect?[a]	type	references
prezygotic					
immigrant inviability	*Hordeum spontaneum* (coast and inland ecotypes)	2–5	mixed	genic	Verhoeven *et al.* (2004)
immigrant inviability	*Mimulus guttatus* (mine and off-mine)	≥1	yes	genic	Macnair (1983) and Smith & Macnair (1998)
immigrant inviability, flowering time	*Mimulus guttatus* (coast and inland ecotypes)	5–16	yes	genic	Hall & Willis (2006) and Hall *et al.* (2006)
flowering time	*Iris fulva*, *I. brevicaulis*	15–17	mixed	genic	Martin *et al.* (2007)
pollinator	*Petunia integrifolia*, *P. axillaris*	≥1	yes	genic	Hoballah *et al.* (2007)
pollinator	*Iris fulva*, *I. brevicaulis*	1–9	yes	genic	Bouck *et al.* (2007) and Martin *et al.* (2008)
pollinator	*Aquilegia* sp.	1	yes	genic	Prazmo (1965)
pollinator	*Aquilegia formosa*, *A. pubescens*	1–2	unkno-wn	genic	Hodges *et al.* (2002)
pollinator	*Mimulus lewisii*, *M. cardinalis*	3–7	yes	genic	Bradshaw *et al.* (1995, 1998), Schemske & Bradshaw (1999) and Bradshaw & Schemske (2003)
pollinator	*Antirrhinum* sp.	1–3	yes	genic	Schwinn *et al.* (2006)
pollinator	*Solanum lycopersicum*, *S. habrochaites*	2–6	mixed	genic	Moyle (2007)
mating system	*Mimulus guttatus*, *M. nasutus*	11–15	no	genic	Fishman *et al.* (2002)
postmating prezygotic					
pollen tube failure	*Arabidopsis thaliana*, *A. lyrata*	n.a.	n.a.	genic	Escobar-Restrepo *et al.* (2007)
postzygotic					
hybrid inviability, hybrid lethality	multiple plant species	2	yes	genic	Bomblies & Weigel (2007)
hybrid lethality	*Arabidopsis thaliana*	2	yes	genic	Bomblies *et al.* (2007)
hybrid lethality	*Iris fulva*, *I. brevicaulis*	4	unclear	genic	Martin *et al.* (2005)
hybrid lethality	*Mimulus guttatus* populations	2	yes	genic	Christie & Macnair (1984) and Christie & Macnair (1987)
hybrid lethality	*Mimulus guttatus* (mine and off-mine)	>2	yes	genic	Macnair & Christie (1983) and Christie & Macnair (1987)
hybrid viability (ecological)	*Helianthus annuus*, *H. petiolaris*	3	no	genic	Lexer *et al.* (2003*a*,*b*)
hybrid sterility	*Mimulus guttatus*, *M. nasutus*	2	yes	genic	Sweigart *et al.* (2006, 2007)
hybrid sterility	*Mimulus guttatus*, *M. nasutus*	2	yes	genic	Fishman & Willis (2006) and Case & Willis (2008)
hybrid sterility	*Solanum lycopersicum*, *S. habrochaites*	8	yes	genic	Moyle & Graham (2005)
hybrid sterility	*Oryza sativa*	2	yes	genic	Li *et al.* (2007)
hybrid sterility	*Clarkia biloba*, *C. lingulata*	n.a.	n.a.	chromosomal rearrangements	Lewis & Roberts (1956) and Gottlieb (2004)
hybrid sterility	*Helianthus annuus*, *H. argophyllus*	>3	yes	chromosomal rearrangements	Quillet *et al.* (1995)
hybrid sterility	*Helianthus* hybrid species	11	yes	genic and chromosomal rearrangements	Lai *et al.* (2005)

[a] A locus was considered to be of major effect if the per cent variance explained or parental divergence is greater than 20%.

