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Hybridization, Introgression, and the Nature of Species Boundaries

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Abstract

Species can be defined as populations that are diagnosably distinct, reproductively isolated, cohesive, or exclusive groups of organisms. Boundaries between species in sympatry are maintained by intrinsic barriers to gene exchange; these boundaries may not be uniform in space, in time, or across the genome. Here, we explore the nature of the species boundary, defined as the phenotypes/genes/genome regions that remain differentiated in the face of potential hybridization and introgression. We emphasize that species boundaries are semipermeable, with permeability (gene exchange) being a function of genome region. The early evidence for semipermeable species boundaries came from data on differential introgression in hybrid zones. This "genic view" of species was common in the hybrid zone literature even when few molecular markers were available to characterize genome-wide patterns of variation. Now, molecular tools allow detailed characterization of differentiation between diverging lineages and patterns of variation across natural hybrid zones, but the questions being asked by evolutionary biologists have remained much the same. Recent data (from DNA sequences and genotypes) reinforce earlier conclusions about the semipermeable nature of most species boundaries. However, debate persists over the nature and extent of genome divergence that accompanies speciation.

Subject areas: *Population structure and phylogeography*

Key words: *genomic divergence, hybrid zones, reproductive isolation, speciation*

Evolutionary and systematic biologists have regularly engaged in prolonged and sometimes acrimonious debates about species concepts and definitions; species have been variously defined as entities that are diagnosably distinct, reproductively isolated, cohesive, or exclusive (monophyletic) groups of organisms. Species concepts that focus on the importance of reproductive isolation (e.g., the Biological Species Concept [BSC] of Mayr and Dobzhansky) have often occupied center stage (Harrison 1998). The BSC has certainly provided the framework for the many empirical studies of speciation that have involved identifying the phenotypes and genotypes responsible for intrinsic barriers to gene exchange (e.g., Coyne and Orr 2004).

But the focus on reproductive isolation has frequently been questioned, and critics have particularly taken aim at the writings of Ernst Mayr. For example, Mallet (2008a) argued that Mayr, by emphasizing discontinuity and complete reproductive isolation, rejected Charles Darwin's (correct) vision of continuity between varieties and species (Darwin 1859) and failed to acknowledge that divergence between lineages can be maintained in the face of gene flow. Similarly, Wu (2001), championing what he termed a "genic view" of species, suggested that Mayr's BSC necessarily implies that reproductive isolation is a "whole-genome concept" and "lose[s] its logical robustness" if we acknowledge that the extent of isolation varies across the genome. According to Wu (2001), the BSC must be a whole-genome concept because Mayr (1963) argued that the genotype is coadapted, or as Mayr (1942) put it, "a 'physiological team'."

Critics of the "isolation view" of species often seem to imply that most students of speciation in the late 20th century were devout disciples of Mayr and that those who defended the BSC also must have been believers in coadapted gene complexes and the sanctity of allopatric speciation. In fact, many evolutionary biologists in that era recognized continuity in degree of divergence, viewed reproductive isolation and barriers to gene exchange as potentially incomplete, and argued that species boundaries can be semipermeable. A "genic view" had already been proposed long before Wu's (2001) article (more on this below). Even Mayr, condemned by Mallet for leading evolutionary biologists astray, apparently softened his views in later years. In *The Growth of Biological Thought* (1982), he discussed hybridization and wrote: "…for it seems as if some part of the genotype of the 2 species is not affected by the hybridization. The 2 species, in such a case, seem to remain "reproductively isolated," in the sense

that they do not fuse into a single population, in spite of the leakage of certain of their genes." (p. 285).

Here, we explore the nature of species boundaries and the importance of hybridization and introgression in defining such boundaries. We examine the notion that species boundaries are semipermeable, with permeability (gene exchange) being a function of genome region. This idea is not new and was widely discussed in the hybrid zone literature long before evolutionary biologists had access to the array of molecular markers that now allow characterization of genome-wide patterns of divergence. Beginning with the application of allozyme and mtDNA data to the study of hybrid zones (see Harrison 1990 for a review), documenting patterns of differential introgression provided strong evidence for the semipermeable nature of species boundaries. The recent introduction of high-throughput sequencing allows characterization of patterns of variation and divergence for multiple markers across the genome; far more detailed views of the species boundary are now becoming available. It is, therefore, timely to examine the history of ideas and data that are relevant to the concept of species boundaries.

Hybridization and Introgression

Natural hybridization can be defined as the interbreeding of individuals from 2 distinct populations or groups of populations. Individuals in those populations must be distinguishable on the basis of one or more heritable characters (Harrison 1990, 1993). Natural hybridization is most easily recognized when previously allopatric populations come together in secondary contact. Renewed sympatry often results in a hybrid zone, with parental types, F_1 hybrids, and multiple generation hybrids and backcrosses present in varying proportions. The presence of diverse genotypes, the product of many generations of recombination, potentially allows fine-scale mapping of genes that contribute to reproductive isolation and estimates of selection on individual alleles (Barton and Hewitt 1985; Barton and Gale 1993). Thus, natural hybrid zones provide data not easily obtained from laboratory crosses because such crosses usually involve relatively few generations of recombination.

Introgression (or "introgressive hybridization") describes the incorporation (usually via hybridization and backcrossing) of alleles from one entity (species) into the gene pool of a second, divergent entity (species) (Anderson and Hubricht 1938; Anderson 1949). Introgression is a relative term; alleles at one locus introgress with respect to alleles at other loci. That is, for the above definition to be applicable, some portion of the gene pool of each of the hybridizing taxa must remain constant and uncontaminated such that we can actually recognize that 2 distinct gene pools exist. As we will discuss below, the genes that define the 2 gene pools and make them distinct are those that comprise the species boundary.

