Speciation Genes

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Glossary

Bateson–Dobzhansky–Muller incompatibility Combination of alleles at two or more loci that interact epistatically to reduce the fitness of hybrid progeny.

***Cis*-regulatory variation** Polymorphisms affecting the transcription of a gene that are located within noncoding DNA sequences that are proximate to the gene itself.

Coalescence The merging of all descendant gene copies within a lineage at a single ancestral gene copy occurring within that lineage going back in time.

Divergent selection Selection that favors different optima for one or multiple traits in alternate environments or niches.

Ecological speciation The process by which barriers to gene flow evolve between populations as a result of ecologically based divergent selection.

Epistasis Dependency of the effects of an allele at one locus on the genotype at one or more additional loci.

Genome editing Targeted alteration of DNA through expression of factors that bind to specific nucleotide sequences and make double strand breaks proximate to them.

Genomic conflict A phenomenon where the proliferation or non-Mendelian inheritance of selfish genetic elements occurs at the expense of the host genome, which may in turn evolve to suppress the deleterious effects of those elements.

High-throughput genotyping Determination of the allelic composition at thousands of loci for hundreds to thousands of individuals using next-generation sequencing or oligonucleotide array-based technologies.

Hybrid zone Geographic region where two species ranges intersect and interbreeding naturally yields hybrid offspring at some frequency.

Incipient species Populations that share a recent common ancestor and that are partially reproductively isolated.

Knockout mutants Organisms carrying mutations that eliminate the function of a given gene.

Linkage disequilibrium Nonrandom association of alleles.

Meiotic drive Distortion of Mendelian inheritance during meiosis by alleles that are preferentially transmitted to the mature gamete pool (e.g., by inactivating sperm or pollen carrying alternate alleles).

Near isogenic lines Accessions generated by repeated backcrossing that have identical genomes except for differences at one or a few narrow genomic blocks.

Reproductive isolating barriers Biological features of organisms that impede the exchange of genes with members of other populations.

RNAi RNA interference; includes several methods by which dsRNA can be introduced to trigger the degradation or inhibit the translation of specific endogenous transcripts.

Sexual conflict A phenomenon where alleles that confer higher fitness to one sex also confer lower fitness to another sex, which may lead to antagonistic coevolution.

SNP Single nucleotide polymorphism.

***Trans*-acting variants** Polymorphisms affecting the transcription of a gene that are not located within or nearby that gene but are instead located elsewhere in the genome.

As speciation proceeds, genetic changes accumulate that restrict and eventually lead to the cessation of gene flow between populations sharing common ancestry, allowing these lineages to become increasingly evolutionarily independent. Because many questions about the nature of the molecular mechanisms and evolutionary forces that drive speciation can only be addressed with knowledge of the specific genes involved, researchers have long sought to identify the genes contributing to this process. However, these loci, dubbed 'speciation genes,' have been understandably quite difficult to identify. Genetic analysis is a formidable challenge when the organisms of interest are naturally recalcitrant to mating or their offspring cannot produce progeny of their own.

Fortunately, through advances in genetic and genomic resources, creative experimental design, and considerable brute force effort, the field of speciation genetics has made rapid progress over the past two decades (Bomblies, 2010; Moyle *et al.*, 2014; Noor and Feder, 2006; Nosil and Schluter, 2011; Orr, 2005; Presgraves, 2010; Rieseberg and Blackman, 2010; Wu and Ting, 2004). Allelic differences between lineages that cause reproductive isolation (RI) by compromising successful fertilization (pre- or postmating prezygotic RI) or the fitness of hybrid progeny (postzygotic RI) have been identified in diverse systems. Moreover, although their number is still modest, these case studies – whether considered individually or as set – have validated, deepened, and at times challenged longstanding ideas as well as promoted new directions for investigation. The aims of this entry are twofold. First, the conceptual history of the term 'speciation gene' and, following from this, empirical approaches for meeting the criteria implied by that definition will be reviewed. Second, insights into the speciation process that have emerged from the study of known speciation genes will be highlighted. Though the present focus is on genic changes that increase RI, how chromosomal rearrangements and polyploidy may also contribute is reviewed elsewhere in this volume.

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What are Speciation Genes and How are They Identified?

An Inclusive Definition

As emphasis on particular forms of RI has shifted over time, how authors have defined the term 'speciation gene' has evolved as well. Most early discussions were restricted in scope, focusing solely on genes contributing to intrinsic postzygotic isolation in sexually reproducing organisms (Orr *et al.*, 2004; Orr, 2005; Orr and Presgraves, 2000). This narrow definition was partly motivated by the argument that although prezygotic barriers or extrinsic postzygotic barriers may be important early in speciation, they may also erode due to shifts in the environment or may be insufficient to maintain divergence when allopatric populations come into secondary contact. In contrast, intrinsic hybrid inviability and sterility are generally predicted to be environment-independent and were considered essential to complete speciation (Muller, 1942). Moreover, the genes underlying intrinsic postzygotic barriers have historically been subject to more fervent attention by geneticists and in the literature due to the greater inherent mystery surrounding their identity (Coyne and Orr, 2004). Traits contributing to prezygotic isolation can be studied within species, and it was expected that the proteins that ordinarily function in the genetic networks regulating these phenotypes within species would also contribute to divergence between species. In contrast, intrinsic hybrid inviability and sterility were predominantly studied only in interspecific crosses. Consequently, beyond the prediction that alleles from each parent genome would interact epistatically (Bateson, 1909; Dobzhansky, 1936; Muller, 1942), few *a priori* expectations existed regarding the functions of genes harboring variants affecting intrinsic postzygotic barriers.