direct effect on the evolution of reproductive isolation (Rieseberg 2001; Gottlieb 2004). This is primarily because underdominant chromosomal rearrangements are only expected to cause hybrid sterility (Stebbins 1958; Grant 1981; Fishman & Willis 2001), which is only one of the many strong barriers to gene flow (figure 1). Recent evidence that hybrid sterility can be caused by genic factors instead of rearrangements (table 2; Fishman & Willis 2001; Sweigart *et al.* 2006; Chase 2007; Moyle 2007; Case & Willis 2008) further supports the conclusion that chromosomal rearrangements have a limited direct role in plant

speciation. Furthermore, even when chromosomal rearrangements are genetically linked to hybrid sterility, it is unclear whether rearrangements are the direct cause of hybrid sterility or whether genic incompatibilities located within rearranged regions are ultimately responsible (Rieseberg 2001). In *Helianthus* hybrids, for example, pollen sterility QTLs map to chromosomal rearrangements, but the direct causes of sterility are unclear (Quillet *et al.* 1995; Lai *et al.* 2005).

4. SYNTHESIS

(a) *Strength of reproductive isolating barriers*

Our review (figure 1; table S1, electronic supplementary material) indicates that, in plants, the combined total strength of prezygotic barriers is, on average, larger than the total strength of postzygotic barriers. This finding, coupled with the generally low strength of extrinsic postzygotic isolation, also suggests that prezygotic reproductive isolation may be the primary form of reproductive isolation that evolves during the early stages of plant speciation. Furthermore, prezygotic isolation is likely to be more important in reducing gene flow between species than our analyses of the individual strengths of barriers suggest. Because reproductive isolating barriers act sequentially, early-acting barriers will contribute more to total isolation than late-acting barriers with the same individual strength (Ramsey *et al.* 2003; Nosil *et al.* 2005). Thus, our calculations probably underestimate the magnitude by which prezygotic barriers outweigh postzygotic barriers in reducing contemporary interspecific gene flow.

It has recently been argued that there should be a positive relationship between immigrant inviability and extrinsic postzygotic isolation (Rundle & Whitlock 2001; Schluter 2001; Rundle 2002; Nosil *et al.* 2005). Unexpectedly, we found little support for this relationship in our analysis. Immigrant inviability was one of the strongest barriers to gene flow, consistent with the findings of Nosil *et al.* (2005). However, extrinsic postzygotic isolation was highly variable, and often weak or negative. This variability may be a general trend in plants, at least in the F_1 stage, where high levels of heterosis in hybrids can mask intrinsic and extrinsic postzygotic isolation that act on F_1 germination, viability and overall fitness (Rundle & Whitlock 2001; Rhode & Cruzan 2005; Lowry *et al.* in press). The high levels of heterosis in the F_1 stage can thus act to facilitate gene flow. It has been argued that extrinsic postzygotic isolation may be stronger in advanced generation hybrids (Rundle & Whitlock 2001). However, as mentioned above, the evaluation of reproductive isolation in advanced generation hybrids is complicated by genetic segregation, and thus the quantification of reproductive isolation in advanced generation hybrids should really be restricted to individual traits and loci (but see Milne *et al.* 2003). More field studies are clearly needed to determine the relative importance of extrinsic postzygotic isolation in the F_1 generation in plants as well as the strength of selection acting against the introgression of individual loci.

Geographical isolation may be a very important barrier in plant speciation, yet it was not estimated for most systems (Schemske 2000; table 1). Geographical isolation, also known as ecogeographic isolation, is defined as reproductive isolation due to limited contact among related taxa as a result of ecological range limits of those taxa (Schemske 2000; Ramsey *et al.* 2003; Angert & Schemske 2005; Lowry *et al.* in press). Both geographical isolation and immigrant inviability result from local adaptation and can thus be tested by reciprocal transplant experiments (Schemske 2000; Coyne & Orr 2004; Nosil *et al.* 2005). However, geographical isolation is a species-wide distributional measurement of reproductive isolation, while immigrant inviability focuses on gene flow among specific populations. A consistent measurement for geographical isolation has yet to be developed, and the quantification of geographical isolation is rare because it requires the tedious collection of species distribution data from the field or herbarium (Ramsey *et al.* 2003; Husband & Sabara 2004; Kay 2006; Lowry *et al.* in press).

