Defining the speciation continuum

Sean Stankowski[1,2,3] iD **and Mark Ravinet[4,5,6]** iD

[1]*Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, United Kingdom*

[2]*Current Address: Sean Stankowski, IST Austria, Klosterneuburg 3400, Austria*

[3]*E-mail: sean.stankowski@ist.ac.at*

[4]*Centre for Ecological and Evolutionary Synthesis, University of Oslo, Oslo 0316, Norway*

[5]*School of Life Sciences, University of Nottingham, Nottingham NG7 2RD, United Kingdom*

[6]*E-mail: mark.ravinet@nottingham.ac.uk*

A primary roadblock to our understanding of speciation is that it usually occurs over a timeframe that is too long to study from start to finish. The idea of a speciation continuum provides something of a solution to this problem; rather than observing the entire process, we can simply reconstruct it from the multitude of speciation events that surround us. But what do we really mean when we talk about the speciation continuum, and can it really help us understand speciation? We explored these questions using a literature review and online survey of speciation researchers. Although most researchers were familiar with the concept and thought it was useful, our survey revealed extensive disagreement about what the speciation continuum actually tells us. This is due partly to the lack of a clear definition. Here, we provide an explicit definition that is compatible with the Biological Species Concept. That is, the speciation continuum is *a continuum of reproductive isolation*. After outlining the logic of the definition in light of alternatives, we explain why attempts to reconstruct the speciation process from present-day populations will ultimately fail. We then outline how we think the speciation continuum concept can continue to act as a foundation for understanding the continuum of reproductive isolation that surrounds us.

A Clear, Intuitive Model of the Speciation Process…Or is it?

When we think about speciation, our conceptual understanding of it is often caught somewhere along a road between two contrasting perspectives. On the one hand, speciation is thought about as a discrete event—a boundary that marks the transition from populations to species. On the other hand, speciation is used to refer to a process that generates the distinct groups that we ultimately recognize as species.

These different views of speciation can be used to draw rough dividing lines in speciation research. For example, studies of speciation with gene flow have largely focused on populations with low levels of reproductive isolation, driven mainly by divergent selection on adaptive traits. The taxa at the center of these studies are often described as "incipient species" that are at an early point in the process (e.g., Turner et al. 2005; Marques et al. 2016). In contrast, much classical work on the genetic basis of hybrid sterility and inviability has focused more toward the end of the speciation process (e.g., Presgraves 2002; Phadnis et al. 2015). These studies require the use of model systems where intrinsic postzygotic isolation has evolved, and so it is tempting to think of speciation as an event that has occurred in the past or as a process that has culminated in the evolution of irreversible reproductive isolation. Similarly, macroevolutionary and comparative phylogenetic analyses typically assume that speciation is a discrete event, which allows its rate to be measured through time (e.g., Rabosky et al. 2013; Cooney et al. 2017).

The idea of a speciation continuum has allowed these apparently paradoxical views of speciation to coexist as part of the same conceptual framework. The concept is perhaps deceptively simple—variation in the degree of speciation observed in the present day is due to some populations having progressed further

Evolution

Table 1. A glossary of key terms.

| |
|---|
| *Barrier locus*: A locus that contributes to reproductive isolation between populations. |
| *Barrier effect*: The reduction in gene flow caused by an isolating barrier. |
| *Complete isolation*: When reproductive isolation = 1. |
| *Chronosequence*: A set of samples arising from the same starting condition but that differ in the time since they formed. |
| *Ecological divergence*: Difference in the ecological niches occupied by populations or species. |
| *Effective migration rate* (m_e): The number of migrants (m) entering a population that contribute genetically to future generations. |
| *Experimental speciation*: Experimental evolution studies of speciation. |
| *Gene flow*: The movement of genetic material between populations. |
| *Genetic divergence*: The accumulation of genetic differences between populations due to any evolutionary process. |
| *Geographic isolation*: A reduction in the migration rate (m) between populations as a result of geographic distance. Geographic isolation is not considered as a form of reproductive isolation because it causes a reduction in m, rather than a reduction in m_e. |
| *Hybrid zone*: A location where divergent populations meet and mate to produce offspring of mixed ancestry (i.e., hybrids). |
| *Isolating barrier*: Any genetically-based factor that causes reproductive isolation between populations. |
| *Local barrier effect*: The localized genomic impact of a barrier resulting from tight linkage disequilibrium between a barrier locus and nonbarrier loci. |
| *Migration* (m): The movement of individuals between populations. |
| *Overall barrier*: The combined effect of a set of isolating barriers. |
| *Phenotypic divergence*: The accumulation and/or level of phenotypic differentiation between populations. |
| *Reproductive isolation* (RI): A reduction in potential gene flow between populations caused by isolating barriers. In a two-deme model, RI can be defined as $1 – (m_e/m)$. |
| *Speciation*: The evolution of reproductive isolation. |
| *Speciation continuum*: A continuum of reproductive isolation. |
| Speciation trajectory: The specific pathway or route taken by a pair of populations as they evolve reproductive isolation. Simple examples of different speciation trajectories include speciation by local adaptation versus speciation by the evolution of intrinsic barriers. |

toward the completion of speciation than others. We can therefore treat each population pair as a "snapshot" of a particular point in the process, and order many pairs in a continuous sequence to understand how the process unfolds, such that "the present is a key to the past" (Lyell 1830).

In the last decade, the speciation continuum concept has become increasingly popular among researchers and is now well-established in the speciation literature, with at least 93 publications using the term in their title or abstract (Fig. 1). However, despite becoming embedded within the language of speciation research, we were surprised to find that the term lacks a clear consensus definition. Given the power of language and concepts to shape and sometimes mislead our thinking (Harrison 2012), we argue that it is high time to critically evaluate the speciation continuum concept and its use. Specifically, what do we mean when we talk about the speciation continuum? Can we develop a definition that is logical and pragmatic? With such a definition in hand, we can then ask deeper, more critical questions. Most importantly, is the speciation continuum a useful concept for helping us understand how speciation unfolds?

Part 1: What is the Speciation Continuum?