The scope of the term 'speciation gene' has since broadened to also include loci contributing to prezygotic barriers and extrinsic postzygotic barriers. As defined by Rieseberg and Blackman (2010), a "speciation gene can be strictly defined as a gene that contributes to the splitting of two lineages by reducing the amount of gene flow between them." Additional authors have advanced similar definitions (Noor and Feder, 2006; Nosil and Schluter, 2011; Wu and Ting, 2004). This more inclusive definition has emerged in part because many have recognized that the speciation process is best understood as continuum proceeding from absent to complete RI. In addition, as the genetics of both prezygotic and postzygotic barriers have received more intense study, it has become clear that postzygotic incompatibilities can diverge contemporaneously with prezygotic barriers, often segregate within species (Cutter, 2012), and may even be genetically related to prezygotic barriers through pleiotropy (Lee *et al.*, 2008).

Genetic Characterization of Speciation Genes

To satisfy the above definition, the contribution of allelic differences to a contemporary barrier phenotype must be characterized with sufficient empirical rigor to make a compelling case for causality. In addition, it has been argued that speciation genes should fulfill several evolutionary criteria that demonstrate these changes contributed to speciation historically, though assessing these requirements can be less than straightforward.

Candidate gene identification

Quests for speciation genes most commonly start with forward genetics approaches in controlled crosses (**Figure 1**). Because winnowing the genomic regions containing causal loci down to segments of tens to several hundred possible candidates requires performing quantitative trait locus (QTL) mapping on panels of related individuals segregating for variation in focal barrier phenotypes, this strategy has often meant working in systems where speciation is incipient and incomplete (**Figures 1(a)** and **1(b)**). That is, existing barriers can be somehow overcome, and F_2 or BC_1 generation progeny descended from partially or fully fertile F_1s are recoverable for at least one of the cross directions between two parental populations. Only classic model organisms, most notably *Drosophila*, where more advanced genetic toolboxes allow for deficiency and introgression mapping in F_1s, have been exceptions to this rule (**Figure 1(d)**; Orr, 2005). When feasible, QTL intervals are further narrowed by fine mapping in advanced-generation crosses (**Figure 1(c)**), by linkage disequilibrium (LD) mapping approaches that exploit historical recombination events in natural populations, or both. Investigators working in systems without sequenced genomes often supplement their mapping efforts with polymorphic markers specifically developed in candidate genes chosen *a priori* for their known functions in the gene networks governing barrier traits (e.g., Kronforst *et al.*, 2006). Even when promising associations between these targeted markers and phenotypic variation are found, fine mapping or LD mapping remain worthwhile pursuits, as they exclude any potential contributions of neighboring genes.

Fueled by advances in array- and sequencing-based methods for high-throughput genotyping, population genomic screens for speciation genes based on patterns of sequence diversity within and among natural populations have become increasingly common alternative strategies to forward genetics approaches (Noor and Feder, 2006; Seehausen *et al.*, 2014). Differentiation studies scour the genomes of incipient species pairs for regions of elevated divergence relative to polymorphism using metrics like Fst (e.g., Chapman *et al.*, 2013). Hybrid zone studies scan for SNPs that do not introgress and thus maintain much steeper allele frequency clines across space relative to genome-wide patterns of introgression (e.g., Teeter *et al.*, 2010). Although powerful, a drawback of these methods is that do not provide any immediate, concrete connection from genotypes back to specific barrier phenotypes, and establishing this link is often challenging. Moreover, positional information alone may be insufficient for assigning highly differentiated SNPs residing in noncoding regions to the genes they functionally affect. In addition, evolutionary causes other than the presence of a speciation gene may produce outliers of high differentiation, leading to false positives (Cruickshank and Hahn, 2014). Consequently, the need to follow up these studies with functional and evolutionary characterization of specific loci is compelling.

Functional characterization of speciation genes

The gold standard for validating candidate speciation genes is experimental manipulation by genetic transformation. Before