While previous studies have frequently found asymmetries in postmating barriers (Tiffin *et al.* 2001), it has been uncertain whether premating barriers are asymmetric and, if so, to what degree. We found small but statistically significant asymmetries in the action of premating barriers. Even so, premating barriers were over three times less asymmetric in their action than postmating barriers. There are many hypotheses that could explain this pattern. Premating barriers, such as immigrant inviability, may only be weakly asymmetric if the two taxon pairs are locally adapted, and trade-offs prevent one taxon from exhibiting the highest fitness across all habitats. Furthermore, it is possible that premating barriers are less asymmetric than postmating barriers because the genetic basis of premating barriers is not dependent on the interaction of uniparentally inherited factors involved in prezygotic pollen–stigma interactions or silencing and cytonuclear interactions in hybrids (Tiffin *et al.* 2001; Fishman & Willis 2006; Turelli & Moyle 2007; Case & Willis 2008).

Large asymmetries in postmating-prezygotic pollen–pistil interactions may evolve as a result of divergence of style lengths, different degrees of pollen competition or perhaps even mate choice (Grant 1995; Tiffin *et al.* 2001; Skogsmyr & Lankinen 2002; Delph & Ashman 2006). Asymmetries in pollen competition, F_1 seed formation and F_1 seed set are probably the result of the evolution of unidirectionally inherited genic changes (Tiffin *et al.* 2001; Turelli & Moyle 2007). The evolution of asymmetries in incompatibilities may be the result of sexual selection on pollen–stigma interactions, genomic conflict (e.g. cytonuclear conflict), drift or a combination of these factors. The resultant asymmetries can be quite large and, in some cases, may actually facilitate hybridization, such as in situations where heterospecific pollen performs better than conspecific pollen (Ramsey *et al.* 2003; Aldridge & Campbell 2006). While the connection between asymmetries of reproductive isolating barriers and directionality of gene flow has yet to be shown in plants, it represents an interesting avenue for future research.

It is important to acknowledge that data were not available for every potential barrier to reproductive isolation in each of the 19 study systems (table 1). Accordingly, future studies should strive to quantify as many potential barriers as possible, even the ones that are thought to be weak, in order to understand how all mechanisms operate together to create reproductive isolation between species. In addition, we did not quantify the strength of postzygotic isolation after the F_1 generation due to difficulties in the interpretation of genetic segregation. However, recessive incompatibilities cannot be detected except in advanced generation hybrids (Coyne & Orr 2004). Furthermore, our quantification of reproductive isolating barriers represents a snapshot in evolutionary time for each study system and, as such, the current strength of barriers for a given pair of species cannot necessarily be interpreted as the order of barrier evolution (Nosil *et al.* 2005).

(b) *Genetic basis of reproductive isolating barriers*

The genetic architecture of reproductive isolating barriers may differ based on the nature of the underlying forces of evolution that cause their fixation. Prezygotic barriers and extrinsic postzygotic barriers are likely to be driven by natural selection (Schluter 2001; Coyne & Orr 2004; Rundle & Nosil 2005), so the genetic architecture of these barriers may reflect the adaptive landscape upon which they evolved (Fishman *et al.* 2002; Orr 2005; Rundle & Nosil 2005). For evolutionary changes in which intermediate phenotypes have a low relative fitness, such as in pollinator isolation, the genetic architecture is predicted to involve a few loci of large effect, coupled with many minor effect loci (Bleiweiss 2001; Colosimo *et al.* 2005; Steiner *et al.* 2007). However, if intermediate phenotypes are favourable, or there is an abundance of standing genetic variation for a particular barrier, such as in mating system evolution, then the genetic architecture may instead involve many loci of small effect (Fishman *et al.* 2002). In comparison, genic-based intrinsic postzygotic barriers should involve the interaction of at least two incompatible loci, as described by the Bateson–Dobzhansky–Muller (BDM) model (Coyne & Orr 2004). Overall, little is known regarding the evolutionary forces that drive the fixation of intrinsic postzygotic barriers in plants (but see Bomblies *et al.* 2007). The determination of the genetic architecture and mechanisms of isolating barriers is the first step towards understanding how these barriers will become fixed over time.