ORIGIN AND USE OF CONTINUUM THINKING IN THE SPECIATION LITERATURE

The speciation continuum concept has enjoyed recent popularity (Fig. 1), but it is not a new idea. In the *Origin of Species* and his other writings, Darwin made it clear that he thought of speciation as a gradual process, occurring through the cumulative action of natural selection. Indeed, he stated clearly that he believed that the processes that gave rise to "varieties" were the same as those that led to the formation of distinct species. Much of Darwin's gradualist thinking was inspired by the geologist Charles Lyell and his popularization of the principle of uniformitarianism—that is, that small, cumulative changes such as erosion or deposition could result in large geological formations over long periods of time. The continuum concept therefore has its roots in this gradualist view of evolution and the problem that arises from it. Specifically, because speciation typically occurs over a timescale far greater than a human lifespan, we cannot directly observe the entire process.
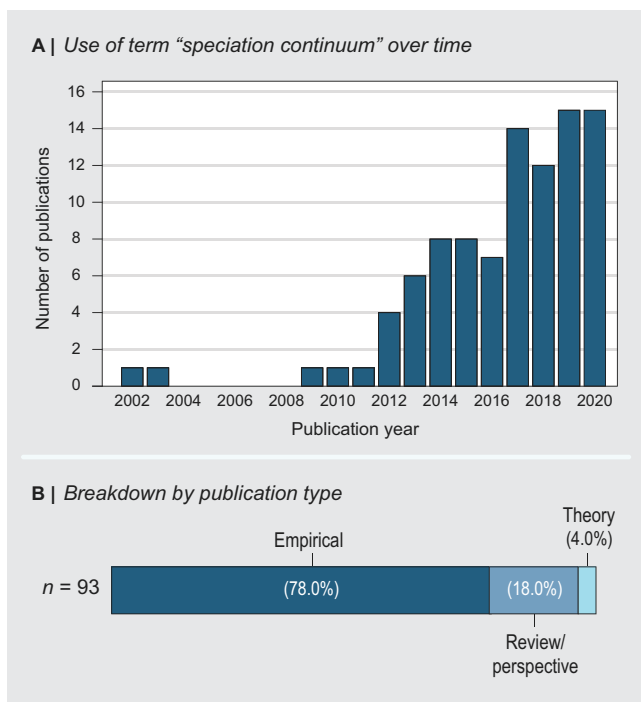
Figure 1. Number of papers using the term "speciation continuum" in their title or abstract. (A) Number of papers each year according to ISI web of Science as of December 20, 2020. (B) Breakdown of paper type as classified by the authors.

The first explicit mention of using multiple pairs of taxa to reconstruct the speciation process is from Walsh (1864) in a study of North American phytophagous insects. Walsh used the transition from childhood to adulthood as an illustrative metaphor and argued that by comparing populations, host races, and species that it was possible to see how ecotypes might continue to diverge until they become species (Walsh 1864; Nosil 2012). Around the same time, Wallace (1865) described six stages of divergence, from polymorphism to species in a manner that would not look out of place in a modern paper on the speciation continuum (Mallet 2009; Lowry 2012). Although he clearly saw these as different steps in the origin of species, he mainly used them as taxonomic ranks for classifying closely related pairs of Malayan Lepidoptera.

Despite these early examples of continuum thinking, the first explicit use of the term "speciation continuum" was by Drès and Mallet (2002), again coincidentally in a paper on phytophagous insects. The authors framed the transition from polymorphism to host races to fully isolated species as distinguishable by a reduction in levels of hybridization and argued that it could be a useful framework for studying the speciation process as a whole. The continuum concept became more popular with growing interest in ecological speciation and the interplay between adaptive divergence and gene flow in the late 2000s, with 91 of the 93 papers

using the term "speciation continuum" being published in 2008 or later (Fig. 1).

Surveying these papers, and others that do not use the term "speciation continuum" explicitly, we recognize four somewhat overlapping problems in speciation research that are rooted in continuum thinking: (i) the factors affecting the progress of speciation, (ii) the genomic changes that occur during speciation, (iii) the rate at which reproductive isolation evolves between populations, and (iv) the order in which different forms of isolation appear (Fig. 2). We briefly outline each of these areas, focusing on just a handful of key examples. In doing so, we intend to give those not already familiar with the concept a broad-brush overview of the types of questions that continuum thinking has been used to address. We reserve any critical thoughts about these ideas and their underlying assumptions for a subsequent section (Part 2).

How different factors influence progress toward speciation

Why do some populations complete the speciation process while others do not? By comparing multiple closely related populations exhibiting different levels of reproductive isolation (or proxies of RI), some authors have suggested that we can identify factors that determine how far speciation proceeds (Hendry 2009; Nosil et al. 2009; Seehausen 2009; Nosil 2012). These factors may include variation in the strength of selection, number of traits and loci under selection, and other factors such as the level of range overlap and demographic history (Hendry 2009; Nosil et al. 2009). An illustrative example of this idea is the continuum of divergence and increased genetic differentiation across multiple threespine stickleback species pairs (Hendry et al. 2009). In general, stronger genetic differentiation is observed between stickleback populations that show greater adaptive divergence, suggesting that ecology may be responsible for driving RI (Räsänen and Hendry 2008; Hendry et al. 2009). However, the stickleback continuum also shows that the speciation process is not just a one-way journey toward increased reproductive isolation; speciation collapse is possible when hybridization breaks down reproductive isolation following a sudden environmental change (Gow et al. 2006).

The genomics of speciation

How do patterns of genome-wide variation evolve over the course of the speciation process? Over the past decade, the speciation continuum concept has been widely referred to in speciation genomic studies, with 48% (37) of papers also having the term "genomic" in their title or abstract. Several papers have used continuum thinking to lay out verbal predictions about how patterns of genomic divergence should accumulate as speciation progresses. For example, the genic view of speciation (Wu 2001; Wu and
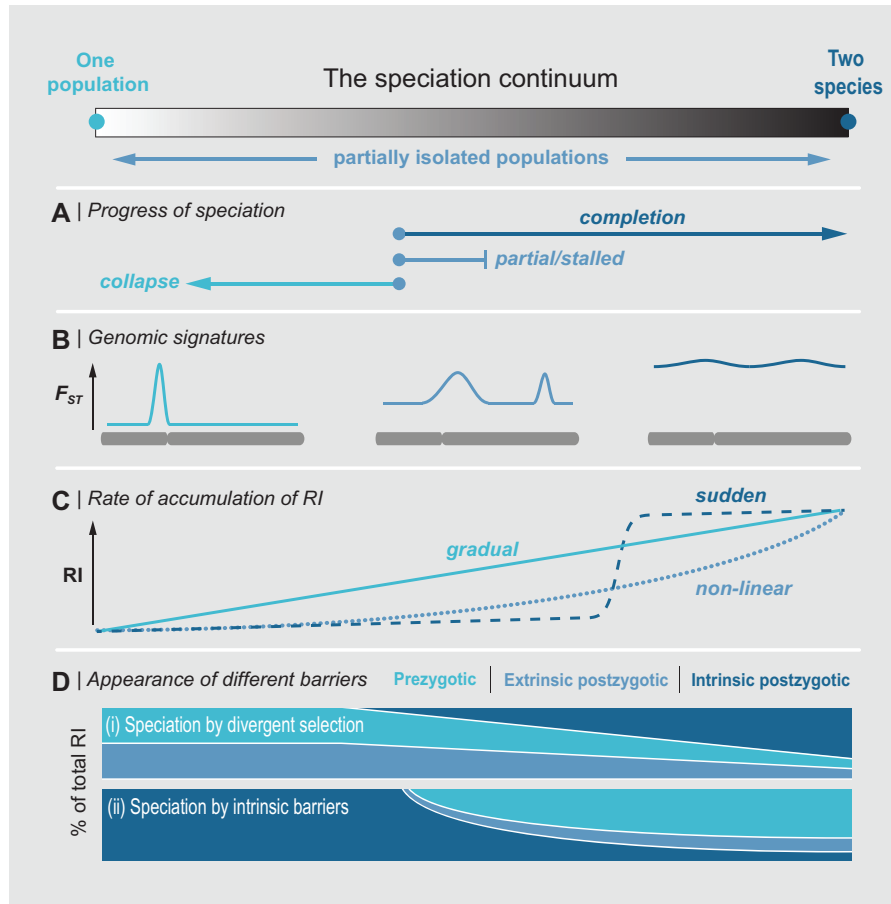
Figure 2. Graphical depiction of the speciation continuum and some problems that it has helped address. The speciation continuum is usually depicted as a gradient with a single population at one end and two species at the other. States in between represent partially isolated populations. (A–C) Some problems in speciation research that regularly employ continuum thinking. (A) Understanding the factors that influence the progress of speciation. The three arrows represent pairs of populations that complete speciation, evolve partial RI either as an optimum or due to a constraint, or collapse when reproductive isolation breaks down. (B) Understanding how patterns of genomic differentiation vary with the progress of speciation. The three lines show the level of allele frequency differentiation (measured by F_{ST}) across a chromosome at three different points along the continuum. (C) The rate of accumulation of reproductive isolation. The curves illustrate rates of accumulation of RI between two populations, including a linear increase, nonlinear (quadratic) increase, and a sudden increase. (D) The order of appearance of different isolating barriers. The curves give hypothetical examples of how the relative contribution (%) of three different barriers may vary along two different routes to speciation: speciation by divergent selection and speciation by intrinsic barriers.