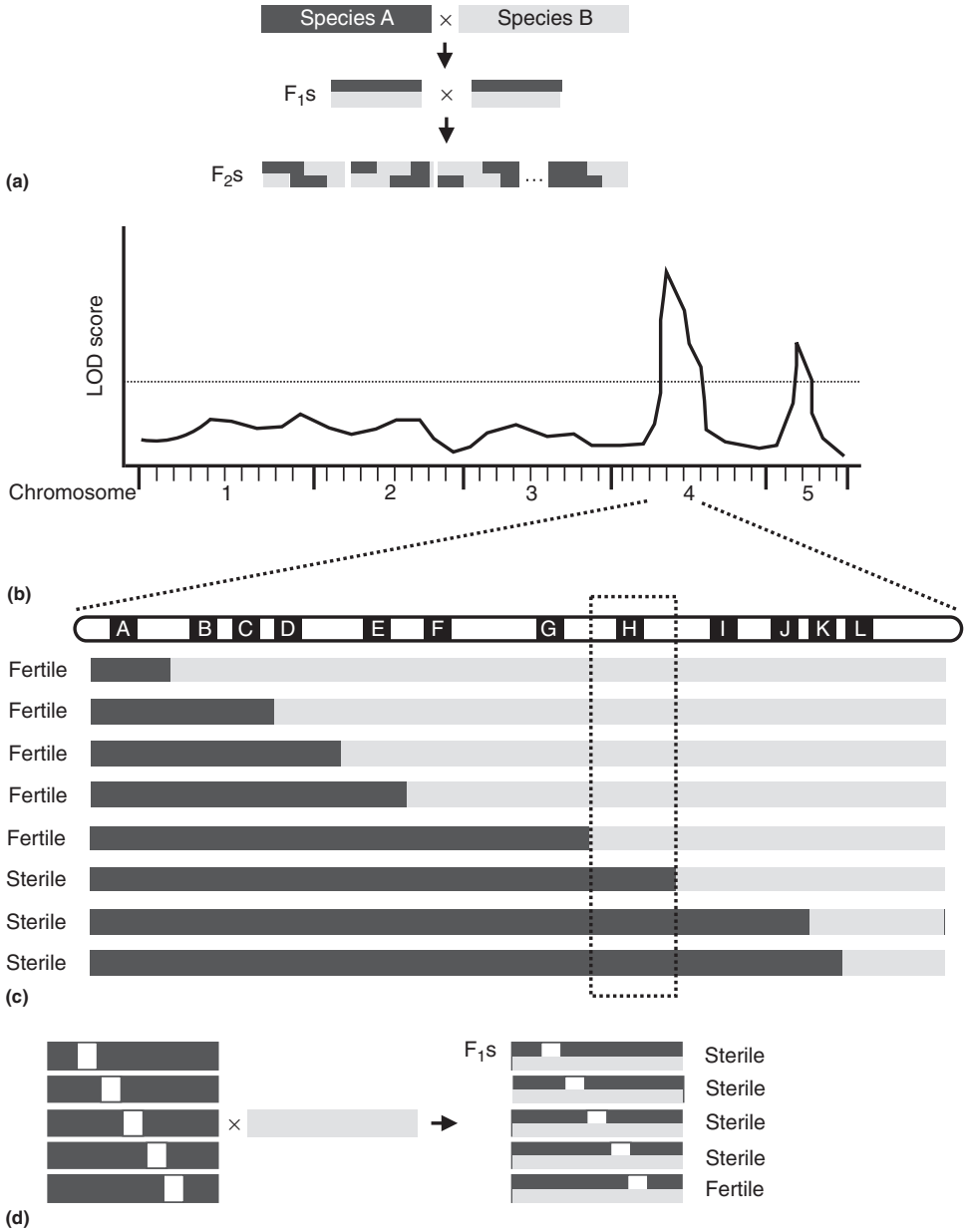

Figure 1 Identifying speciation genes by forward genetics. (a) Genetic mapping requires generating recombinant progeny, a prospect that may be impossible because reproductive isolating barriers in interspecific crosses prevent production of F_1s or leave them infertile. If RI barriers can be overcome, then the phenotypes of recombinants segregating for parental genomic segments can be obtained in the F_2 (shown) or BC_1 generation (not shown). Note that only effects detectable when comparing the heterozygous genotype to the backcross parent homozygote can be studied in a BC_1 generation while effects differentiating the heterozygote and either parental homozygote genotype can be studied in an F_2 generation. (b) Recombinant progeny are genotyped for polymorphic markers throughout the genome, and the effect of the genotype across a coarse genomic interval on segregating variation in a barrier trait is then detected by QTL mapping. The LOD score is the $\log_{10}$ likelihood ratio comparing a model with a QTL present in the interval to a model with no QTL present. (c) Finally, fine mapping may be conducted in the current panel and/or subsequent generations. The QTL interval is narrowed to a region containing one or a handful of genes by genotyping of the subset of progeny recombinant in that interval for additional markers. In the schematic, the region of chromosome 4 contained in the dashed line box is the minimal interval defined by recombination breakpoints affecting hybrid sterility, strongly implicating gene H as the causal locus. (d) In species with libraries of deletions or knockout lines, deficiency mapping can be performed for genes causing F_1 hybrid dysfunction. In the schematic, the genomic interval of Species A spanned by the rightmost deficiency segment contains a hybrid sterility allele.

proceeding with these studies, comparisons of parental coding sequences and gene expression levels in relevant tissues may provide circumstantial evidence useful for reducing the candidate gene set and bolstering support for particular genes in systems where transformation is prohibitively difficult. Allele-specific expression patterns in F_1 hybrids may be evaluated to corroborate that observed expression differences derive wholly or in part from *cis*-regulatory variation affecting the gene itself

rather than *trans*-acting variants located elsewhere in the genome (e.g., Hopkins and Rausher, 2011; Klahre *et al.*, 2011). Network-centered approaches that integrate mapping of barrier traits and gene expression (i.e., QTL and eQTL mapping, respectively) may facilitate description of complex gene interactions often characteristic of postzygotic isolation phenotypes as well (Turner *et al.*, 2014).

Allelic replacement of one species' sequence with that of another, and vice versa, by genetic transformation is the most rigorous experiment for validating a speciation gene. Such tests have historically been possible in only a few model systems, but new genome editing approaches promise to extend the capability for allelic replacement to any transformable system (Turner, 2014). Even when conversion of one species' sequence to that of another is not possible, alternative manipulative experiments may yield solid support. For instance, if crosses involving knockout mutants or individuals carrying RNAi constructs that knockdown expression of candidate gene exhibit reduced RI relative to control crosses, then the candidate gene is necessary for the isolating barrier (e.g., Presgraves *et al.*, 2003). Likewise, if introducing another species' sequence for a candidate gene causes increased RI in an intraspecific cross, then the candidate gene is sufficient for enhancing the isolating barrier (e.g., Phadnis and Orr, 2009). For genes whose protein function can be assessed biochemically, the effects of interspecific substitutions can be determined by *in vitro* assays (e.g., Wessinger and Rausher, 2015). Although not sufficiently rigorous to show causality of a specific gene since contributions of linked variation cannot be excluded, near isogenic lines carrying alternate species alleles in either background can be particularly advantageous for demonstrating a genomic region's effect on RI in field settings (e.g., Bradshaw and Schemske, 2003).