Differential introgression, a phenomenon documented in many hybrid zones, refers to the observation that alleles at some loci introgress more than others. In theory, globally advantageous alleles will tend to introgress easily ("adaptive introgression"; e.g., see Whitney et al. 2006; Pardo-Diaz et al. 2012; Hedrick 2013); neutral alleles will introgress to varying extents, but linkage to genes that contribute to local adaptation or reproductive isolation will inhibit their movement (Barton 1979). Alleles will introgress little or not at all when they represent variants at loci subject to divergent directional selection and/or loci that determine speciation phenotypes (phenotypes that are responsible for reproductive isolation; see Shaw and Mullen 2011). Thus, patterns of differential introgression across hybrid zones potentially allow identification of genes or genome regions that are important for local adaptation and speciation (Payseur 2010; Nachman and Payseur 2012).

The geographic pattern and spatial scale of introgression will depend on many factors, including the environmental context in which hybridization occurs, how far individuals disperse, and the nature of natural selection (e.g., contrast clinal "tension zones" with mosaic hybrid zones embedded in a patchy environment). Some authors (e.g., Heiser 1973) have differentiated between localized and dispersed introgression, distinguished by whether introgressed alleles are found only where the 2 parental types occur together (and hybridize) or whether alleles of one species flow into otherwise pure populations of the "other" species that may be geographically far from a hybrid zone.

Species Boundaries

The term "species boundary" has been used frequently in the evolutionary biology literature. Introgression is often described as occurring "across species boundaries" (e.g., the introduction of techniques for mtDNA restriction fragment length polymorphism (RFLP) analysis led to a spate of papers that discussed mtDNA gene flow across the species boundary; Ferris et al. 1983; Powell 1983; Harrison et al. 1987). Recent articles refer to resolving, delimiting, or mapping species boundaries (Bouck et al. 2005; Lemmon et al. 2007; Roe and Sperling 2007; Wagner et al. 2013). However, exactly what the species boundary represents is not always made clear. It is certainly the case that the boundary in some way reflects the fact that gene flow between species is limited or prevented in nature by a set of intrinsic barriers. These barriers reflect phenotypic differences between species that impact whether individuals mate assortatively, whether after mating (or spawning or pollen release) gametes get together to form zygotes, or whether the zygotes thus formed give rise to viable and fertile adults.

The term species boundary can be used to refer to the geographic boundary between parapatric taxa. Although of obvious importance for understanding the ecology and recent history of the taxa, spatial boundaries are not what students of speciation mean when they use the term "species boundaries." In many cases, delimiting or resolving species boundaries refers to "boundaries" that might be visualized in tree space. For species with relatively old divergence times, a phylogenetic approach can give straightforward results, where species boundaries are defined by the presence of exclusive or reciprocally monophyletic groups (although allopatric monophyletic groupings may not be recognized as species).

However, for pairs or groups of species that are products of recent divergence, or that continue to exchange genes, species boundaries can be difficult to define not only because there is little differentiation but also because there may be discordance among character sets or among different gene trees. Discordance can reflect differential introgression (see below) but is also expected because of ancestral polymorphism, random lineage sorting, and the long time required for many or most loci to achieve reciprocal monophyly (e.g., see Hudson and Coyne 2002). Discordance among individual gene trees has been documented in many different pairs or groups of species (Beltran et al. 2002; Machado and Hey 2003; Dopman et al. 2005; Putnam et al. 2007; Andrés et al. 2008; Nachman and Payseur 2012). In many cases, a provisional set of boundaries can be defined on the basis of phenotype (morphological, behavioral, or ecological traits). If a significant fraction of gene trees are concordant with the provisional tree, then those markers are often assumed to mark the species boundary, with discordant trees explained by shared ancestral polymorphism or ongoing gene exchange. In the most problematic cases, increasing the amount of molecular data can lead to resolution. Thus, Wagner et al. (2013) demonstrate that high-throughput DNA sequence data from restriction site–associated DNA (RAD) markers provide "unprecedented resolution of species boundaries" in Lake Victoria cichlid fish, a group for which previous phylogenetic analyses had consistently revealed extensive allele sharing between putative morphs or species.

Phylogenetic approaches assume that species should be exclusive or monophyletic groups, at least for some part of the genome. These approaches work equally well for taxa that are allopatric and those that are sympatric or parapatric. However, patterns of exclusivity for allopatric taxa provide no guarantee that these taxa would remain distinct in sympatry; exclusivity can arise simply as a product of geographic isolation over time (e.g., due to genetic drift), without necessarily impacting the potential for gene exchange when taxa become sympatric. In contrast, exclusive phylogenetic relationships for taxa that occur together (either broadly sympatric or in narrow hybrid zones) suggest that the taxa are indeed distinct species, in the sense that gene flow between them does not lead to fusion or homogenization. In these situations, the focus is on what maintains species boundaries. Mayr (1963) clearly recognized the importance of geographic context in defining species. He emphasized what he called a "nondimensional" species concept, "characterized by the non-interbreeding of 2 coexisting demes, uncomplicated by the dimensions of space and time." (Mayr 1963, p. 669).

Thus, we might consider the species boundary to be defined by the phenotypes/genes/genome regions (or some subset thereof) that remain differentiated in the face of potential hybridization and introgression (i.e., when the entities in question are locally sympatric). This definition acknowledges that species boundaries do not necessarily extend across the entire genome, that alleles at some (perhaps many) loci can be exchanged between species, that species boundaries are semipermeable or porous, and that species boundaries can vary geographically. The words "or some subset thereof" are included because not all genome regions that remain distinct when taxa are sympatric necessarily contribute to reproductive isolation. In an extreme case, allelic differences at a single locus could result in perfect positive assortative mating or hybrid lethality and would prevent gene exchange across the entire genome; yet, the "species boundary" might be thought of as defined by a single locus.