Ting 2004)—where reproductive isolation begins at a single locus but increases in its genomic extent over the course of speciation—is described in stages along a continuum. Other papers have expanded on this idea by considering how divergent selection, physical linkage, and linkage disequilibrium interact to increase the level and extent of genomic differentiation as speciation proceeds (Feder et al. 2012, Nosil and Feder 2012; Seehausen et al. 2014). These papers have motivated a number of empirical studies aimed at testing these predictions by reconstructing a speciation continuum from multiple pairs of related taxa and studying patterns of genomic differentiation along it (Martin et al. 2013; Burri et al. 2015; Supple et al. 2015). Empirical evidence can provide insight

as to whether the same genomic regions underlie parallel adaptation and speciation (Roesti et al. 2014; Marques et al. 2016) and how patterns of genomic differentiation are shaped by intrinsic genomic properties, like the density of functional elements and local recombination rate (Burri et al. 2015; Stankowski et al. 2019).

The rate at which reproductive isolation accumulates
How long does it take for strong RI to evolve between populations? By using the level of genetic distance as a proxy for the divergence time, several comparative studies have attempted to address this question using multiple pairs of taxa that vary in

their strength of RI (Coyne and Orr 1989, 1997; Presgraves 2002; Price and Bouvier 2002; Russell 2003; Moyle et al. 2004; Moyle and Nakazato 2010; Stelkens et al. 2010, 2015). To date, the most taxonomically inclusive and in-depth study in this area was conducted by Roux et al. (2016). They used genomic data and an approximate Bayesian Computation (ABC) framework to estimate the probability that gene flow was still occurring (hereafter, $P_{geneflow}$) for 61 independent speciation events spanning a broad range of the animal kingdom. After plotting $P_{geneflow}$ against the genetic distance (d_a) for each pair, they found that speciation was generally complete beyond a threshold of about 2% sequence divergence. Although it is difficult—even inappropriate—to convert this into an estimate of time, because mutation rates, generation times, and levels of gene flow vary broadly across the taxa studied, the results suggest that roughly the same relative number of genetic differences is needed to ensure that gene flow between any two populations is strongly suppressed. However, at intermediate levels of genetic divergence ($d_a = 0.05–2\%$) $P_{geneflow}$ spans from very low to very high, indicating that populations can accumulate RI at very different rates.

Order of appearance of different forms of RI

How does the relative importance of different isolating barriers change over the course and context of speciation? By dividing RI into three forms—(i) extrinsic postzygotic, (ii) intrinsic postzygotic, and (iii) prezygotic—Seehausen et al. (2014) use the continuum concept to roughly outline some predictions for their order of appearance for two alternative routes to speciation (see also Lowry and Gould 2016). In the first verbal model—speciation driven by divergent selection—prezygotic and extrinsic postzygotic barriers are predicted to evolve first, and may interact to reduce gene flow between populations. However, intrinsic postzygotic barriers may evolve later, resulting in two irreversibly isolated species. In the alternative model—speciation driven by intrinsic barriers—RI is initially caused by intrinsic postzygotic barriers, with premating barriers evolving later to reinforce RI. To date, some of the best empirical studies considering the order of appearance of different barriers have made use of the continuum of RI in the genus *Drosophila* (Coyne and Orr 1989, 1997; Yukilevich 2012; Nosil 2013). For example, in their classic study, Coyne and Orr (1989) found evidence that premating barriers evolve faster than intrinsic postmating barriers in sympatry—a pattern that may reflect the reinforcement of RI following the secondary contact of previously allopatric taxa. Additional studies, using updated versions of the *Drosophila* data set, have uncovered additional signatures of reinforcement (Yukilevich 2012; Nosil 2013) suggesting that between 60% and 83% of all sympatric *Drosophila* have taken this route to speciation.
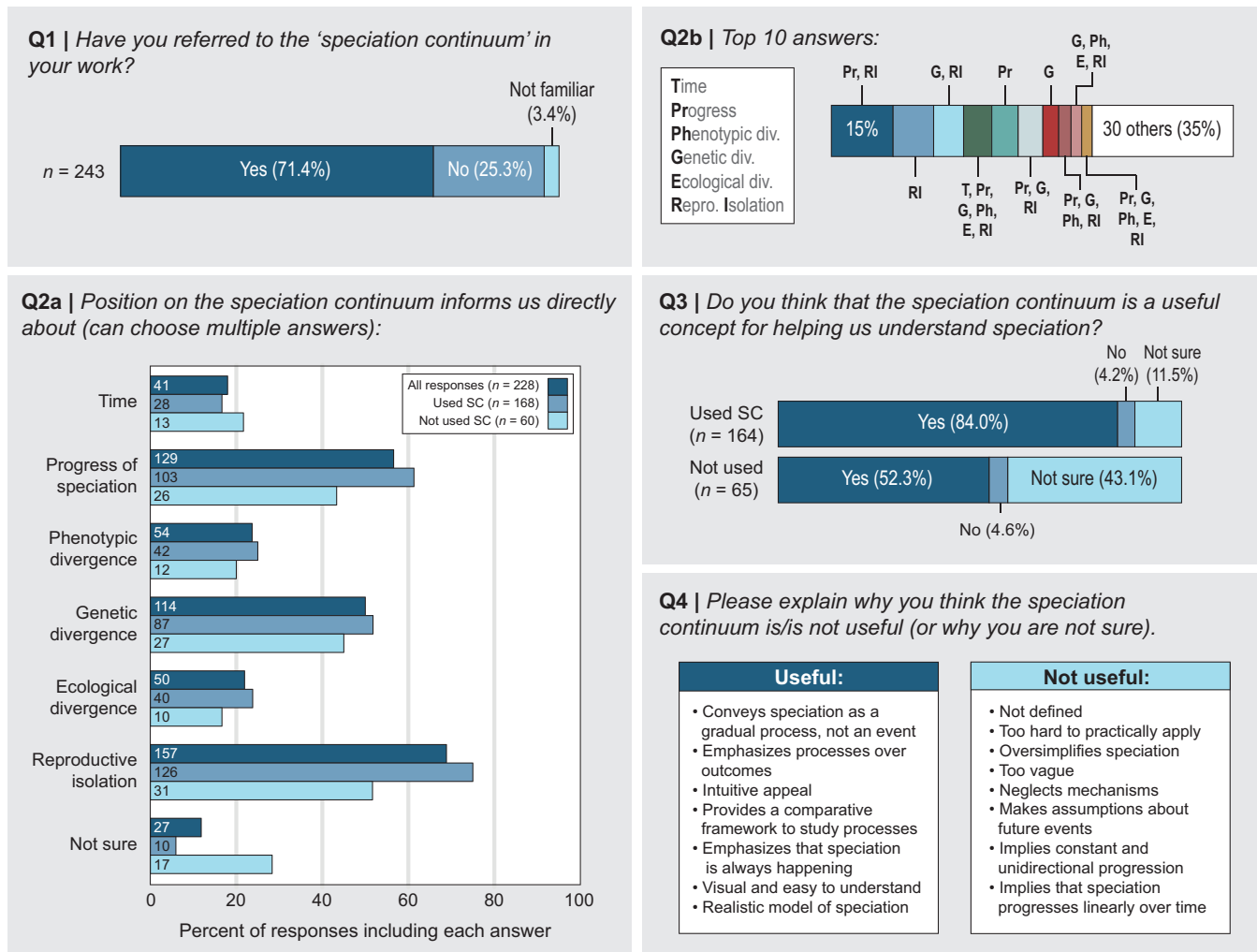
EXISTING DEFINITIONS OF THE SPECIATION CONTINUUM

