Speciation Continuum

DB Lowry and BA Gould, Michigan State University, East Lansing, MI, USA

Glossary

Allopatry   It refers to geographically isolated populations.

Allopolyploid   A new species resulting from hybridization between two parental species, where the hybrid species has twice the chromosome number of the parental species.

Assortative mating   When individuals with similar genotypes mate preferentially with each other at greater frequency than if mating was random.

Autopolyploid   A new species resulting from the doubling of the genome of one parental species is called autopolypoid.

Chromosome doubling test   A test conducted to determine if chromosome rearrangements are involved in hybrid sterility. Diploid hybrid fertility is compared to artificial allopolyploid hybrid fertility. If the allopolyploid hybrids have increased fertility, then it can be concluded that chromosomal rearrangements are involved in the sterility of hybrids.

Dobzhansky–Muller incompatibility   Hybrid sterility, inviability, or lethality that typically results from a negative epistatic interaction between two or more genetic loci. These incompatibilities are thought to evolve in allopatry.

Ecogeographic isolation   Reproductive isolation that occurs when two ecotypes or species are geographically isolated as a result of adaptation to local environmental conditions.

Gametic isolation   A post-mating prezygotic reproductive isolating barrier caused by the failure of the gametes of two species to generate a fertilized embryo.

Homoploid hybrid speciation   A new species resulting from hybridization between two parental species where the hybrid species has the same chromosome number as the parental species.

Parallel speciation   Repeated evolution of reproductively isolated populations by the same mechanisms as a result of similar selection pressures in different geographic regions.

Reciprocal monophyly   When phylogenetic analysis reveals that two groups form distinct monophyletic clades.

Reinforcement   The process by which prezygotic isolation evolves by natural selection to avoid costly mating between species that results from postzygotic isolation.

Sympatry   It refers to populations with overlapping geographic ranges.

Introduction

The biological diversity that we observe on earth is the product of a chain of speciation events stretching back to the origin of life. But what do we mean by a speciation event? The formation of a new species is not instantaneous. Rather, each speciation 'event' is a complex continuum that occurs over time as interbreeding populations bifurcate into different species and eventually, distinct phylogenetic lineages. This process generally takes considerable time and thus we can only observe brief snapshots of the continuum in a human lifespan. Over the past century and a half, many evolutionary biologists have attempted to understand the speciation continuum, but many questions still remain (Sobel et al., 2010; Baack et al., 2015).

One of the approaches that evolutionary biologists have adopted is to classify parts of the continuum as stages in the process of speciation. Historically, there were three major points when evolutionary biologists divided the speciation continuum into distinct stages (reviewed in Lowry, 2012). Alfred R. Wallace was the first to clearly articulate the idea of stages in the process of speciation (Wallace, 1865). During the middle part of the twentieth century, multiple plant evolutionary biologists, most notably Gote Turesson and Jens Clausen, developed their own scheme for describing stages in the process of speciation (Turesson, 1922; Clausen, 1951). Recently, a number of zoologists have resurrected the idea of stages in the process of speciation (Wu, 2001; Nosil et al., 2009; Seehausen et al., 2014). By focusing on different stages of the continuum of speciation, it is possible to gain insights into the different evolutionary mechanisms involved along the entire continuum. Recent studies now regularly split the speciation continuum into stages to understand the entire process better (Nosil et al., 2009; Hendry et al., 2009; Powell et al., 2013; Conflitti et al., 2014; Seehausen et al., 2014).

Mathematical modeling can be very useful for clarifying the details of individual stages of the speciation continuum, especially when tailored to particular biological systems (Gavrilets et al., 2014). However, the entire process of speciation is not easily captured in a single model. Instead, the process of speciation is more often constructed as a verbal narrative, whose story arc is revised and edited in response to the positive feedback between modeling and new empirical data. This article explores the reproductive isolating mechanisms that accumulate along the speciation continuum, how the process of speciation is completed, and why disagreements between species concepts could be the result of the continuous nature of speciation.