Evolutionary Characterization of Speciation Genes

Recognizing that the evolution of RI is a temporally dynamic process that begins prior to and continues after speciation, the definition advanced above also requires that the gene 'contribute to the splitting of two lineages.' Genetic changes that contribute to contemporary barriers to reproduction between fully isolated species may have accumulated long after speciation was already complete. Likewise, the relative importance of different barriers to gene flow, and consequently the impact of particular alleles on total RI, may change over time. Therefore, it has been argued that speciation genes must meet two additional evolutionary criteria (Nosil and Schluter, 2011). First, divergence at the locus must have occurred before speciation was complete. Second, the gene should have had a measurable effect on RI at the time it diverged. Obtaining empirical data addressing the first criterion can be straightforward, but demonstrating a gene meets the second criterion is potentially far more challenging.

The divergence time criterion is automatically met for genes that contribute to barriers between incipient species. For species pairs that are already completely isolated, divergence time can be assessed with gene trees or genealogies (Nosil and Schluter, 2011). If divergence at a putative speciation gene occurred prior to full cessation of gene flow, then its genealogy should be discordant with genealogies of unlinked neutral loci, which are more likely to show shallower coalescence and greater evidence of gene flow. Such tests can be problematic, however, in that they may yield false negatives depending on the evolutionary process driving divergence (Lessios, 2011). For instance, RI between species often arises as a by-product of accelerated sequence evolution driven by arms races mediated by sexual or genomic conflict within species that continue well after speciation is complete. Thus, a pattern of coalescence within species, rather than a pattern of coalescence prior to the timing of species divergence, may be expected and has been observed for some speciation genes evolving in this manner (Palumbi, 2009).

As for the second criterion, it is unclear whether a speciation gene's effect on RI relative to all other current barriers at the time of its divergence can be rigorously estimated for any species. The absolute effect of allelic divergence at a speciation gene on the strength of a contemporary RI barrier may be readily estimated by the methods discussed above. Moreover, a speciation gene's relative contribution to contemporary RI as a whole may also be estimated to the extent that the full genetic architecture for the barrier trait and the relative strength of that barrier relative to other barrier traits on RI are known. All these parameters are important because isolating barriers, to a large extent, act sequentially to prevent successful fertilization or to impair hybrid fitness, and hence it is possible that loci with major effects on later acting barriers (e.g., hybrid dysfunction) may only have minor contributions to overall RI.

By extension then, estimating a speciation gene's contribution to historical levels of RI would require not only knowledge of the temporal dynamics of evolution at a given locus (and interacting loci if RI is caused by epistatic incompatibilities) in one or both lineages, but also the past history of all other loci contributing to the cessation of gene flow. Thus, meeting this criteria may only be possible in rare systems where speciation in action can be followed over observable time scales. Alternatively, in species pairs isolated by few barriers with tractable genetics, it is conceivable that the historical series of genotypes at speciation loci could be reconstructed if the order of substitutions causing RI were inferable from genes trees or observable in ancient DNA time series. Estimates of absolute and relative contributions to contemporary RI may help determine the bounds to paths historically possible in other systems. However, given the pragmatic hurdles to determining historical dynamics (irrespective of any complexity introduced by gene $\times$ environment effects), it seems sufficient to demonstrate a speciation gene affects contemporary isolation and acknowledge the caveat that the specific contribution to total RI at the time of divergence is unknown.

What Have We Learned from Known Speciation Genes?

Beyond important basic knowledge of whether the types of genes or mutations involved follow predictable functional patterns, identifying speciation genes allows many models about the molecular and evolutionary mechanisms that drive speciation to be tested. In addition, while some questions are

addressable without knowing the underlying loci, having causal allelic sequences in hand is often a boon. For instance, patterns of intraspecific polymorphism for RI can be more efficiently assessed by sequencing-based surveys of allelic diversity than through expansive crossing schemes in most systems. Although only a modest set of speciation genes and other strong candidates is known (**Table 1**), the emerging picture supports a plurality of mechanisms while also revealing some broad patterns.

The Identities of Speciation Genes are Predictable for Some Isolating Barriers

As noted above, speciation genes for prezygotic barriers were broadly predicted to be members of networks that govern these phenotypes within species. Although the number of genes known remains insufficient to test this prediction for some barriers (e.g., temporal and mechanical isolation), identification of speciation genes affecting certain forms of RI in multiple systems has proven informative and largely confirms the expectation (**Table 1**). Pollinator isolation stands out in this regard (Yuan *et al.*, 2013a). For floral color and scent, enzymes in pigment and volatile biosynthetic pathways and components of the transcriptional complexes that regulate their expression (often R2R3-MYB transcription factors) are responsible for species differences (Byers *et al.*, 2014; Hoballah *et al.*, 2007; Hopkins and Rausher, 2011; Klahre *et al.*, 2011; Streisfeld *et al.*, 2013; Yuan *et al.*, 2013b). One particularly impressive example of convergence at the genetic level is in the genus *Penstemon*, where function-compromising variants in F3′5′H contribute to flower color shifts in 13 independent transitions from bee to hummingbird pollination (Wessinger and Rausher, 2015). The repeated observation of rapid protein evolution affecting gamete recognition systems in free-spawning marine invertebrates represents a similar case of predictable speciation gene identity (Lessios, 2011).