Based on our literature review, it is clear that continuum thinking has played an important role in several areas of speciation research, acting both as a conceptual model for formulating predictions and as an empirical framework for testing them. However, we also think that the concept lacks a clear, consensus definition. Although some early descriptions of the speciation continuum were quite detailed (Drès and Mallet 2002; Hendry 2009; Hendry et al. 2009; Nosil et al. 2009; Nosil 2012), none of them defined the term explicitly. However, we did find two different definitions, both posed in 2014. First, Shaw and Mullen (2014) defined the speciation continuum as "*the continuous sequence of genetically-based changes that occur as two lineages diverge from one another on the pathway to reproductive isolation*." In an alternative definition, Seehausen et al. (2014) defined it as "*variation in the strength of reproductive isolation between two incipient species either in different locations or in different species pairs that belong to the same evolutionary lineage and that diverge in similar ways*" (also see Seehausen 2009).

These views of the speciation continuum seem to differ from one another in several respects. For example, Shaw and Mullen see the speciation continuum as the trajectory taken by a single pair of diverging lineages, whereas Seehausen et al. (2014) describe it as an axis of variability that emerges through the study of multiple closely related population pairs. Perhaps more importantly, they also seem to differ in terms of what position on the speciation continuum tells us. Shaw and Mullen (2014) focus on the accumulation of genetic changes between lineages that ultimately become reproductively isolated, whereas Seehausen et al. (2014) focus on variation in the strength of RI itself. Coupled with varying descriptions of the concept, these alternative definitions may help to explain why empirical studies have used the concept in different ways. For example, some studies have arranged population pairs along a continuum of genetic divergence (Ravinet et al. 2018; Stankowski et al. 2019), whereas others have ordered them based on the strength of RI (Merrill et al. 2011; Merot et al. 2017). Although these and other approaches have merit, substantial disagreement or confusion about the meaning of the speciation continuum concept will ultimately limit its utility as a tool for advancing our general understanding of speciation.

INSIGHTS FROM A SURVEY OF SPECIATION RESEARCHERS

Concerned that the meaning and use of the speciation continuum varies substantially among researchers, we used an anonymous online survey to gain direct insight into thoughts about the meaning and value of the speciation continuum concept. The sur-

Q1 | *Have you referred to the 'speciation continuum' in your work?*

Not familiar (3.4%)

n = 243 — Yes (71.4%) | No (25.3%)

Q2b | *Top 10 answers:*

Time
Progress
Phenotypic div.
Genetic div.
Ecological div.
Repro. **I**solation

Pr, RI | G, RI | Pr | G | G, Ph, E, RI | 15% | 30 others (35%)

RI | T, Pr, G, Ph, E, RI | Pr, G, RI | Pr, G, Ph, RI | Pr, G, Ph, E, RI

Q2a | *Position on the speciation continuum informs us directly about (can choose multiple answers):*

Time: 41 / 28 / 13
Progress of speciation: 129 / 103 / 26
Phenotypic divergence: 54 / 42 / 12
Genetic divergence: 114 / 87 / 27
Ecological divergence: 50 / 40 / 10
Reproductive isolation: 157 / 126 / 31
Not sure: 27 / 10 / 17

All responses (n = 228)
Used SC (n = 168)
Not used SC (n = 60)

Percent of responses including each answer

Q3 | *Do you think that the speciation continuum is a useful concept for helping us understand speciation?*

No (4.2%) Not sure (11.5%)

Used SC (n = 164) — Yes (84.0%)

Not used (n = 65) — Yes (52.3%) | Not sure (43.1%)

No (4.6%)

Q4 | *Please explain why you think the speciation continuum is/is not useful (or why you are not sure).*

Useful:
• Conveys speciation as a gradual process, not an event
• Emphasizes processes over outcomes
• Intuitive appeal
• Provides a comparative framework to study processes
• Emphasizes that speciation is always happening
• Visual and easy to understand
• Realistic model of speciation

Not useful:
• Not defined
• Too hard to practically apply
• Oversimplifies speciation
• Too vague
• Neglects mechanisms
• Makes assumptions about future events
• Implies constant and unidirectional progression
• Implies that speciation progresses linearly over time