Reproductive Isolating Barriers along the Continuum

Mechanisms that Initiate the Speciation Process

The process of speciation always begins with the evolution of one or more reproductive isolating barriers. Isolating barriers

are typically categorized as prezygotic or postzygotic, acting before or after the formation of a hybrid zygote. Barriers are also divided between extrinsic (dependent on the external environment) or intrinsic (independent of environment) mechanisms. Prezygotic extrinsic barriers cause *assortative mating* between populations, ecotypes, or species. These barriers typically result from adaptive evolutionary changes in behavior, habitat, and/or pollinators. In contrast, intrinsic postzygotic barriers cause hybrid inviability, sterility, and lethality. Among intrinsic and extrinsic isolating mechanisms, postzygotic barriers tend to be weaker than prezygotic barriers early in speciation (Nosil et al., 2005; Lowry et al., 2008a; Sobel et al., 2010). The initiation of the speciation process occurs most often in groups that are spatially or temporally isolated due to geography and/or ecology. Following this initial isolation, incipient species progress through the speciation continuum by accumulating stronger reproductive isolating mechanisms over time. Much of contemporary speciation research aims to determine the order in which isolating mechanisms evolve throughout the process (Seehausen et al., 2014).

The Genetic Basis of Isolating Barriers

Early theory on the population genetic basis of reproductive isolation recognized that epistatic interactions between two or more loci, which evolved in allopatry, could cause intrinsic postzygotic isolation (Bateson, 1909; Dobzhansky, 1934; Muller, 1942). *Dobzhansky–Muller incompatibilities* constitute mutations that reduce hybrid fitness when populations come into secondary contact with each other following a period of allopatry. Chromosomal rearrangements can cause hybrid sterility through the production of unbalanced gametes in meiosis. While intrinsic barriers can evolve by selection or drift, extrinsic barriers to gene flow result from the evolution of traits in response to selection. Extrinsic barriers can thus result from any gene that has evolved in response to natural or sexual selection.

The genetic basis of several reproductive isolating barriers has been determined in recent years (Presgraves, 2010;

Rieseberg and Blackman, 2010; Seehausen et al., 2014). This research has confirmed the Dobzhansky–Muller model of intrinsic postzygotic isolation by identifying the genes that interact epistatically to cause reductions in hybrid fitness. The evolution of incompatibility genes is thought to be driven by an array of mechanisms including genomic conflict, selfish genetic elements, and rapidly evolving immunity genes. Further, ecological adaptations can drive the spread of incompatibilities when the loci under natural selection are genetically linked to incompatibility loci (Wright et al., 2013). Very few studies have identified genes involved in extrinsic barriers to reproductive isolation. Successful gene identification has only occurred in cases where the genetic architecture underlying the barrier was simple (e.g., Hopkins and Rausher, 2011). The dearth of known ecological reproductive isolation genes may reflect the complex genetics underlying the evolution of morphological and physiological traits involved in most extrinsic barriers.

Different Narratives of Speciation

The Selection-Based Narrative of Speciation

Most of the evolutionary biologists who have divided speciation into stages have focused on scenarios in which the initial divergence leading to speciation is driven by selection. We describe the narrative here for speciation driven by selection (Figure 1):

The first stage of speciation in this narrative occurs when different populations diverge due to selection. We now have strong evidence that natural and sexual selection can lead to assortative mating, and thus drive reproductive isolation between populations. This is especially true for studies of *parallel speciation*, where reproductive isolation occurs repeatedly between independent pairs of populations due to strong ecological selection. An excellent example of the early stages of parallel speciation is found in the stick insect species *Timema cristinae* (Figures 2(a) and 2(b)). These insects repeatedly form
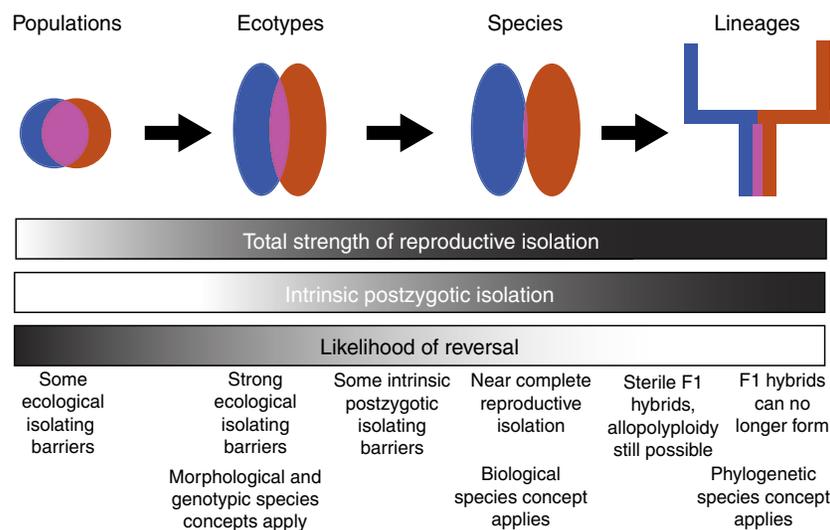