Our analysis of the genetic basis of reproductive isolation indicates that traits associated with reproductive isolating barriers in plants are controlled by a variable number of loci and often involve individual loci that account for more than 20% of the variation or parental divergence for a given phenotype. Independently acting pairs of BDM incompatibilities are often sufficient to cause genetic incompatibilities in plants (Sweigart *et al.* 2006; Bomblies & Weigel 2007; Bomblies *et al.* 2007; Case & Willis 2008). This contrasts with hybrid male sterility in animals, which very frequently requires complex interactions (Coyne & Orr 2004). However, it should be noted that the limited number of studies makes it difficult to draw any major conclusions regarding the genetic basis of reproductive isolation in plants. Furthermore, studies of the genetic architecture of traits associated with reproductive isolation may be compromised by small sample sizes, which can prevent the detection of small-effect QTLs and elevate the effect of large QTLs (Beavis 1994).

The genetic basis of some barriers, such as pollinator isolation, may actually be more complex than they initially appear because these barriers involve multiple traits. Recent studies suggest a simple genetic basis for pollinator isolation (Schwinn *et al.* 2006; Hoballah *et al.* 2007). However, these studies focused on the genetic basis of flower colour, while other factors such as morphology and nectar volume may also play a key role (Bradshaw *et al.* 1998; Schemske & Bradshaw 1999; Cozzolino & Scopece 2008). Field experiments are clearly necessary, as QTLs identified for floral traits in the laboratory do not necessarily correspond to pollinator preference QTLs in the field (Bouck *et al.* 2007; Martin *et al.* 2008). Furthermore, once genes underlying barriers are cloned, follow-up studies should aim to quantify the effect of particular alleles on the strength of reproductive isolating barriers in the field.

Immigrant inviability may have a very complex genetic architecture as local adaptation may involve multiple independent changes to a suite of environmental factors. In order to map and identify the genes involved in immigrant inviability, both the genotype and phenotype of recombinant hybrids need to be assessed in a reciprocal transplant design, so that associations between particular loci and survival are made in parental habitats. To our knowledge, only one study has carried out QTL mapping in a reciprocal transplant experiment (Verhoeven *et al.* 2004). In this study, two to five QTLs influenced immigrant viability, depending on the field site. The use of nearly isogenic lines in reciprocal transplants promises to be valuable for demonstrating the effect of particular genomic regions or loci on immigrant inviability.

Detecting trends in the evolution of postzygotic isolation can be difficult. Foremost, it is often unclear whether particular incompatibility loci actually contribute to speciation, since many of the incompatibility alleles thus far identified are highly polymorphic within and among populations (Christie & Macnair 1987; Bomblies & Weigel 2007; Bomblies *et al.* 2007; Sweigart *et al.* 2007; Case & Willis 2008; see also Lexer & Widmer 2008). Future studies should aim to determine whether the same types of genes are involved in segregating incompatibilities among populations when compared with those that are fixed among species. There may also be an ascertainment bias in our survey as the identification of simple two-locus incompatibilities, as most studies did not have sufficient power to detect complex epistatic interactions. Regardless, it should be kept in mind that simple two-locus incompatibilities will be much more effective in reducing gene flow, because a greater percentage of hybrids will be affected than for more complex incompatibilities. Finally, our analysis of the strength of reproductive isolating barriers (figure 1)

indicates that hybrid lethality and inviability, for which there are the most data (Macnair & Christie 1983; Bomblies & Weigel 2007; Bomblies *et al.* 2007), may play a limited role in overall reproductive isolation among pairs of taxa. As implied by the trends in relative importance of different types of postzygotic barriers (figure 1), future studies should focus on the genetics of reproductive isolation involving F_1 seed formation and F_1 fertility. The genetic basis of isolating barriers due to pollen–pistil interactions is virtually unknown and should also be the focus of future studies.