Figure 3. What speciation researchers think about the speciation continuum, determined using an online survey. We distributed the survey using email lists for several speciation-focused conferences and workshops (Gordon Conference of Speciation 2019, Ventura, California; SMBE Speciation Genomics 2019, Tjärnö, Sweden; Speciation Genomics Conference, 2018 Cambridge, UK), by targeted email, on Twitter, and on Evoldir. Responses were accepted from July 26, 2019 to September 10, 2019. We received 402 responses, but here focus only on the 243 people who identified themselves as speciation researchers. We also gathered information about whether people had referred to the speciation continuum in their work (Q1), what position on the speciation continuum informs us directly about (Q2a and Q2b), whether respondents thought the speciation continuum was useful or not (Q3), and why (Q4). Written answers used in Q4 are available in Table S1. Note that the number of answers for some questions is less than the number of respondents because answers were occasionally not provided.

vey was distributed to the attendees of several speciation-focused conferences and workshops, in targeted emails, on Twitter, and on Evoldir (see Fig. 3 caption for more details). Here, we focus on answers from 243 respondents who answered yes to the question "*do you study speciation?*" When asked, "*have you referred to the speciation continuum in your work?*" (Fig. 3, Q1), 169 (71%) respondents indicated that they had used the term, while 60 (25%) indicated that they had not used it. Only eight respondents (3%) indicated that they were not familiar with the concept, showing that it is indeed widely used and recognized among speciation researchers.

We next asked people to complete the statement "*position on the speciation continuum informs us directly about*" by choosing up to seven of the following answers: (i) time, (ii) progress of speciation, (iii) level of phenotypic divergence, (iv) level of genetic divergence, (v) level of ecological divergence, (vi) strength of reproductive isolation, and (vii) I'm not sure (Fig. 3, Q2a). After excluding participants who were not familiar with the concept (n = 8), we found that no single answer was used in all 228 responses to this question. The most common answer was reproductive isolation, selected by 69% of respondents. "Progress of speciation" and "level of genetic divergence" were also common

answers, selected in 57% and 50% of responses, respectively. "Time", "level of phenotypic divergence", and "level of ecolical divergence" were selected less frequently (17–23% of responses). Interestingly, the proportions of these six answers were similar regardless of whether people had or had not referred to the speciation continuum in their work (chi-squared = 3.288, df = 5, P = 0.65). However, people who had not referred to the concept tended to answer "I'm not sure" more frequently (28% compared with 6%), meaning that the overall frequency of answers was quite different when this group of researchers was considered (chi-squared = 27.107, df = 6, P = 0.00013).

Because people were able to make multiple choices, we also examined the specific combinations of answers about what position on the speciation continuum informs us directly about (Fig. 3, Q2b). We observed 40 unique responses, consisting of between one and six answers. The most common response included two answers: "progress of speciation" and "strength of reproductive isolation ". However, this combination only represented 15% (35) of the total. Only two other answers were observed more than 10% of the time. These were both single-answer responses: "I'm not sure" and "strength of reproductive isolation", each with 23 responses. Sixteen people (7%) chose all of the answers except for "I'm not sure". Overall, the top 10 answers (excluding "I'm not sure") accounted for roughly 65% of responses. The remaining 30 answers were quite rare (<2.2% of the total), although together accounted for more than a third (35%) of the total answers. In summary, the answers to Q2 revealed broad disagreement about what position on the speciation continuum actually tells us.

Next, we asked "*do you think that the speciation continuum is a useful concept for studying speciation?*" (Fig. 3, Q3). Of those who had referred to the speciation continuum, 84% thought that it was useful. Despite having used it, 4.2% of respondents thought that it was not useful, with a further 12% indicating that they were not sure if it was useful. These proportions were very different when compared with the respondents who had not used the concept (chi-squared = 28.79, df = 2, $P = 5.6 \times 10^{-7}$); just over half thought that it was useful, 4.6% thought that it was not useful, and 43% were not sure if it was useful.

Finally, we asked respondents to "*Please explain why you think the speciation continuum is/is not useful (or why you are not sure)*" (Fig. 3, Q4). These written answers were variable (Table S1), but we identified several recurring themes regarding the perceived strengths and weaknesses of the concept—some of which were directly contradictory. The most common reasons that people thought it was useful were that it conveys speciation as a continuous process rather than discrete event, and that it emphasizes process over outcome. In addition, some respondents thought that it has intuitive appeal, is a visual model that is easy to understand, is realistic, and provides a comparative framework

for studying speciation. Respondents thought that it was not useful because it has not been clearly defined, is too vague, is too simple to apply generally, is too difficult to apply practically, and neglects underlying mechanisms. Respondents also argued that the concept is potentially misleading because it makes untenable assumptions about future events, portrays speciation as a unidirectional process, and implies that speciation progresses linearly over time.

SO WHAT IS THE SPECIATION CONTINUUM?

Although most speciation researchers are familiar with the speciation continuum and think that it is useful for helping us understand the speciation process, we found surprisingly little consensus about what it is and some concern about its overall value. We suspect that this is for two reasons. First, the concept has rarely been defined in the literature and has been used in a variety of ways since it was first introduced and popularized. Second, views about species and speciation are highly variable (Hey 2001; Stankowski and Ravinet, In Press).

Regardless of the root cause of the disagreement, clearer definitions will help reduce confusion and ease communication between researchers with different perspectives on speciation. With this in mind, we next offer an explicit definition of the speciation continuum that is compatible with the Biological Species Concept (BSC)—a widely used species concept that equates speciation with the evolution of reproductive isolation (Mayr 1942; Coyne and Orr 2004). We then outline the logical basis of the definition and discuss some potential limitations. In the final section, we consider what continuum-based studies can and cannot tell us, and give some suggestions for how the concept may be used as a framework for advancing our general understanding of speciation.

Before sharing our perspective, we want to state that it is not our intention to persuade other researchers to abandon their own fundamental views on speciation. Our definition is aimed primarily at researchers that have similar views to our own. However, we think that many of our more general points about the value and potential pitfalls of continuum thinking are also relevant to those with different perspectives.
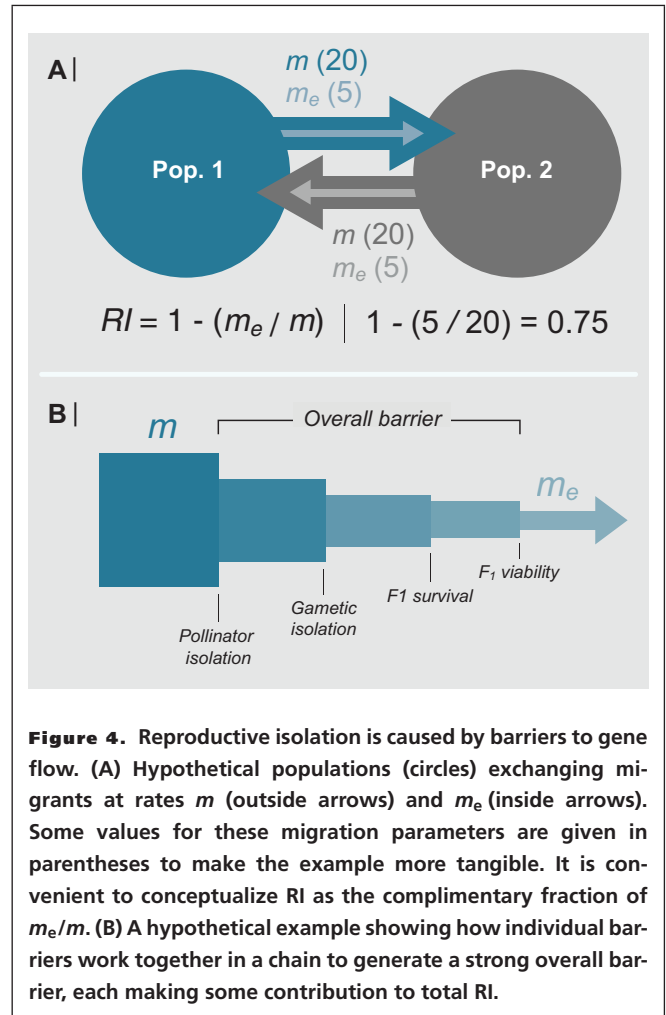
Part 2: An Explicit Definition of the Speciation Continuum