Populations   Ecotypes   Species   Lineages

Total strength of reproductive isolation

Intrinsic postzygotic isolation

Likelihood of reversal

Some ecological isolating barriers	Strong ecological isolating barriers	Some intrinsic postzygotic isolating barriers	Near complete reproductive isolation	Sterile F1 hybrids, allopolyploidy still possible	F1 hybrids can no longer form
	Morphological and genotypic species concepts apply		Biological species concept applies		Phylogenetic species concept applies

Figure 1   A selection-based narrative of the continuum of speciation. Orange and blue shapes represent diverging groups, with pink indicating potential gene flow between those groups.
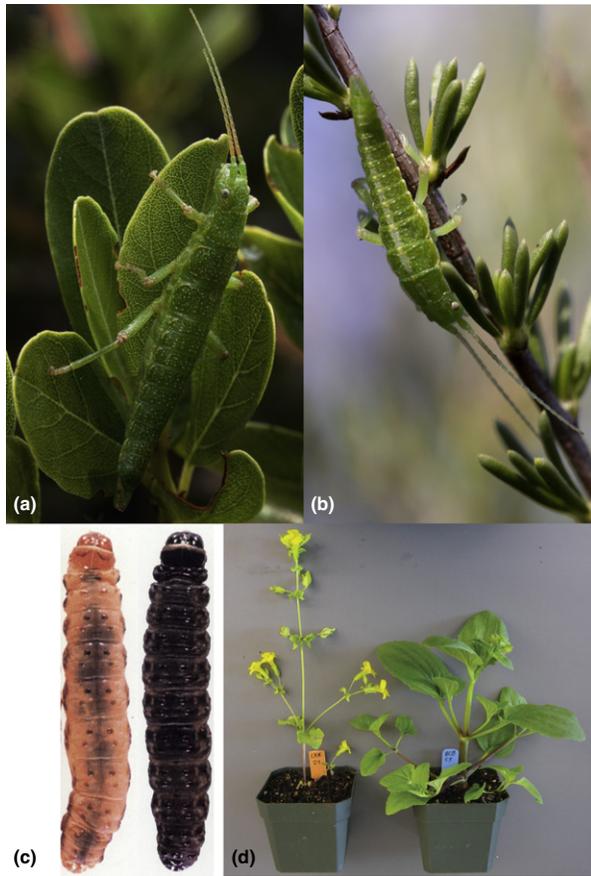
Figure 2 Divergent populations and ecotypes. Parallel patterns of reproductive isolation have occurred between nearby populations of *Timema cristinae* stick insects that are adapted to (a) *Ceanothus* and (b) *Adenostoma* host plants (see Soria-Carrasco *et al.*, 2014; photo credit: A. Comeault). (c) Ecotypes of the larch budmoth *Zeiraphera diniana* adapted to pine (left) and larch (right) trees (see Emelianov *et al.*, 2004; photo credit: W. Baltensweiler and J. Mallet). (d) Inland annual (left) and coastal perennial (right) ecotypes of the yellow monkeyflower *Mimulus guttatus* (see Lowry, 2012; photo credit: D. Lowry).

reproductively isolated pairs of adjacent populations that are adapted to living on different plant host species (Soria-Carrasco *et al.*, 2014).

The next stage of speciation is the formation of ecotypes, which encompass multiple populations that share a set of traits in common (**Figure 2**). Ecotypes have gone by different names, including ecological races, host races, varieties, and subspecies (Clausen, 1951; Mayr, 1982; Dres and Mallet, 2002; Nosil, 2012). The discrepancy in the names of these groups reflects differences in the interests of various biologists and what evolutionary forces are thought to be responsible for the evolution of these groups. Since ecotypes involve multitrait divergence, they are best characterized by the principal components of the set of traits that define them (Lowry, 2012). Ecotypes are not distinct species. While they have some reproductive isolation that prevents gene flow between them, it is not strong enough to prevent collapse through mating if environmental conditions change or sexual selection shifts. An example of ecotypes can be found in the yellow

monkeyflower, *Mimulus guttatus* (Lowry *et al.*, 2008b). Within *M. guttatus*, a geographically widespread perennial ecotype occurs in cool-wet coastal habitats which differs strongly from a closely related annual ecotype adapted to hot and dry inland habitats (**Figure 2(d)**).