Semipermeable Species Boundaries

The "genic view" of species advocated by Wu (2001) was not a new idea, but his review article brought the idea to the attention of a larger community. The notion that gene flow and reproductive isolation are characteristics of genome regions, not entire genomes, was already well established in the hybrid zone literature in the 1980s. Key (1968, p. 19), in discussing hybridization in Morabine grasshoppers, wrote: "Thus the tension zones act like semipermeable membranes, holding back some genes and chromosomal rearrangements to varying degrees, but permitting others rather free passage." And Bazykin (1969), commenting on models of sympatric speciation, introduced the concept of "isolation for part of the gene pool." In an early review of hybrid zones, Barton and Hewitt (1981, p. 119), citing Bazykin 1969, wrote that "Strict application of the biological species concept might lead to different results for different loci; perhaps one can only define 'groups of actually or potentially interbreeding natural populations' … at the gene level." A figure in that review shows an example of how sequences on a chromosome become homogenized over time by gene flow, except in small regions surrounding genes subject to divergent directional selection (Figure 1). In a subsequent review of hybrid zones, Harrison (1990, pp. 98–99) wrote that "Boundaries [between species] are, therefore, semi-permeable, the permeability depending on the genetic marker …. Genetic isolation must be considered as a property of individual genes (or chromosome segments), not as a characteristic of entire genome."

Some authors prefer to characterize the species boundary as "porous" rather than semipermeable. Porous is a synonym of permeable and means "easily crossed or penetrated" (www.thefreedictionary.com/) or "easy to pass or get through" (www.merriam-webster.com/dictionary/). In cell biology, semipermeable is a term used to describe membranes that are selective in allowing only certain molecules or ions to pass through. A semipermeable boundary between species implies that differential introgression is the result of a selective process, with alleles at some loci able to cross the boundary, whereas alleles at other loci cannot. The term porous does not imply selectivity.

The early evidence for semipermeable species boundaries came primarily from data on patterns of differential introgression across hybrid zones. In the 1980s, available molecular markers were few (allozyme markers were first used in evolutionary biology in 1966, mtDNA data [RFLPs] first appeared in 1979). Nonetheless, comparisons using those markers, together with observations of morphological or

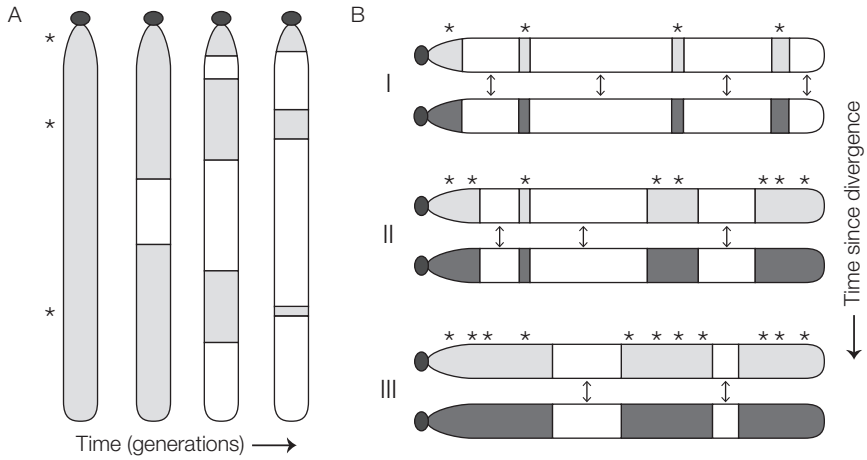

Figure 1. Classic illustrations of the semipermeable nature of species boundaries. (**A**) Gene flow following secondary contact as depicted by Barton and Hewitt (1981). The vertical bars represent a chromosome with 3 loci contributing to reproductive barriers (indicated by *). Immediately after contact, linkage disequilibrium along the chromosome will be high, but over time recombination breaks down associations among loci. Barrier genes or genes under divergent selection will remain differentiated (light gray regions), whereas alleles at loci that are neutral (white regions) will be exchanged between species. Many generations of recombination in hybrid zones allow fine-scale mapping of genes contributing to reproductive isolation and estimates of the strength of selection on individual alleles. (**B**) The idea that the genomes of diverging lineages become less permeable over time, as shown by Wu (2001). Each pair of horizontal bars represents chromosomes of 2 diverging lineages. Very recently diverged species (pair I) may have few genes contributing to reproductive isolation (indicated by *). These regions will remain differentiated (represented by light and dark gray regions), whereas gene exchange can occur in other parts of the genome (white regions). With increasing genetic divergence (chromosome pairs II and III), an increasing number of loci contribute to reproductive barriers, thus restricting gene flow for a greater proportion of the genome.

behavioral traits, supported the view that some markers (or sorts of markers) introgress further/faster than others (Table 1 in Harrison 1990). The observation that the extent of introgression of mtDNA markers was often greater than that of nuclear encoded markers was explained by the fact that mtDNA sequences are unlinked to the nuclear genome and, therefore, unlinked to genes that contribute to reproductive isolation (Barton and Jones 1983; Harrison 1989).

By combining many variable molecular markers (random amplified polymorphic DNAs) with a linkage map of those markers, Rieseberg et al. (1999) were able to identify chromosomal segments with reduced introgression across 3 replicate hybrid zones between 2 sunflower species (genus *Helianthus*). The consistent patterns for the 3 presumably independent hybrid zones strongly suggested that reduced introgression was the product of deterministic forces (i.e., selection), and indeed, many of the chromosomal blocks with reduced introgression were shown to be associated with hybrid pollen sterility (an important barrier to gene exchange in *Helianthus*). This article provided the first detailed analysis of differential introgression in the context of a genetic map and remains a classic in the hybrid zone literature.

Given the rapid advances in DNA sequencing and genotyping technology, patterns of differentiation and introgression for multiple markers can now be assayed relatively easily, even for organisms that lack substantial genomic resources. Methods for estimating the extent of introgression and for interpreting observed patterns have similarly made important advances (Gompert and Buerkle 2009, 2011, 2012; Payseur 2010; Fitzpatrick 2013). These methods can be divided into 2 categories: 1) those that analyze geographic clines (how allele and genotype frequencies change over space) and 2) those that employ genomic clines, in which changes in genotype frequencies for individual loci are examined "along a genomic admixture gradient" (Gompert and Buerkle 2009, p. 1207). Both approaches can define patterns of differential introgression, but a genomic cline approach is particularly useful in mosaic hybrid zones, where it may not be possible to define a simple geographic transect, except at very fine spatial scales.