We define the speciation continuum as *a continuum of reproductive isolation*. Under this definition, the position of a pair of populations on the speciation continuum is only directly informative about the strength of RI between them (see Box 1). The definition emphasizes that reproductive isolation is a continuous property and that speciation is a process rather than a discrete event.

We define reproductive isolation (RI) as the reduction in potential gene flow between populations caused by isolating barriers. To explain what we mean in more detail, we will use a simple two-deme model (Fig. 4). Imagine two populations that exchange individuals at a rate of m migrants per generation. In a case where barriers to gene flow are absent (and assuming that all individuals mate), the number of migrants that contribute genetically to future generations—the effective migration rate, m_e—is equal to the actual migration rate, m. However, if we add an isolating barrier, then some fraction of migrants will fail to successfully reproduce, meaning that the rate of gene flow into the population does not correspond to the real migration rate (i.e., $m_e < m$). Barton and Bengtsson (1986) defined the ratio of the effective to the actual migration rate, m_e/m, as the "gene flow factor" (*gff*), because it measures the penetrability of a barrier to incoming genes. For example, if the *gff* $= 0.25$, then effective gene flow in the presence of a barrier is 25% of the potential gene flow. It is therefore convenient to conceptualize RI as the complementary fraction to the gene flow factor, $1 - (m_e/m)$, which is the reduction in potential gene flow caused by a barrier, in the above case, RI $= 0.75$. It is, however, important to note that reproductive isolation is more difficult to define in reality, because the strength of RI can vary across the genome (see Box 3) and because populations are distributed across continuous geography rather than existing as two discrete demes (Barton and Bengtsson 1986).

RI is caused by isolating barriers, which we define as any genetically determined factor that causes a reduction in effective gene flow between two populations. Isolating barriers can reduce gene flow at any point in the lifecycle of an organism and can include intrinsic and extrinsic factors. For example, in a flowering plant, potential isolating barriers include, but are not limited to, differences in flowering time, pollinator isolation, gametic isolation, seed set rate, germination rate, survival rate, and the viability of hybrid offspring. These individual barriers reduce gene flow in a sequence, each contributing to the overall barrier and total RI (Sobel and Chen 2014). Consistent with our definition, we do not consider spatial isolation alone as an isolating barrier, because it causes a reduction in the migration rate (m) between populations rather than a decrease in the effective migration rate (m_e). Exceptions to this rule include cases where spatial isolation can be shown to be the result of intrinsic biological factors such as ecogeographic isolation or habitat choice (Sobel et al. 2010; Sobel 2014).

Figure 4. Reproductive isolation is caused by barriers to gene flow. (A) Hypothetical populations (circles) exchanging migrants at rates m (outside arrows) and m_e (inside arrows). Some values for these migration parameters are given in parentheses to make the example more tangible. It is convenient to conceptualize RI as the complimentary fraction of m_e/m. (B) A hypothetical example showing how individual barriers work together in a chain to generate a strong overall barrier, each making some contribution to total RI.

JUSTIFICATION, LOGICAL BASIS, AND CAVEATS OF THE DEFINITION

Our decision to define the speciation continuum in terms of the amount of RI (Box 1) between populations is based on the consideration of several factors. First, our own approach to speciation research is rooted in the Biological Species Concept (BSC; Mayr 1942; Coyne and Orr 2004), which seeks to explain the occurrence of distinct clusters of sexually reproducing organisms based on RI between them. Of course, distinct groups can form or be maintained in other ways—and in organisms that lack regular sexual reproduction—which has led to the formulation of alternative species concepts (Hey 2001). Researchers that follow an alternative concept may not find our definition of the speciation continuum very useful, and could simply refer to it as the "continuum of RI." Second, our definition of the speciation continuum is most compatible with existing perceptions of the concept, as the majority of respondents to our survey indicated that position on the continuum informs us directly about the strength of RI. Furthermore, it is in line with some of the more detailed descriptions of the concept in the literature, which also empha-

size the importance of RI (e.g., Hendry et al. 2009; Seehausen 2009; Nosil 2012; Seehausen et al. 2014). Finally, although we define the speciation continuum exclusively in terms of RI, we acknowledge that other factors play an important role in speciation. Rather than defining the continuum based on these factors, we see the concept as a framework for understanding how they shape the observable continuum of RI that surrounds us.

We think that the most logical place to draw the upper bound of the speciation continuum is RI = 1 (i.e., complete isolation), because this is the maximum RI that can act between two populations in nature (Sobel and Chen 2014). We note that some versions of the BSC take a more relaxed approach to deciding when speciation ends, stating that RI need not be complete (Coyne and Orr 2004). Although we think that this approach has merit in some cases (e.g., for the taxonomic recognition of species), we argue that complete isolation is the most conceptually and practically useful criterion to define the end of the continuum when it comes to studying the evolution of RI. Not only is this point nonarbitrary, meaning that it can be applied consistently across systems, but the presence of any ongoing gene flow between populations means that there is still something to be learned about how RI evolves.

In our definition, position on the continuum only indicates the current strength of RI, not its irreversibility. Whether the evolution of RI is reversible is of general interest in speciation research, because it may have some bearing on whether species persist in the future. For example, although the maximum RI that can be realized between two populations is 1, additional barriers may continue to evolve after RI = 1; if current barriers cease to act, these additional barriers may begin to contribute to RI. Similarly, some types of barriers may be more stable in the face of environmental perturbation than others. Although we agree that it is important to understand which factors influence the potential for speciation reversal, this potential has no bearing on current levels of gene flow and depends on future events that may or may not occur. Our definition therefore emphasizes the importance of understanding current variation in the strength of RI between populations, rather than speculating about their future.