Once reproductive isolation reaches a very high level, divergent populations have reached the species stage. Gene flow is rare at this stage, but is still possible. Reversal of speciation is also much less likely at this point. Unlike ecotypes, the species stage is far less controversial and most biologists agree that species exist (Coyne and Orr, 2004). A good example of the species stage is represented by *Helianthus annuus* and *Helianthus petiolaris*, which are two widespread species of sunflowers that have partially overlapping ranges in North America. Reproductive isolation is extremely high between these two species. However, reproductive isolation is not fully complete and recent gene flow has been detected between these sunflower species through molecular methods (Sambatti *et al.*, 2012).

Some biologists assume species is the final stage in the process of speciation, but this view ignores the fact that gene flow often does still occur between many species. Gene flow can also reverse the process of speciation and lead to species collapse if environmental conditions change. Therefore, the final stage of the speciation continuum is two distinct phylogenetic lineages, which will no longer exchange genes in the future through sexual reproduction. To achieve those distinct irreversible lineages requires complete intrinsic postzygotic isolation. While not all evolutionary biologists currently agree, a renewed focus on the continuum of speciation has revived the view that complete intrinsic postzygotic isolation is necessary to complete the process (Seehausen *et al.*, 2014).

Alternatives to the Selection-Based Narrative of Speciation

Speciation that results from natural selection and sexual selection has come to dominate the field of evolutionary biology over the past 20 years. There is now sufficient empirical evidence to suggest that the general selection narrative (**Figure 1**) described above applies to many biological systems (Sobel *et al.*, 2010; Lowry, 2012; Soria-Carrasco *et al.*, 2014; Baack *et al.*, 2015). However, there are many alternative narratives by which speciation can occur.

One major alternative narrative for speciation to occur is through the initial accumulation of strong intrinsic postzygotic reproductive isolation (**Figure 3**). In this scenario, two populations become geographically isolated from each other. Over time, those two populations accumulate genetic changes through drift or selection that contribute to intrinsic postzygotic isolation. When those species come back into secondary contact, the two species can no longer produce fertile offspring. This is the classic allopatric model of speciation and is still a common narrative for the speciation continuum (Grant, 1981; Coyne and Orr, 2004).

While biologists have constructed different verbal narratives of the speciation process, most speciation events likely defy simple classification. It has long been recognized that the entire process of speciation often involves the simultaneous divergences of morphology and physiology with the accumulation of reproductive isolating barriers (Clausen, 1951, p. 90).
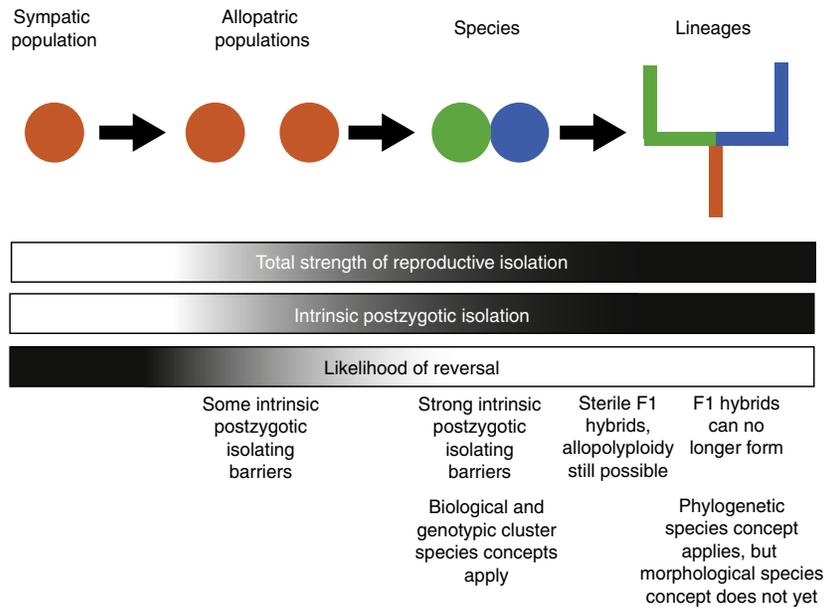
Figure 3   A geographic isolation narrative of the speciation continuum, where most reproductive isolation is the result of intrinsic postzygotic isolation accumulated in allopatry by selection or genetic drift.
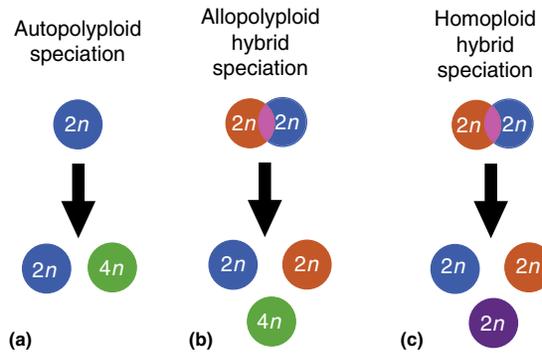