More surprisingly, the molecular basis of some postzygotic barrier traits in plants also appears predictable. Intra- and interspecific crosses in various groups often yield progeny exhibiting a form of hybrid inviability – hybrid necrosis – in which F_1 or F_2 hybrids show lesioning and compromised growth (Bomblies and Weigel, 2007). Across several systems, the vast majority of loci involved are immune receptors whose faulty epistatic interactions trigger errant autoimmune responses (Bomblies *et al.*, 2007; Chae *et al.*, 2014; Chen *et al.*, 2014; Krüger *et al.*, 2002; Rooney *et al.*, 2005; Todesco *et al.*, 2010). The genetic basis of cytoplasmic male sterility (CMS) barriers appears similarly predictable (Rieseberg and Blackman, 2010). Chimeric mitochondrial transcripts that cause sterility evolve as selfish elements, and the majority of nuclear fertility restorer genes identified to date are members of the pentatricopeptide repeat (PPR) gene family. Although most genetic studies of CMS have been conducted in crosses of cultivated plants to wild species, exciting work in fully wild crosses signals these results will bear out for natural populations as well (Barr and Fishman, 2010; Case and Willis, 2008).

Similar patterns of repeated involvement of common genes or gene families have not emerged for postzygotic isolation barriers in animals or fungi (Presgraves, 2010). An absence of trends here may signal that disruptions to viability or fertility in hybrids are more phenotypically idiosyncratic and taxon-specific in these groups relative to plants, where hybrid necrosis and CMS are widespread isolating barriers. Alternatively, the number of genes identified to date may be too small to reveal trends, but existing examples that implicate particular complexes of proteins regulating recombination, transposable element suppression, and chromosome segregation may prove general (**Table 1**). For instance, allelic differences in the histone methyltransferase PRDM9, a major determinant of recombination hotspots in mammals, have been shown to cause hybrid male sterility in some crosses between subspecies of *Mus musculus* (Flachs *et al.*, 2012; Mihola *et al.*, 2009). Signatures of positive selection altering the number and sequence of zinc finger domains across diverse rodent, primate, and other metazoan species raise the possibility that PRDM9 gene contributes to hybrid sterility in diverse systems (Oliver *et al.*, 2009).

Hybrid Incompatibilities Evolve by Diverse Paths and are Often Polymorphic

The Bateson–Dobzhansky–Muller (BDM) model of reproductive incompatibilities provides a simple and powerful explanatory framework for how alleles that cause hybrid inviability or sterility evolve among lineages that recently shared freely interbreeding ancestors (Bateson, 1909; Dobzhansky, 1936; Muller, 1942). In its most commonly expressed form, Lineage 1 fixes a derived allele at Locus A and Lineage 2 fixes a derived allele at Locus B, but when newly brought onto the same genomic background through hybridization, negative epistatic interactions leading to dysfunction emerge (**Figure 2(a)**). Empirical examples of such BDM incompatibilities have been described (e.g., *Lhr*/*Hmr*; Brideau *et al.*, 2006), as have additional mechanisms. For instance, recent work has described how substitutions of multiple derived alleles in a single lineage can lead to ancestral-derived incompatibilities between lineages involving two unlinked loci (e.g., Krüger *et al.*, 2002; Phadnis and Orr, 2009; Rooney *et al.*, 2005; **Figure 2(b)**) or multiple alleles of a single locus (e.g., Chae *et al.*, 2014; Todesco *et al.*, 2010; **Figure 2(c)**).

In the classic BDM model, the time required for fixation of derived alleles is assumed to be instantaneous, relative to the time spent in allopatry, for mathematical convenience (Orr, 1995; Cutter, 2012). However, numerous empirical studies involving many well-known speciation genes have found that alleles involved in BDM incompatibilities are polymorphic (e.g., Brideau *et al.*, 2006; Mihola *et al.*, 2009; Phadnis and Orr, 2009). In other words, only some strains will produce dysfunctional hybrids in interspecific crosses because the contributing alleles segregate within species. Moreover, intraspecific standing variation in BDM incompatibilities is often found (e.g., Bomblies *et al.*, 2007; Bikard *et al.*, 2009). Describing the evolutionary causes of these polymorphisms and determining whether variable reproductive isolation maintained within an ancestral lineage eventually contributes to postzygotic RI among lineages are major theoretical and empirical challenges in the field of speciation genetics (Cutter, 2012).