The Genetic and Genomic Architecture of Species Boundaries

The "genetic architecture of species boundaries" refers to the number, effect size, and chromosomal distribution of the genes that encode phenotypes that result in barriers to gene exchange (speciation phenotypes). Discussions of genetic and genomic architectures have proliferated in recent years, as new and more efficient DNA sequencing and genotyping technologies have emerged. Comparisons between individuals from sister species or from races/strains/subspecies that are in the early stages of divergence can now be made at the level of whole-genome sequences, sequences from targeted regions of reduced complexity (e.g., transcriptome sequences

or RAD sequences), or for hundreds to hundreds of thousands of single-nucleotide polymorphisms (SNPs).

One of the early articles to make such a comparison, between 2 forms (now named species) of mosquitoes in the genus *Anopheles*, found that divergence appeared to be restricted to 3 regions of the genome and labeled these regions "genomic islands of speciation" (Turner et al. 2005). The term "genomic islands" stuck, and the subsequent literature has elaborated on the geographic/topographic imagery, although in some cases "islands of differentiation" has replaced "islands of speciation" (e.g., Nosil et al. 2009). Invoking similar imagery, some have suggested that a more common pattern may be "archipelagoes" or "continents" of speciation (Michel et al. 2010). It is not clear that the imagery of oceans, sea level, and terrestrial topography provides a useful context for discussing genetic architecture (Harrison 2012). Indeed, simply identifying regions that are significantly elevated in divergence remains a challenge and depends on (often unstated) assumptions about historical demography.

We are interested in how and where genes that determine speciation phenotypes are arrayed on chromosomes. We are also interested in how selection influences allele frequencies at these loci and in the impact of that selection on surrounding chromosome regions. The expected size of genome regions that remain differentiated in the face of some gene flow will depend on selection and recombination. It will also depend on the frequency of individuals heterozygous for population-specific markers (positive assortative mating will reduce this frequency) and on the reproductive success of those individuals. It is only in such individuals that recombination between population-specific alleles can occur. Via and West (2008) coined the term "divergence hitchhiking" to describe the fact that when there is the potential for hybridization between diverging populations, divergent selection and nonrandom mating reduce effective recombination rates (from those expected based simply on map distance). Whether "divergence hitchhiking" should result in larger islands of differentiation, as claimed by Via and West (2008), remains controversial (Nosil et al. 2009; Via 2009, 2012; Feder and Nosil 2010; Feder et al. 2012; Flaxman et al. 2012).

Many recent studies have characterized genome-wide patterns of divergence between closely related species. Table 1 summarizes data collected from a diversity of taxa. These studies represent a range of approaches for surveying patterns of variation across the genome and for identifying regions that exhibit excess divergence. However, the majority of studies have relied on estimates of F_{ST} (e.g., an "F_{ST} outlier" approach; Beaumont and Balding 2004) or other relative measures of divergence. Because relative measures of divergence (including F_{ST}) depend both on divergence between and variation within populations, elevated F_{ST} can be due to reduced nucleotide diversity within populations (Charlesworth 1998; Nachman and Payseur 2012; Cruickshank and Hahn 2014). As a consequence, high F_{ST} values could reflect loss of variation within populations (e.g., as a result of a selective sweep), rather than excess divergence. It is, therefore, worth revisiting case histories for which F_{ST} outliers have been identified to determine the cause of elevated F_{ST} (see Cruikshank and Hahn 2014). Future studies need to include comparisons based on absolute measures of sequence divergence.

Genome-wide comparisons between recently diverged forms or species suggest that divergence is not restricted to a few discrete regions. Several studies that compare ecologically distinct but morphologically indistinguishable forms claim that there is widespread, but heterogeneous, divergence across the genome. These comparisons include the M and S forms of *Anopheles gambiae* (Lawniczak et al. 2010) and host races of *Rhagoletis pomonella* (Michel et al. 2010). Similarly, divergences between hybridizing flycatchers (*Ficedula*) and between oceanic and freshwater threespine sticklebacks are also highly heterogeneous across the genome, with many "divergence islands" (Hohenlohe et al. 2010; Ellegren et al. 2012). However, exactly what is meant by "widespread" or "heterogeneous" divergence remains unclear. Indeed, few generalizations are yet possible; this is not surprising, given the very recent development of efficient sequencing/genotyping methods, the importance of having a reference genome or a dense linkage map, and the additional difficulty of making comparisons when very different sets of markers and analyses have been used for different pairs of taxa (Table 1). Among the clear patterns that have emerged are the observations that differentiation is greater in regions of low recombination (Nachman and Payseur 2012) and very often on sex chromosomes (Carneiro et al. 2010; Lawniczak et al. 2010; Ellegren et al. 2013). In fact, a number of models predict the accumulation of barrier genes in regions of restricted recombination (e.g., within inversions, adjacent to centromeres) (Noor et al. 2001; Rieseberg 2001; Navarro and Barton 2003).

Genome-wide comparisons (ranging from modest numbers of microsatellite loci to full genome sequences) also can provide important insights into the evolutionary history of recent speciation/diversification events. An increasing number of studies have revealed evidence of hybridization among diverging lineages (Patterson et al. 2006; Putnam et al. 2007; Grant and Grant 2010; Garrigan et al. 2012; Cui et al. 2013; Keller et al. 2013; Nadeau et al. 2013; The Heliconius Genome Consortium 2012; Prüfer et al. 2014). These observations are consistent with a variety of scenarios for diversification in the face of at least episodic gene flow and lend support to the notion that hybridization allows introgression of adaptive traits (see below) and can, in some cases, lead to the origin of novel traits.