Figure 4   Diagram of three modes of rapid speciation: (a) autopolyploidy, (b) allopolyploid hybrid speciation, and (c) homoploid hybrid speciation. Diploids are represented by $2n$ and tetraploids by $4n$.

Overall, while there are general patterns of speciation, such as the progressive accumulation of reproductive isolation over time, the process is most commonly the result of a complex combination of multiple selective forces and chance events. The specific details of individual speciation narratives are likely as unique as snowflakes.

The length of time for the speciation process can also be considerably shorter in cases of polyploidy and hybrid speciation (Figure 4). Autopolyploid speciation occurs through spontaneous genome doubling, with a tetraploid daughter species instantaneously reproductively isolated from a parental diploid species (Soltis et al., 2007). Hybrid speciation occurs when related species successfully reproduce with the result being the formation of a new species. Allopolyploid hybrid speciation is very frequent in plants and occurs when the hybridization between two diploid species results in a tetraploid (or higher ploidy) daughter species. Reproductive isolation is often immediately strong between diploid parent and polyploid daughter species because hybrids are triploid and can be sterile.

Further, both autopolyploids and allopolyploids can have different ecological niches than parents simply as a by-product of changes in genome complement and those differences can lead to immediate habitat isolation (Ramsey, 2011). Homoploid hybrid speciation occurs, for example, when the hybridization between two diploid species results in a new species that is also diploid. There are few good examples of homoploid hybrid speciation and so it is thought to be far less common than allopolyploid hybrid speciation (Schumer et al., 2014).

Geography and the Continuum

The geographic context of speciation is a key component of the speciation. Allopatric separation is thought to occur at least at one point during the process in most of cases of speciation. Allopatry can occur early in speciation and be followed by a secondary contact phase in which speciation is completed. Alternatively, initial ecological divergences among sympatric

or parapatric populations could facilitate a subsequent phase of allopatry in which the accumulation of intrinsic postzygotic isolating barriers completes the process. There are many documented cases where local adaptation to different habitats maintains the allopatric distribution of populations and thus could lead to the further accumulation of reproductive isolating barriers. Allopatry that is maintained by ecological divergence is called *ecogeographic isolation*, and is thought to be very important in speciation (Schemske, 2000; Sobel, 2014).

Alternatively, speciation could occur without an allopatric phase if natural selection was strong enough to restrict gene flow between diverging taxa. Parapatric speciation along environmental gradients likely occurs often and may be the major mode of speciation in the ocean (Bowen *et al.*, 2013). Theoretical models overwhelmingly support the likelihood of parapatric speciation (Coyne and Orr, 2004; Gavrilets, 2014). However, proving that any given speciation event was entirely parapatric, without an allopatric phase, is difficult. Empirical evidence for speciation in *sympatry* is also sparse. There are only a handful of compelling cases of completely sympatric speciation (Barluenga *et al.*, 2006; Savolainen *et al.*, 2006) and those have been brought into question by modeling tailored to those systems (Gavrilets, 2014). Further, classic cases of sympatric speciation, mostly notably *Rhagoletis* fruit flies, now appear to involve genetic variation that first arose in allopatry (Feder *et al.*, 2005).