Table 1 Representative speciation genes and candidates

Stage	*Isolation type*	*Gene(s)*	*Species*	*Phenotype(s)*	*References*
Prezygotic, Premating	Habitat	Odorant-binding proteins *OBP57d/OBP57e*	*Drosophila sechellia/ Drosophila simulans*	Oviposition preference	Matsuo *et al.*, 2007
		Ectodysplasin (Eda)	Marine and freshwater *Gasterosteus aculeatus*	Lateral plate number and growth rate*	Colosimo *et al.*, 2005; Barrett *et al.*, 2009
	Temporal	*FLOWERING LOCUS C* (*FLC*)	*Arabidopsis suecica/ Arabidopsis arenosa,* and *Arabidopsis thaliana*	Delayed flowering of allopolyploid	Wang *et al.*, 2006
	Pollinator/ behavioral	*R2R3-* and *R3-MYB* transcription factors (e.g., *anthocyanin2* and *ROSE INTENSITY 1*)	Various *Petunia, Antirrhinum, Phlox,* and *Mimulus* species pairs	Flower color differences leading to pollinator shift	Quattrocchio *et al.*, 1999; Hoballah *et al.*, 2007; Schwinn *et al.*, 2006; Hopkins and Rausher 2011, 2012; Streisfeld *et al.*, 2013; Yuan *et al.*, 2013a, 2013b
		Flavonoid 3′,5′-hydroxylase (F3′5′H)	Various *Penstemon* species; *Phlox drummondii/Phlox cuspidata*	Flower color differences leading to pollinator shift	Hopkins and Rausher 2011, 2012; Wessinger and Rausher 2014, 2015
		Methyl-branched CHC-specific fatty acid synthase (*mFAS*)	*Drosophila serrata/ Drosophila birchii*	Cuticular hydrocarbon profile differences affect mate choice	Chung *et al.*, 2014
		ODORANT1	*Petunia axillaris/ Petunia exserta*	Floral scent differences leading to pollinator shift	Klahre *et al.*, 2011
		Stearoyl-acyl carrier protein desaturases 1 and 2 (*SAD1/SAD2)*	Various *Ophyrus* species	Floral scent differences leading to pollinator shift	Xu *et al.*, 2012
		OCIMENE SYNTHASE	*Mimulus lewisii Mimulus cardinalis*	Floral scent differences leading to pollinator shift	Byers *et al.*, 2014
		optix	Various *Heliconius* species	Wing color pattern leading to assortative mating*	Heliconius Genome Consortium, 2012; Reed *et al.*, 2011
Prezygotic, Postmating	Gametic	*ui1.1* (Cullin 1)*; ui6.1* (S-Locus F-box)	Various *Solanum* species	Unilateral incompatibility accompanying mating system shift	Li and Chetelat, 2010, 2015
		Bindin	Various sea urchin genera	Species-specific fertilization	Palumbi 2009; Lessios 2011
		Lysin	*Haliotis rufescens/ Haliotis corrugata*	Species-specific fertilization	Palumbi 2009; Lessios 2011
		Cysteine-rice peptide *LURE1*	*Torenia concolor/ Torenia fournieri; A. thaliana/ Arabidopsis lyrata*	Species-specific chemoattraction of pollen tubes	Kanaoka *et al.*, 2011; Takeuchi and Higashiyama, 2012
Postzygotic, Extrinsic	Ecological	*optix*	Various *Heliconius* species	Wing color pattern intermediates suffer greater predation*	Heliconius Genome Consortium 2012; Reed *et al.*, 2011
	Pollinator/ behavioral	*R2R3-MYB* transcription factor	*Phlox cuspidate/ Phlox drummondii*	Flower color intermediates less attractive to pollinators	Hopkins and Rausher, 2011, 2012

(*Continued*)

Table 1 Continued

Stage	*Isolation type*	*Gene(s)*	*Species*	*Phenotype(s)*	*References*
Postzygotic, Intrinsic	Hybrid Inviability	Various NLR immune receptors (e.g., *DANGEROUS MIX 1*)	*A. thaliana* (intraspecific); *Oryza rufipogon/ Oryza sativa*; *Solanum lycopersicum/ Solanum pimpinellifolium*	Hybrid necrosis	Krüger *et al.*, 2002; Bomblies *et al.*, 2007; Todesco *et al.*, 2010; Chae *et al.*, 2014; Chen *et al.*, 2014
		HISTIDINOL-PHOSPHATE AMINO-TRANSFERASES 1 and 2 (*HPA1/HAP2*)	*A. thaliana* (intraspecific)	Arrest of seed development	Bikard *et al.*, 2009
		Nucleoporins Nup96 and Nup160	*D. melanogaster/ D. simulans*	Hybrid lethality	Presgraves *et al.*, 2003; Tang and Presgraves, 2009
		Lethal hybrid rescue (Lhr)/ Hybrid male rescue (Hmr)	*D. melanogaster/ D. simulans*	Hybrid lethality	Barbash *et al.*, 2003; Brideau *et al.*, 2006; Thomae *et al.*, 2013; Satyaki *et al.*, 2014
	Hybrid Sterility	*Odysseus (Ods)*	*D. simulans/ D. mauritiana*	Hybrid male sterility	Ting *et al.*, 1998; Sun *et al.*, 2004; Bayes and Malik, 2009
		JYAlpha	*D. melanogaster/ D. simulans*	Hybrid male sterility	Masly *et al.*, 2006
		PR domain-containing 9 (PRDM9)	*Mus musculus musculus/Mus musculus domesticus*	Hybrid male sterility	Mihola *et al.*, 2009
		Overdrive	*Drosophila pseudoobscura pseudoobscura/ Drosophila pseudoobscura bogotana*	Hybrid male sterility	Phadnis and Orr, 2009

Genes were selected to illustrate the breadth of isolation barriers for which speciation genes or strong candidates have been characterized, and the table is not intended to be comprehensive. Although compelling evidence linking genotype to phenotype has been provided in all cases, the direct impact of allelic variation on RI may not be fully demonstrated for some candidates (asterisks (*)). That is, an impact on the prevention of fertilization or hybrid dysfunction is assumed based on the phenotypes changed by these alleles but reproductive isolation has not been explicitly examined following allelic replacement by transformation or introgression for these candidates.