Chromosomal rearrangements were long thought to play a major role in plant speciation (Stebbins 1950, 1958; Grant 1981; Rieseberg 2001). The underdominant nature of incompatibilities caused by chromosomal rearrangements means that they most probably will spread through drift and inbreeding, and thus are unlikely to disperse via gene flow over large geographical scales (Lande 1979, 1985). Therefore, if chromosomal rearrangements are the prevailing mechanism of plant speciation, then speciation would have to be initiated and completed locally, as has been suggested by Levin (1993, 1995). However, underdominant rearrangements do not appear to play a major role in overall reproductive isolation in plants, primarily because they can only directly cause one reproductive isolating barrier, hybrid sterility. Even for hybrid sterility, additional studies are needed to determine the relative role of chromosomal rearrangements versus genic factors. The preponderance of genic factors in plant speciation, which can disperse more readily over a large geographical range (Kane & Rieseberg 2007), suggests that even if plant speciation is initiated locally, it can be completed at a larger geographical scale (Clausen 1951; Lexer & Widmer 2008; Lowry *et al.* in press).

Although the direct effect of underdominant chromosomal rearrangements may be relatively minimal, rearrangements that suppress recombination may facilitate the accumulation of genic incompatibilities, and thereby influence the evolution of reproductive isolating barriers. For instance, chromosomal inversions between closely related species can eliminate recombination within a local genomic region, thereby facilitating the persistence of reproductive isolating alleles in the face of gene flow (Noor *et al.* 2001; Rieseberg 2001; Kirkpatrick & Barton 2006). The relative importance of this indirect involvement of chromosomal rearrangements in the evolution of reproductive isolation remains unknown. Finally, the relative role of gene duplication and transposition (Werth & Windham 1991; Lynch & Force 2000; Masly *et al.* 2006) versus nucleotide changes as the cause of BDM incompatibilities is an important question that can only be resolved with the cloning of more reproductive isolating genes.

5. CONCLUDING REMARKS

Understanding the origin of species is contingent on determining the forces and mechanisms involved in the evolution of reproductive isolation. A critical next stage in plant speciation research will be to determine how prezygotic and postzygotic reproductive isolation alleles spread within species at various geographical scales to complete the process of speciation. Initial steps in this direction have been made with studies of polymorphism in incompatibility alleles in *Mimulus* and *Arabidopsis* (Christie & Macnair 1987; Bomblies *et al.* 2007; Sweigart *et al.* 2007; Case & Willis 2008; see also Lexer & Widmer 2008). The cloning of genes involved in plant speciation, followed by molecular population genetic analysis, will allow us to reach our ultimate goal: an understanding of the role of microevolutionary forces in the evolution of reproductive isolation.

Finally, efforts are needed to evaluate the effects of individual loci on reproductive isolating barriers under field conditions. Field-based studies investigating the genetics of pollinator isolation (Schemske & Bradshaw 1999; Bradshaw & Schemske 2003; Martin *et al.* 2008) and immigrant inviability (Verhoeven *et al.* 2004) have made notable progress, but have yet to demonstrate the involvement of particular genes in these barriers. Postzygotic isolation can be context dependent, and thus must also be examined under field conditions (Rundle & Whitlock 2001). Ultimately, we hope that researchers will be able to identify and quantify the contribution of individual DNA polymorphisms to changes in the strength of reproductive isolating barriers under field conditions. Experimentation in the field is critical, as the effect of loci can vary with environment (Weinig *et al.* 2003; Li *et al.* 2006). This is likely to be a difficult and long-term endeavour, as multiple genes can underlie single QTLs (Davis & Wu 1996) and multiple genetic changes may be required at each of those genes for trait evolution (McGregor *et al.* 2007). Fortunately, there are many excellent emerging model plant systems that offer the ideal combination of short generation times, genomic resources and ease of manipulation in the field (Wu *et al.* 2008; Lexer & Widmer 2008).

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