Mechanisms that Complete the Speciation Process

The Reversibility of the Speciation Continuum

The process of speciation is unstable until new species reach a point at which the process can no longer be reversed (Figures 1 and 3). While extrinsic ecological barriers to gene flow can be quite strong, they are contingent on current environmental conditions. If those environmental conditions change, ecological barriers can disappear leading to high levels of gene flow and subsequent species collapse. Reversals along the speciation continuum, linked to environmental changes, have now been documented in multiple fish species. For example, eutrophication has been linked with recurrent declines in differentiation between divergent sympatric lake whitefish subspecies (Vonlanthen *et al.*, 2012) and cichlid fish in Lake Victoria, Africa (Seehausen *et al.*, 2008). Similarly, stickleback fish researchers have documented the collapse of two species into a hybrid swarm following the introduction of an exotic crayfish (Taylor *et al.*, 2006). It should be mentioned that reversals in the speciation process could also occur for intrinsic postzygotic barriers, but those reversals are yet to be documented.

Reinforcement

While intrinsic postzygotic isolating barriers are ultimately responsible for the completion of the speciation process, the accumulation of those postzygotic barriers can actually drive the evolution of strong prezygotic barriers near the end of speciation. Reinforcement is the process by which new prezygotic barriers are selected for in order to avoid costly hybridization between species. Avoidance of hybridization is advantageous because hybrids are less fit than progeny produced by crosses within parental ecotypes or species. While reinforcement was controversial for much of the twentieth century, there are now many examples of reinforcement in both plants and animals (Yukilevich, 2012; Hopkins, 2013). Reinforcement is thought to typically occur when previously allopatric species come into secondary contact. Once in secondary contact, reinforcement is predicted to occur in regions of sympatry but not allopatry. One can assess the occurrence of reinforcement by comparing the strength of prezygotic isolating barriers in allopatric versus sympatric populations.

Hopkins and Rausher's (2012) recent research on *Phlox* identified the first genes to be involved in the process of reinforcement. *Phlox cuspidata* and *Phlox drummondii* come in contact with each other in central Texas, USA. These species produce highly sterile hybrids when they are crossed with each other. In the sympatric zone of these species' range, *P. drummondii* takes on a bright red coloration, which reduces deleterious hybridization with *P. cuspidata*. Two genes in the anthocyanin pigmentation pathway are ultimately responsible for the transition between pink and red flowers (Hopkins and Rausher, 2012). The *Drosophila* of the island of São Tomé, Africa provide an excellent example of how reinforcement can evolve without changes in mating preference. Both *Drosophilia yakuba* and *Drosophilia santomea* form a hybrid zone on the island and male hybrids between the two species are sterile. Matute (2010) found that these *Drosophilia* species evolved reinforcement through the evolution of *gametic isolation* in sympatric populations. Thus, reproductive isolation that occurs after mating, but before fertilization, can evolve through reinforcement.

Mechanisms Involved in the Completion of Speciation

While recent studies have established that natural selection can initiate the process of speciation through the formation of divergent populations and ecotypes, we have a poor understanding of how diverging groups subsequently acquire intrinsic postzygotic isolation to complete the speciation process. It is largely unknown how often the same environmental factors driving the formation of initial ecological barriers also directly drive the evolution of intrinsic postzygotic barriers. Intrinsic isolating barriers can result from both intrinsic and extrinsic selective forces. Further, intrinsic barriers can spread by genetic drift within populations that are already reproductively isolated by strong ecological barriers. It would be particularly interesting to determine whether ecogeographic isolation could be a major factor facilitating the spread of intrinsic barriers, since this barrier functions by promoting allopatry.

Dobzhansky–Muller incompatibilities have been localized in different types of organisms and are thus thought to be frequently involved in speciation (Presgraves, 2010; Rieseberg and Blackman, 2010). There is also strong evidence that chromosomal rearrangements are commonly involved in plant speciation. This conclusion comes from studies of species that have a high level of sterility in F1 hybrids. When the genomes of those species are artificially doubled to make allopolyploid hybrids, hybrid fertility is often restored. The proposed mechanism for restored fertility is that, rearranged chromosomes no longer pair

at meiosis and unbalanced gametes are in turn no longer produced. Ramsey and Schemske (2002) surveyed multiple highly reproductively isolated plant species and found that diploid hybrids only had on average 17% fertility. When synthetic tetraploid hybrids were made between these same species, fertility was elevated to 71%. A major conclusion of these *chromosome doubling tests* is that rearrangements are largely responsible for hybrid sterility in the later stages of plant speciation. While chromosomal reproductive isolation is common in plants, it does not appear to be as common in animals. There are multiple hypotheses for why chromosomal reproductive isolation is more common in plants than animals (Coyne and Orr, 2004, pp. 266–267), but this question has yet to be resolved.