Diverse Evolutionary Models Supported

Because many evolutionary models in the field of speciation genetics are grounded in specific molecular mechanisms, identifying speciation genes is essential to test their biological merit. In addition, knowing key sequences facilitates the application of population genetic tests to determine whether drift, selection, and/or migration have been the predominant forces driving the evolution of RI. The speciation genes identified to date lend support to diverse processes.

Ecological speciation

A classic model of speciation – ecological speciation – postulates that RI evolves as a by-product of ecologically based divergent natural selection (Schluter and Conte, 2009). In other words, the mechanism by which individual populations differentially adapt to local selection pressures is genetically linked to the evolution of RI between populations. Pleiotropy, tight physical linkage, capture by a chromosomal inversion, or one-allele assortative mating all represent alternative mechanisms by which recombination between the loci contributing to locally adaptive phenotypes and the loci causing RI may be sufficiently frustrated to allow the joint evolution of both traits (Nosil, 2012).

Speciation genes provide ample empirical evidence that supports this model for prezygotic or extrinsic postzygotic barriers. For instance, *cis*-regulatory changes that eliminate expression of mFAS, a fatty acid synthase responsible for methyl-branched cuticular hydrocarbon production, in the humid habitat specialist *Drosophila birchii* relative to the habitat generalist *Drosophila serrata* are pleiotropic (Chung *et al.*, 2014; Chung and Carroll, 2015). They alter both desiccation sensitivity and mate choice. Likewise, allelic differences that specialize plants to different pollinators or to alternative mating systems necessarily also reduce gene flow between plant species (e.g., Hoballah *et al.*, 2007; Byers *et al.*, 2014; Li and Chetelat, 2015). In *Heliconius* butterflies, wing color patterns are essential for both predation avoidance through mimicry and mate choice (Jiggins *et al.*, 2001). Thus, genetic differences that have evolved in response to divergent selection favoring

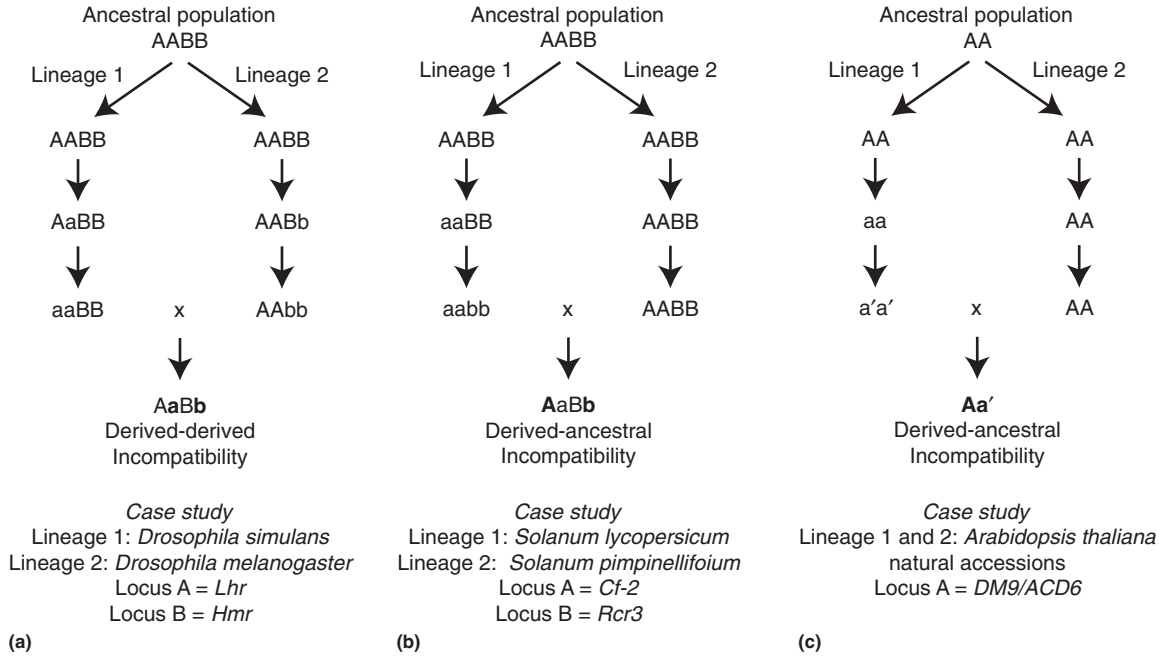

Figure 2 BDM incompatibilities evolve by multiple evolutionary mechanisms. (a) Classic BDM model where incompatible derived substitutions fix at alternative loci in each lineage, as observed for F_1 hybrid male lethality in crosses of two *Drosophila* species (Brideau *et al.*, 2006). (b) Substitution of a derived allele at Locus A permits the evolution of a second derived allele at Locus B in the same lineage, leading to incompatibility with the ancestral allele at Locus A. In the case study, hybrid necrosis arises in 3/16 of F_2 progeny of crosses between wild tomato species, because bb homozygotes are incompatible with AA or Aa genotypes (Rooney *et al.*, 2005). (c) A succession of substitutions at a single locus leads to the evolution of an incompatibility between a derived and ancestral allele. Some loci involved in incompatibilities that cause hybrid necrosis in intraspecific crosses among *Arabidopsis thaliana* accessions likely evolved by this mechanism (Chae *et al.*, 2014; Todesco *et al.*, 2010). Incompatible alleles are highlighted in bold.

alternative mimicry patterns (Kronforst *et al.*, 2006; Kronforst and Papa, 2015) may lead to behavioral isolation between color pattern races as well as extrinsic postzygotic isolation since hybrids with intermediate, non-mimicking color patterns are more susceptible to predation.