Although the speciation continuum has often been used to simultaneously represent divergence along multiple axes of biological variation (e.g., Hendry 2009; Nosil 2012), we have followed Seehausen et al. (2014) in defining it exclusively based on RI. Our reason for this is that RI can evolve largely independently of phenotypic, ecological, or genetic divergence, making it impossible to represent these aspects together on a one-dimensional axis. Take, for example, the level of genetic divergence between populations. Although genetic divergence is generally expected to be higher between populations that are more reproductively isolated from one another—both as a cause and consequence of the level of RI—the relationship across multiple population pairs is weak

when divergence times are short (Coyne and Orr 2004; Roux et al. 2016). Genetic divergence can also be strongly impacted by factors aside from RI, including, but not limited to, differences in effective population size (N_e) (Kimura 1962; Kimura and Ohta 1969), mating system variation (Nordborg and Donnelly 1997), isolation-by-distance (Wright 1943; Rousset 1997), local population bottlenecks (Tajima 1989), selection at nonbarrier loci (Barton 2000; Cruickshank and Hahn 2014; Ravinet et al. 2017), and geographic isolation. RI may also evolve rapidly, controlled by a small number of loci, long before genome-wide genetic divergence can accumulate as a consequence of a strong barrier. As a result, measures of genetic divergence (e.g., F_{ST}) are unlikely to provide a reliable proxy for the amount of RI between two populations below a certain threshold. Similarly, variation in the strength of RI is only weakly correlated with phenotypic or ecological divergence. This is highlighted by numerous studies of hybrid zones, where barriers to gene flow have been characterized between taxa that are morphologically and ecologically cryptic (e.g., Phillips et al. 2004) or highly divergent (e.g., Grahame et al. 2006).

The above difficulty of projecting multiple measures of variation on a single axis did prompt us to consider proposing a multidimensional definition of the speciation continuum. For example, Dieckmann et al. (2004) proposed the idea of a "speciation cube," comprising three axes: ecological differentiation, mating differentiation, and spatial differentiation (see Butlin et al. 2008). This model is appealing, because it allows multiple population pairs to be projected in a three-dimensional space based on several variables that are relevant to speciation. However, we also see an obvious problem; that is, the three axes of the speciation cube (which was originally proposed as a model of "adaptive speciation") are only some of the factors that are relevant to speciation, so a general multidimensional definition would need to include many additional axes. This would make it impractical as a conceptual model, and would also draw focus away from the axis that is most fundamental to understanding speciation under the BSC. Rather than including other variables as part of the definition, we think that our one-dimensional definition motivates us to understand how these other factors shape variation in RI (see Part 3).

Our definition avoids making an explicit connection between position on the speciation continuum and time. Unlike time, the evolution of RI need not be unidirectional, as the amount of RI between populations may increase, decrease, or remain constant over time (Taylor et al. 2006; Servedio and Hermisson 2019). In models where RI does increase over time, it typically does not do so at a constant rate. For example, theoretical and empirical studies suggest that the strength of intrinsic postmating RI between allopatric populations increases quadratically rather than linearly, essentially "snowballing" over time (Orr and Turelli 2001; but

see Gourbière and Mallet 2010). Other models of speciation with gene flow indicate that the strength of RI may increase very suddenly, coinciding with the coupling of multiple relatively weak barrier effects into a strong overall barrier (Barton 1983; Barton and De Cara 2009; Flaxman et al. 2013). In summary, the ability of RI to increase, decrease, and evolve at different rates means that position on the speciation continuum cannot jointly inform us about the strength of RI and time.

Many early descriptions of the speciation continuum (or papers that use continuum thinking) portray or discuss it in terms of steps, stages, or phases (Wu 2001; Drès and Mallet 2002; Hendry 2009; Hendry et al. 2009; Nosil et al. 2009; Feder et al. 2012; Lowry 2012; Lowry and Gould 2016; Kulmuni et al. 2020). We have deliberately avoided using these terms in our definition for two reasons. First, steps and stages imply that there is a series of generally recognizable states that a pair of diverging populations must sequentially pass through to evolve complete isolation. Because speciation can occur via many different routes (e.g., different initial conditions, contrasting demographic histories, and varying importance of different types of barriers), we think it is unhelpful to view the speciation continuum as some general roadmap of the speciation process (also see Part 3). The second issue is that speciation is a continuous process, so dividing it into stages (usually for convenience) gives a false impression and may inadvertently cause researchers to classify cases of speciation based on arbitrary criteria rather than focusing on underlying mechanisms. The kinds of problems that can arise from the categorization of continuous variation into categories were highlighted by Butlin et al. (2008) in reference to geographic modes of speciation. In short, researchers have spent considerable effort classifying cases as allopatric, parapatric, or sympatric speciation rather than focusing on the degree of geographic separation (which is continuous) and its relationship to other factors that shape barriers to gene flow (Butlin et al. 2008). The power of continuum thinking—and the speciation continuum as a concept—is that it moves us beyond a need for categorization toward a more process-based understanding of how speciation unfolds. This view clearly resonates with researchers who took the survey, as the most common reason for which respondents thought that the concept was useful is that it acknowledges the continuous nature of speciation (Fig. 3, Q4).

The final point that we considered when forming our definition was whether it is useful to think about position on the continuum as a measure of the progress of speciation. In our survey, the most popular answer to the question "*position on the speciation continuum informs us directly about…*" was the strength of reproductive isolation and progress of speciation (15% of answers) (Fig. 3, Q2b). Unlike some of the other answers (Fig. 3, Q2b), this combination makes logical sense, assuming that progress is simply a way of referring to how much RI has evolved relative to the

maximum amount possible (i.e., RI = 1). However, "progress" is a somewhat loaded term, as it is often used to describe movement toward an improved state (New Oxford American Dictionary, 3rd ed.). This has caused some researchers to argue that the term has no place in evolutionary thinking (e.g., Gould and Nitecki 1988; Gould 1990). Taking this and the survey results into account, we think it is worth noting that the evolution of complete RI may not always be evolutionarily optimal (Nosil et al. 2009; Barton 2020). This was demonstrated in a recent simulation study by Servedio and Hermisson (2019), which showed that partial RI can be the adaptive optimum across a broad range of scenarios and conditions. The term progress also carries the implication that RI = 1 is a destination, drawing focus away from the process of speciation as the accumulation of RI, blind to its final outcome. It is, indeed, much more likely that many partially isolated population pairs are not "incipient species" (Butlin et al. 2008), as they will never evolve complete isolation, yet they are still important for understanding how RI evolves.

Part 3: How the Continuum Concept can Advance our Understanding of Speciation

With a clear definition of the speciation continuum established, we next consider how we can use it to advance our understanding of how reproductive isolation evolves. Overall, we agree with the majority of researchers who took our survey (Fig. 2), as we also think that the speciation continuum has been, and will continue to be, a useful conceptual and empirical framework for studying the speciation process. However, we also sympathize with people who had reservations about its conceptual and/or practical utility (Fig. 2). Coupled with a clearer definition, we argue that many of the concerns raised in the survey can be addressed by (*i*) being realistic about what studies of the speciation continuum can and cannot tell us, and (ii) by working together to measure and collate estimates of RI in a way that maximizes their value to other researchers.