Although comparisons between recently diverged allopatric lineages can document the genomic landscape of genetic differentiation between species, observed differences between allopatric populations may or may not persist if populations come into contact. In contrast, hybrid zones allow us to examine directly the maintenance of genetic differentiation between sympatric or parapatric taxa. Often the product of secondary contact between forms that have been allopatric for at least some of their recent history (Barton and Hewitt 1985; Harrison 1990), natural hybrid zones provide direct information on patterns of differential introgression. In most study systems, alleles at some loci introgress

Table 1 Studies of genome-wide patterns of differentiation between recently diverged pairs of taxa that are isolated by reproductive barriers

Taxa compared	Organism	Time	Barriers	Populations (sample size)	Markers (library type)	F_{ST}	Outliers	References
Acyrthosiphon pisum "alfalfa," "red clover," and "pea"	Pea aphid	0.008–0.016 Ma	Habitat isolation	3 host plants from 3 regions (N = 180)	390 microsatellites (whole genome)	0.069–0.17[a]	2.8%	Jaquiéry et al. (2012), also Via and Hawthorne (2001), Via and West (2008), Smadja et al. (2012), and Via et al. (2012)
Anopheles gambiae "M," "S," and "Bamako"	Mosquito	—	Habitat isolation, assortative mating	1 sympatric (N = 60)	400 000 SNPs (whole genome)	—	—	Lawniczak et al. (2010), Neafsey et al. (2010) also Turner et al. (2005), Turner and Hahn (2007), White et al. (2010), and Weetman et al. (2011)
Coregonus clupeaformis "normal" and "dwarf"	Lake whitefish	0.06 Ma	Habitat isolation	1 sympatric (N = 24)	2203 SNPs (exon capture)	0.046	12%	Hebert et al. (2013), also Campbell and Bernatchez (2004), Renaut et al. (2010, 2011), and Gagnaire et al. (2013)
Ficedula albicollis and *Ficedula hypoleuca*	Flycatcher	>2 Ma	Assortative mating, hybrid sterility	2 allopatric (N = 20)	3.81 M SNPs (whole genome)	0.357	2.7%	Ellegren et al. (2013)
Gasterosteus aculeatus "freshwater," "oceanic," "benthic," and "limnetic"	Stickleback	0.012 Ma	Habitat isolation, assortative mating	34 allopatric (N = 196)	1159 SNPs (EST)	0.193 (0.031–0.383)	4.0%[b]	Jones, Grabherr, et al. (2012), also Hohenlohe et al. (2010, 2012) and Jones, Chan, et al. (2012)
Gasterosteus aculeatus "lake" and "stream"	Stickleback	0.012 Ma	Habitat isolation, assortative mating	8 allopatric (N = 216)	4127–8417 SNPs (RADseq)	0–0.149[a]	—	Roesti et al. (2012), also Deagle et al. (2012)
Gryllus firmus and *Gryllus pennsylvanicus*	Field cricket	0.2 Ma	Habitat isolation, assortative mating, postmating prezygotic	2 allopatric (N = 30)	9731 SNPs (RNAseq)	—	—	Andrés et al. (2013)
Helianthus petiolaris "dune" and "non-dune"	Sunflower	0.01 Ma	Habitat isolation	20 allopatric (N = 100)	19 539 SNPs (RADseq)	0.121	1.7%	Andrew and Rieseberg (2013)
Helianthus annuus and *H. petiolaris*	Sunflower	1.8 Ma	Habitat isolation, hybrid sterility	10 allopatric (N = 20)	27 994 SNPs (RADseq)	0.316	—	Andrew and Rieseberg (2013), also Yatabe et al. (2007), Strausberg et al. (2009), Gompert and Buerkle (2009), and Kane et al. (2009)
Helianthus annuus ssp. *annuus*, *H. annuus* ssp. *texanus* and *Helianthus debilis*	Sunflower	—	Habitat isolation, hybrid sterility	13 allopatric (N = 378)	88 microsatellites	—	3.4%	Scascitelli et al. (2010)
Heliconius melpomene ssp., *Heliconius timareta* ssp., *Heliconius heurippa*, *Heliconius cydno* ssp., and *Heliconius hecale*	Butterfly	—	Assortative mating, hybrid inviability	5 sympatric, 2 parapatric (N = 60)	4078 SNPs (RADseq)	—	7.0%	Nadeau et al. (2013) also Nadeau et al. (2012) and The Heliconius Genome Consortium (2013)

Table 1 Continued

Taxa compared	Organism	Time	Barriers	Populations (sample size)	Markers (library type)	F_{ST}	Outliers	References
Howea belmoreana and *Howea forsteriana*	Palms	6.9 Ma	Habitat isolation, assortative mating	—	274 AFLPs	0.31	1.5%	Savolainen et al. (2006)
Littorina saxatilis "crab" and "wave"	Marine snail	0.009 Ma	Habitat isolation, assortative mating	6 allopatric, 3 regions (N = 32)	614 AFLPs	0–0.027[a]	1.8–8.3%	Butlin et al. (2014), also Wilding et al. (2001), Grahame et al. (2006), Wood et al. (2008), and Galindo et al. 2010)
Mus musculus domesticus and *M. m. musculus*	House mouse	0.5 Ma	Assortative mating, hybrid sterility	2 allopatric (N = 15)	10 265 SNPs (whole genome)	—	—	Harr (2006)
Neochlamisus bebbinae "willow" and "maple"	Leaf beetle	—	Habitat isolation, assortative mating	5 allopatric (N = 165)	447 AFLPs	0.0363–0.1060[a]	4.0–8.1%	Egan et al. (2008)
Populus alba and *Populus tremula*	Poplar	—	Assortative mating, postzygotic isolation	2 allopatric (N = 14)	38 525 SNPs (RADseq)	0.634	—	Stölting et al. (2012)
Quercus rober and *Quercus petraea*	Oak	—	Habitat isolation	14–20 allopatric (N = 50–1190)	389 markers (isozymes, AFLPs, SCARs, microsatellites, SNPs)	0.0357	12%	Scotti-Saintagne et al. (2004)
Rhagoletis pomonella "apple" and "hawthorne"	Apple maggot	>0.001 Ma	Habitat isolation, assortative mating	5 allopatric (N = 508 micro satellites/1419 allozymes)	39 microsatellites/ allozymes	0.0141–0.0066[a]	7.7%	Michel et al. (2010), also Schwarz et al. (2009)
Silene latifolia and *Silene dioica*	Campion flower	—	Habitat isolation, assortative mating	6 allopatric (N = 180)	305 AFLPs	0.39–0.58[a]	9.6%	Minder and Widmer (2008)
Timema cristinae "*Ceanothus*" and "*Adenostoma*"	Walking-stick insect	—	Habitat isolation, assortative mating	8 allopatric (N = 161)	86 130 SNPs (RADseq)	0.111	17.6%	Nosil, Parchman, et al. (2012), also Nosil et al. (2008)
Zeiraphera diniana "larch" and "pine"	Larch budmoth	—	Habitat isolation, assortative mating	5 allopatric (N = 92)	1291 AFLPs	0.216	17.7%	Emelianov et al. (2004)