Another profound implication of the chromosome doubling test is that as long as two species can make F1 hybrids there still may be potential for those species to combine to form a new allopolyploid hybrid species. Therefore, the gradual accumulation of postzygotic isolation barriers will not only lead to the formation of two separate phylogenetic lineages, but will also create the potential to instantaneously form a third hybrid lineage through allopolyploidization. As a result of this observation, Clausen (1951) argued that the continuum of speciation is only finally complete when two species can no longer produce any viable hybrids. For some organisms, it may take a very long time until F1 hybrids can no longer be produced. A recent study identified a natural F1 hybrid fern that had resulted from the hybridization between two species that had been separated for ∼60 million years (Rothfels *et al.*, 2015).

Species Concepts and the Continuum of Speciation

Most of the twentieth century was filled with fierce debate over the definition of species. However, much of the differences between species definitions can be attributed to biologists focusing on particular points of the speciation continuum. This controversy is analogous to the Indian parable of the blind men and the elephant. Each of the blind men had very different descriptions of the elephant because they had only touched one particular part of the elephant's body: ears, trunk, tail, tusks, etc. This resulted in a major disagreement between the men about the true nature of the elephant. The disagreement is only settled when a wise man informs the men that they are all correct because the elephant contained all the elements that the men had described.

Viewed as a continuum, it is quite easy to see how the major species concepts have arisen out of a focus on particular points along the continuum. This is clearly illustrated by revisiting the selection narrative of speciation (Figure 1). The morphological species concept is often used by taxonomists and primarily relies on a combination of traits quantified from museum specimens to define species. Many taxonomists prefer the morphological species concept because it does not require extensive additional research that other species concepts require. The genotypic cluster species concept defines species as a 'distinguishable groups of individuals which have few or no intermediates when in contact' (Mallet, 1995). A similar definition has recently been proposed for the term ecotype (Lowry, 2012). The reliance of the morphological and genotypic cluster species concepts on distinct traits is likely to bias them toward the earlier points along the

speciation continuum. The biological species concept utilizes a strict criterion of reproductive isolation to define species and therefore corresponds well with the species stage of the continuum (Figure 1). The phylogenetic species concept defines species by *reciprocal monophyly* between taxa, at least at a large portion of loci across the genome (Baum and Donoghue, 1995; Queiroz, 2007). In other words, species are only recognizable by the phylogenetic species concept when gene flow has ceased long enough that allelic differences become fixed between species. Such a situation will only occur after complete reproductive isolation has occurred between taxa for numerous generations, as shared ancestral polymorphism must be eliminated before gene tree topologies will reflect actual species trees (Cruickshank and Hahn, 2014; Fontaine *et al.*, 2014). Thus, the phylogenetic species concept will, by definition, be biased toward the very end of the speciation continuum.

If speciation only occurred by a single type of narrative, it would be clear that different species concepts simply refer to different points along the continuum of speciation. However, because there are many different narratives of speciation, confusion still persists over different species concepts. For example, imagine a scenario where speciation primarily occurs through the accumulation of intrinsic postzygotic isolation in allopatry without much morphological divergence (Figure 3). In this case, different species would be readily identifiable by the biological species concept, and perhaps even the phylogenetic species concept, before those species would be identifiable by the morphological species concept. Essentially, they would be considered as cryptic species. The key take away from this thought exercise is that viewing speciation as a continuum, without assuming any particular narrative, can free us from the need for rigid species concepts.

Going forward, researchers should try to build a more comprehensive understanding of the different stages of speciation. Studies over the past two decades have produced many new insights regarding the role of ecological and sexual selection in the early stages of speciation. A major challenge in the future will be to understand the mechanisms that complete the speciation process and forever maintain distinct non-recombining phylogenetic lineages. A key component of this future research will include the interrogation of the causes of hybrid sterility through combining the chromosome doubling test with modern techniques (e.g., Stathos and Fishman, 2014) as well as the examination of what eventually prevents the formation of F1 hybrids. Overall, we must always keep in mind that there are many routes to producing new species and that no one model will capture the diversity of forms of the speciation continuum.

See also: Reproductive Isolation, Postzygotic. Reproductive Isolation, Prezygotic. Species Concepts and Speciation

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