Theoretical models and experimental evolution studies both provide strong evidence that intrinsic postzygotic RI can evolve through ecological speciation as well (Dettman *et al.*, 2007; Gavrilets, 2004; Schluter and Conte, 2009). Although several speciation genes exhibit evolutionary patterns consistent with this process, their histories may also be consistent with other models or their contribution to interspecific barriers remains to be fully affirmed (Chae *et al.*, 2014; Lee *et al.*, 2008).

Evolutionary arms races, mutation pressure, and hybrid incompatibilities

Although multiple processes may drive the fixation of alleles within lineages that cause postzygotic incompatibilities between lineages, incompatibilities are predicted to arise more rapidly if selection is involved. Consistent with this expectation, many speciation genes known to be involved in BDM incompatibilities appear to rapidly evolve within lineages due to ongoing evolutionary arms races. These patterns of substitution and counter-substitution seem to be largely driven by mutation pressure and selection to resolve genomic conflicts rather than ecological pressures (Presgraves, 2010). Within lineages, selfish genetic elements may parasitize host genomes through elevated transposition rates or biasing transmission in their favor through meiotic drive and gamete-killing segregation distortion. Host genomes respond to these strong selection pressures by evolving mechanisms that suppress these activities or compensate for their deleterious effects. If parasitic elements are separated from their corresponding suppressors or if different host genome compensatory mechanisms are incompatible in hybrid genetic backgrounds, dysfunction may manifest.

For instance, the speciation genes *Lhr* and *Hmr* interact as part of a protein complex that represses satellite DNA and transposable elements (TEs), and epistatic interactions between *Lhr* and *Hmr* in *Drosophila melanogaster* × *Drosophila simulans* hybrids lead to mis-expression of TEs and consequently hybrid lethality (Brideau *et al.*, 2006; Satyaki *et al.*, 2014; Thomae *et al.*, 2013). Rapid coevolution of repetitive DNA regions and the proteins that regulate their segregation during meiosis and mitosis, and other systems that compensate for mechanisms that distort chromosomal segregation patterns, have also been implicated in intrinsic postzygotic barriers (Bayes and Malik, 2009; Ferree and Barbash, 2009; Fishman and Saunders, 2008; Phadnis and Orr, 2009), providing support for a prescient mechanistic model (Henikoff *et al.*, 2001). The evolution of CMS and restorer genes reflects a similar case of postzygotic RI that evolves due to genomic conflict within lineages (Rieseberg and Blackman, 2010). The speciation genes involved in these types of arms races – as well as host–pathogen systems related to hybrid necrosis – exhibit high levels of coding sequence divergence, often in tandem

with copy number evolution. Whether the cumulative effects of all substitutions are necessary for BDM incompatibilities, or whether substitutions that cause incompatibilities are rare and only these types of genes evolve rapidly enough to hit upon them, remains an open question.

Relaxation of purifying selection, particularly following gene or genome duplication, may also accelerate rates of substitution through genetic drift. In the case of gene or genome duplication, if resolution of functional redundancy among recent duplicates occurs by gene silencing or subfunctionalization within populations such that different descendant lineages maintain function in separate map locations, postzygotic RI may also result (Lynch and Force, 2000; Werth and Windham, 1991). In this scenario, F_1 gametophytes or F_2 generation hybrids may inherit a chromosome set that lacks either a full complement of ancestral functions or any functional copy period. Examples of speciation genes consistent with this form of RI arising from gene movement driven by passive mutational silencing of gene duplicates have been observed in intraspecific crosses in *Arabidopsis* and interspecific crosses among rice and *Drosophila* species (Bikard *et al.*, 2009; Masly *et al.*, 2006; Yamagata *et al.*, 2010).

Hybridization as a driver of speciation

Reinforcement, allopolyploid speciation, and homoploid hybrid speciation are documented ways that hybridization may foster speciation. Speciation gene sequences have revealed an additional, perhaps surprising way that hybridization may actually promote the cessation of gene flow. In several cases, gene flow has facilitated the spread of alleles contributing to the evolution of RI between lineages. For instance, *optix* sequences involved in wing pattern divergence have been passed among *Heliconius* species and repeatedly reused in the independent evolution of mimetic races (Heliconius Genome Consortium, 2012; Reed *et al.*, 2011). In the threespine stickleback, the *Eda* allele that confers adaptation to freshwater through loss of lateral plates, and that may contribute to behavioral isolation between marine and freshwater populations due to effects on growth rate in a species that assortatively mates based on size, was likely transported to many freshwater populations worldwide through the marine gene pool (Barrett *et al.*, 2009; Colosimo *et al.*, 2005; McKinnon *et al.*, 2004; Schluter and Conte, 2009). It remains to be determined whether alleles that initiate or resolve genomic conflicts similarly introgress among species and, if so, whether gene flow of such alleles would facilitate or impede the speciation process.

See also: Ecological Speciation and Its Consequences. Reproductive Isolation, Postzygotic. Reproductive Isolation, Prezygotic. Speciation Continuum. Speciation Genomics

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