For each pair, the table not only summarizes data from the most recent study but also includes references to earlier genome scans. Only studies that estimated divergence for >20 markers are included. The columns provide information on the estimated time since divergence (Time), known barriers to gene exchange (Barriers), the number of populations sampled and total number of individuals genotyped (Populations/sample size), the number/type of markers and their source (Markers/library type), the average F_{ST} between the taxon pairs (F_{ST}), and the number of outlier loci identified in each study (Outliers). Ma = million of years ago; N = sample size; AFLP = amplified fragment length polymorphism; EST = expressed sequence tag; RADseq = restriction site–associated DNA sequencing; RNAseq = transcriptome sequencing; SCAR = sequence characterized amplified region.

[a] The range of mean F_{ST} observed in comparisons of multiple species or population pairs.

[b] Outliers were estimated between "benthic" and "limnetic" population pairs.

significantly more than expected; for other loci, introgression is much less than expected. Figure 2 shows an example of data from a field cricket hybrid zone, in which SNPs are chosen because they exhibit major allele frequency differences between allopatric populations that reveal variable patterns of introgression (Larson et al. 2013; Larson et al. 2014). A subset of SNPs show limited introgression in 2 very different regions of the hybrid zone and at very different spatial scales. SNPs that show consistent patterns of restricted introgression across multiple transects or contacts may mark genome regions that are components of a "universal" species boundary. Indeed, differential introgression is characteristic of all hybrid zones for which multiple markers have been studied (Table 2). Regions/alleles that introgress more than expected may be examples of adaptive introgression; regions with restricted introgression may be associated with divergent directional selection, hybrid unfitness, and or positive assortment, that is, they may harbor genes that determine speciation phenotypes. Recent comparisons of whole-genome sequences from humans and Neanderthals have revealed evidence of hybridization and differential introgression between the 2 lineages. These studies document both reduced introgression on the X chromosome (perhaps associated with the presence of male sterility genes on the X; Sankararaman et al. 2014) and signatures of adaptive introgression for genes that determine skin phenotypes (Vernot and Akey 2014).

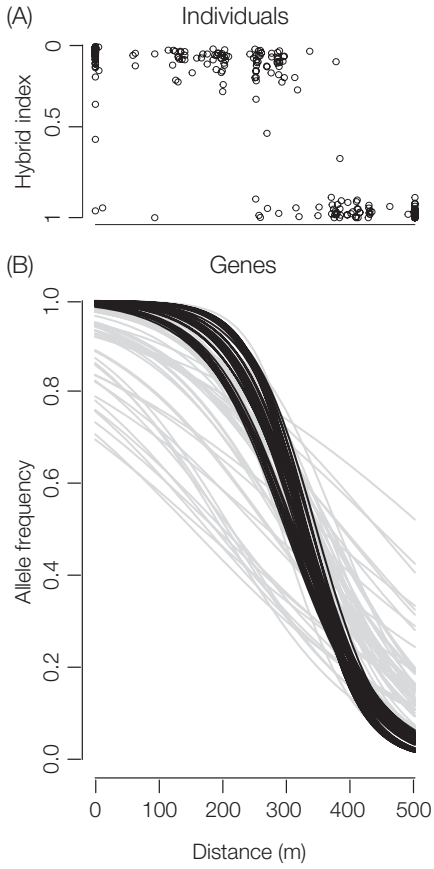

Figure 2. Differential introgression across a hybrid zone between the field crickets *Gryllus firmus* and *Gryllus pennsylvanicus*. The hybrid zone was sampled along a 500-m transect that spans the boundary between habitat patches (sand and loam soils). (**A**) The hybrid index (HI, estimated from 110 diagnostic SNPs) for each cricket ($N = 260$) plotted against the distance along the transect. There is an abrupt transition from *G. pennsylvanicus*–like (left, HI = 0) to *G. firmus*–like (right, HI = 1) crickets at approximately 320 m. (**B**) The change in *G. pennsylvanicus* allele frequencies for 110 markers along the transect. Many loci have gradual changes in allele frequencies, whereas others have relatively abrupt changes that coincide with the transition between species seen in panel (A). These loci have restricted introgression relative to the other loci and represent regions of the genome that define the species boundary.

The Species Boundary as a Continuum

Are species discrete entities and what is their relationship to varieties, races, and subspecies? In confronting this question, Mallet et al. (2007) and Mallet (2008a, 2008b) have repeatedly stated that Darwin (1859) got it right and that Mayr (1942, 1963) got it wrong. The essence of the argument is that Darwin emphasized continuity between varieties and species, whereas Mayr emphasized that species are real and discrete entities. Without wading into the murky waters of interpreting exactly what each of these prominent evolutionary biologists had to say, it is evident that there is truth in both points of view.

Geographic populations of the "same" species can be distinct in many ways, and such distinct populations are often recognized as races, strains, subspecies, or semispecies. A classic example of varying amounts of divergence between allopatric populations is the *Drosophila willistoni* group in South America, a group studied closely by Dobzhansky and his students (e.g., Ayala et al. 1974). Using data from 36 allozyme loci, Ayala et al. (1974) demonstrated increasing genetic differentiation (increasing proportion of loci showing significant allele frequency differences) in comparisons of conspecific geographic populations, subspecies, semispecies, sibling species, and morphologically distinct species. For these flies, and perhaps for many examples of allopatric divergence, the species boundary would seem to increase with time since divergence. An important consequence is that hybrid zone interactions can reflect a continuum of times and stages of taxon divergence. That is, secondary contact following geographic isolation can occur at varying times subsequent to the vicariance or dispersal event that led to isolation.

More problematic is whether sympatric populations also reveal the same continuum. If divergence with gene flow occurs easily/regularly, then the species boundary will grow in situ, and again, we might expect a continuum. Some recent studies address this issue by comparing patterns of differentiation between ecotypes with patterns of differentiation

Table 2 Studies of introgression across hybrid zones

Taxa compared	Organism	Time	Barriers	Populations (sample size)	Struct.	Markers (library type)	Method	Cline width	Diff	Asym	References
Cottus perifretum and *Cottus rhenanus*	Sculpin	1–2 Ma	Possible hybrid inviability	3 allo (N = 136), 2 sym (N = 344)	Clinal	858 micros	Genomic	NA	Y	N	Nolte et al. (2009), also Nolte et al. (2006)
Gryllus firmus and *Gryllus pennsylvanicus*	Field cricket	0.2 Ma	Habitat isolation, assortative mating, postmating prezygotic	6 allo (N = 71), 2 sym (N = 561)	Mosaic	168 SNPs (RNAseq)	Genomic/ geographic	162–861 m	Y	Y	Larson, White, et al. (2013), also Larson, Andrés, et al. (2013)
Helianthus annuus and *Helianthus petiolaris*	Sunflower	1.8 Ma	Habitat isolation, hybrid sterility	6 allo (N = 69), 4 sym (N = 228)	Mosaic	110 RAPDs (linkage map)	Genomic	NA	Y	N	Buerkle and Rieseberg (2001), Gompert and Buerkle (2009), also Rieseberg et al. (1999)
Lycaeides idas and *Lycaeides melissa*	Butterfly	2.4 Ma	Habitat isolation, possible premating barriers	2 allo (N = 192), 1 sym (N = 186)	Patchy	119 677 SNPs (RADseq)	Genomic	NA	Y	Y	Gompert et al. (2012)
Manacus candei and *Manacus vitellinus*	Manakin	—	Assortative mating	2 allo (N = 100), 1 sym (N = 104)	Clinal	119 677 SNPs (RADseq)	Genomic	NA	Y	N	Parchman et al. (2013)
Mus musculus domesticus and *M. m. musculus*	Mouse	0.5 Ma	Assortative mating, hybrid sterility	2 allo (N = 14), 2 sym (N = 679)	Clinal	59 100 SNPs (whole genome)	Genomic/ geographic	6.4–341.8 km[a]	Y	Y	Janousek et al. (2012), also Payseur et al. (2004), Teeter et al. (2008, 2010), and Macholán et al. (2011)
Oryctolagus c. cuniculus and *Oryctolagus c. algirus*	Rabbit	1.8 Ma	Unknown	Transect (N = 1078)	Clinal	1401 SNPs (whole genome)	Geographic	10–545 km	Y	N	Carneiro et al. (2013), also Carneiro et al. (2010)
Picea sitchensis and *Picea glauca*	Spruce	—	Habitat isolation	2 allo (N = 66), 29 sym (N = 721)	Clinal	22 SNPs (candidate genes)	Genomic	NA	Y	Y	Hamilton et al. (2013a, 2013b)
Populus alba and *Populus tremula*	Poplar	—	Habitat isolation, possible post-mating barriers	6 allo (N = 248), 3 sym (N = 436)	Mosaic	268 micros (whole genome)	Genomic	NA	Y	Y	Lindtke et al. (2012), also Lexer et al. (2007, 2010)
Timema cristinae "*Ceanothus*" and "*Adenostoma*"	Walking-stick insect	—	Habitat isolation, assortative mating	2 allo (N = 42), 2 para (N = 84)	Parapatric	38 304 SNPs (RADseq)	Genomic	NA	Y	N	Nosil, Gompert, et al. (2012)

For each hybrid zone, data from the most recent study are summarized, but references to earlier studies are included. Only hybrid zones for which >20 markers were analyzed are included. For each pair of hybridizing taxa, the table includes estimated divergence time (Time), documented barriers to gene exchange (Barriers), the number of populations sampled and total number of individuals genotyped (Populations), the hybrid zone structure (Struct.), the number/type of markers and their source (Markers/library type), the method of estimating introgression (Method), the estimated geographic cline width (cline width), whether the studies found evidence of differential introgression (Diff) or asymmetric introgression (Asym) and references. N = sample size; allo = allopatric populations; sym = sympatric populations; para = parapatric populations; micros = microsatellite loci; RADseq = restriction site–associated DNA sequencing; RAPD = random amplified polymorphic DNA.

[a] Cline width estimated from Teeter et al. (2008).

between species (e.g., Andrew and Rieseberg 2013). Because Mayr did not believe that sympatric speciation occurred (except in a few cases, e.g., allopolyploid hybrid speciation in plants), he emphasized discontinuity. In fact, species histories are generally more complex than simple allopatric or sympatric scenarios might have us believe, and many taxa have probably experienced alternating periods of isolation and gene flow. In the end, it seems that one does not have to take sides in the Darwin versus Mayr debate to recognize the species boundary as a continuum.

Hybrid Speciation, Adaptive Introgression, and the Origin of Novel Traits

Hybridization may contribute directly to the origin of species, either as a result of reinforcement or hybrid speciation (Servedio and Noor 2003; Mallet 2007; Abbott et al. 2010, 2013). Some proponents of this view, like many of their colleagues, invoke the specter of Mayr (1942) and suggest that hybridization has traditionally been viewed as an "evolutionary dead end" (Seehausen 2013), or together with gene flow, as "mainly destructive forces with little evolutionary consequence" (Saetre 2013). Homoploid hybrid speciation involves the formation of novel genetic combinations and novel adaptations that allow persistence of the hybrid lineage, often in an environment distinct from that of either parent. Recognized as a common phenomenon in plants (Arnold 1997; Abbott et al. 2010), homoploid hybrid speciation has more recently gained support as a speciation mechanism in animals (Gompert et al. 2006; Mallet 2007). However, this potentially "constructive" role for hybridization remains controversial and many think that homoploid hybrid speciation will not turn out to be an important mode of speciation in animals (Barton 2013; Servedio et al. 2013; Schumer et al. 2014).

One of the contentious issues is the relative contribution of hybridization (vs. mutation) as a source of novel alleles or genotypes. Hybridization allows introgression of combinations of alleles that have already been "tested" by natural selection. Moreover, because of the greater genetic differences between (as opposed to within) hybridizing taxa, one outcome of hybridization may be the appearance of transgressive phenotypes (extreme phenotypes not seen in either of the parents), which is a source of evolutionary novelty. But it can also be argued that in a set of populations subdivided by hybrid zones, novel adaptations will appear no faster than if the entire set of populations was panmictic (Barton 2013). Similarly, hybridization tends to make 2 populations more similar (not less) and therefore must (at some level) oppose divergence of the hybridizing lineages (Servedio et al. 2013). It is possible that adaptive introgression of traits from species A into species B might lead to splitting of B into B and B', that is, the introgression of traits from A may render some individuals of B sufficiently different from others that they are now effectively 2 species. This appears to be the case in *Heliconius* butterflies, where alleles at loci encoding wing color patterns have introgressed (Pardo-Diaz et al. 2012). Numerous examples of adaptive introgression have been reported, but few result in speciation events.

Human-Mediated Secondary Contact

Semipermeable species boundaries have important implications for human-mediated secondary contact. Such contact may occur as a consequence of environmental disturbance, accidental introductions, or intentional introductions of wild populations, crop plants, or domestic animals. Thus, introduced species may overlap with and potentially interbreed with congeners, and in some of these cases, there is evidence for differential introgression (Abbott et al. 2003; McDonald et al. 2008; Feulner et al. 2013; Goedbloed et al. 2013; Hohenlohe et al. 2013). Gene flow may carry alleles in both directions (from introduced into native and vice versa), and the consequences of gene flow may be problematic. For example, transgenes or other alleles from crop plants can make their way into populations of wild relatives. These alleles may increase the fitness of the wild plants, and thus, natural selection will drive introgression (Ellstrand 2003; Snow et al. 2010; Snow 2012). Similarly, hybridization between domesticated sheep and their wild relatives has resulted in changes in coat color and pattern in the wild sheep, changes that appear to be adaptive (Feulner et al. 2013).

But "adaptive" changes in wild populations may not be desirable; alleles that confer resistance to pesticides or herbicides may endow insects or plants with properties that allow them to flourish but that we view with concern (e.g., a weedy plant becomes resistant to herbivory; Yang et al. 2011). The probability that such transfers will occur depends not only on the selective advantage/disadvantage conferred by a particular allele but also on the genomic location of the gene and its linkage relationship to other genes. In situations where human-mediated secondary contact allows for hybridization and introgression between species that previously were not connected by gene flow, genome scans define the genomic context in which potentially invasive alleles are embedded and thereby provide information about the likelihood of introgression (Hohenlohe et al. 2013).

Conclusions

Patterns of differentiation between recently diverged taxa and patterns of variation in hybrid zones provide important insights into the genetic architecture of species boundaries. For the past 40 years, evolutionary biologists have been using molecular markers to characterize differentiation between species and races and to define allele and genotype frequencies across natural hybrid zones. In the beginning, markers were few and reference genomes unimagined. Today, markers are virtually unlimited in number and reference genomes relatively easy to obtain. More and better genetic data can be obtained in a single Illumina Hi-Seq run than could be obtained over many years of using RFLPs or other indirect

methods for assaying DNA sequence variation. These data now allow genome-wide patterns of divergence or differential introgression to be described in remarkable detail, although in few cases are convincing explanations for these patterns available.

In contrast to the major advances in data generation and analysis, the questions being asked by evolutionary biologists have remained much the same. Recent data reinforce conclusions based on many fewer loci: species boundaries are semipermeable, with permeability varying as a function of genome region. Thus, hybridizing taxa often remain distinct for only part of the genome. The proportion of the genome that is resistant to introgression varies among taxa and, in some cases, patterns of introgression appear to be different when data are available for multiple transects across the "same" hybrid zone (e.g., Teeter et al. 2010). This suggests that the species boundary may vary geographically, perhaps a result of local adaptation in heterogeneous environments. Genome regions that consistently show reduced introgression between pairs of hybridizing taxa likely harbor genes that contribute to barriers that are independent of environmental variation. Working with sunflowers, Rieseberg et al. (1999) clearly documented such a pattern, and more recent work on a field cricket hybrid zone has identified a set of markers that exhibit reduced introgression in 2 distinct regions of the hybrid zone (Larson et al. 2014).

Genomic divergence and differential introgression are likely taxon specific, but some consistent patterns have begun to emerge. The massive amounts of data that are now being produced in a wide variety of natural systems promise that we may soon have a clearer picture of the details of species boundaries. Comparisons of diverging lineages provide static views of patterns of differentiation across the genome, but with more data, we ultimately will be able to define the dynamics of species boundaries, how boundaries become less (or more) permeable over time.

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