WHAT EMPIRICAL STUDIES OF THE SPECIATION CONTINUUM CAN AND CANNOT TELL US

Setting realistic expectations about what we can and cannot learn from continuum-based studies is necessary to ensure that we ask the right questions and arrive at conclusions that are not misleading. In this respect, we question the validity of a key premise of the continuum concept: that we can reconstruct the speciation process using many different pairs of populations that differ in their level of RI. The idea of reconstructing long-term processes from contemporary observations is not limited to evolutionary biology and is more generally referred to as chronose-
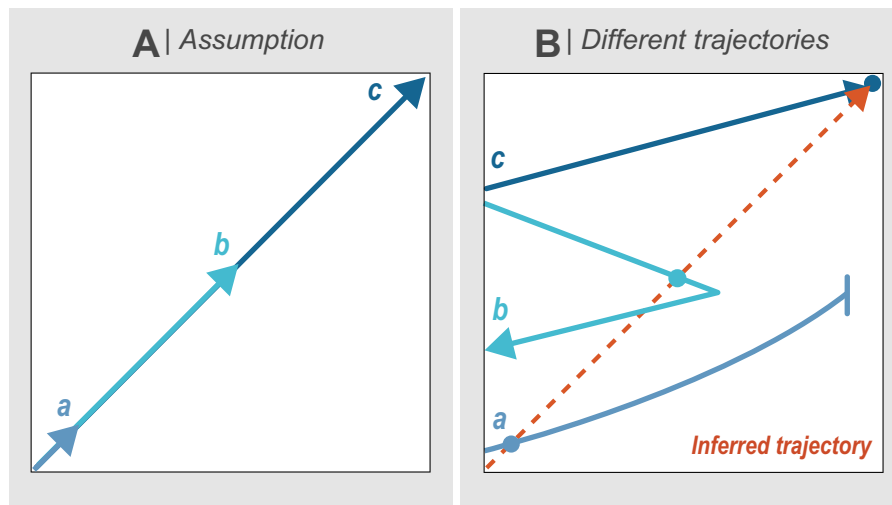
Figure 5. The assumptions of chronosequence analysis. (A) A process can only be reconstructed from independent contemporary samples if its trajectory is highly predictable across space and time. This is the case in A, where samples taken from three independent runs of a process (a, b, and c) are on the same trajectory through an arbitrary two-dimensional space, differing only in their position along it. However, many processes are highly stochastic, unstable, or can occur via multiple routes. (B) When this is the case, the critical assumption of chronosequence analysis is violated, meaning that the inferred trajectory has no predictive power. However, note that the dashed line is the line of best fit between the two variables, *x* and *y*, which is highly informative about the relationship between them without making assumptions about the past or future.

quence analysis (Walker et al. 2010; Phillips 2015). Textbook examples include classic ecological studies of plant community succession, in which chronosequences were constructed by comparing vegetation communities across many sites that differ in their age since a common initial condition (e.g., formation of a sand dune, the retreat of a glacier, or time since a disturbance) (Johnson and Miyanishi 2008; Walker et al. 2010). Change across these chronosequences has often been adopted as a model indicating the trajectory that any site starting the process will inevitably take if given sufficient time (Walker et al. 2010; Phillips 2015).

Despite being a commonly used device for inference in both the earth and life sciences, chronosequence analysis has been widely criticized in the literature because it makes the critical assumption that each sample included in the sequence is on the same trajectory, differing from other samples only in their position along it (Johnson and Miyanishi 2008; Walker et al. 2010) (Fig. 5A). If this assumption is violated, meaning that there is no successional link between the samples being compared, then we will arrive at erroneous conclusions about the mechanisms, factors, and temporal dynamics underlying the processes that we are trying to understand (Walker et al. 2010) (Fig. 5B). In the case of plant community succession, new empirical data have rejected models of vegetation change inferred from classic chronosequence studies, as intrinsic differences between sites have ultimately caused them to take different routes of succession (Johnson and Miyanishi 2008).

Thus, while it is easy to conceptualize the speciation continuum as a form of chronosequence—with pairs of populations arranged in a sequence of increasing RI—we think it is unrealistic to assume that each pair has followed the same trajectory up to its present point and will continue along that trajectory in the future. There are simply too many different ways to arrive at any given level of RI (Coyne and Orr 2004; Smadja and Butlin 2011). Some authors have argued that differences in speciation history will be largely minimized by restricting continuum-based analyses to populations within a single closely related group (e.g., sticklebacks) (Nosil et al. 2009; Seehausen et al. 2014), However, even closely related population pairs can vary markedly in their evolutionary histories, and it is difficult to test whether the critical assumption of a common trajectory holds for a given set of natural populations. Therefore, we agree with Merot et al. (2017): it is unreasonable to see continuum-based analyses as a means to reconstruct a feasible route to speciation, either retro- or prospectively, for a given speciation event. Laboratory studies of experimental speciation offer an exception to this, because they allow us to repeatedly measure levels of RI between the same two lineages at different time points in the process. Unlike chronosequence analysis, this provides an actual record of the speciation trajectory, rather than a reconstruction (White et al. 2020). We will discuss the potential utility of experimental speciation studies further when we consider questions that continuum-based studies can help address.

What, then, can we expect empirical studies to tell us? Rather than attempting to reconstruct speciation trajectories, we think that continuum-based studies should focus on identifying factors that explain variation in the level of RI and components of isolation across many population pairs. In other words, we see the speciation continuum as an underutilized axis of variation that can be used to test the generality of relationships between the strength of RI and potential factors that may cause it. Like chronosequence analysis, this approach makes use of the observable variation in RI across many case studies of speciation (i.e., the continuum), but does so without making untenable assumptions about past or future events (a criticism raised in the survey). Funk et al. (2006) provide a nice example of a continuum-based study that employs this comparative approach, first used by Coyne and Orr (1989) (also see Coughlan and Matute 2020 for a more recent example). Using data from more than 500 population pairs, including closely related plant, vertebrate, and invertebrate taxa, they tested for evidence of stronger premating RI between populations inhabiting more different habitats. In agreement with their prediction, they found consistent positive correlations between premating RI and habitat difference after correcting for divergence time, which supports the hypothesis that ecological adaptation plays a fundamental and taxonomically general role in the speciation process.

Comparative analyses, like the one conducted by Funk et al. (2006), also have limits to what they can tell us. The major limitation, common to all comparative analyses, is that the presence of correlation does not imply a causal relationship or indicate the direction of causality between the variables being considered (Harvey and Pagel 1991). For example, in the study by Funk et al. (2006), the authors note that the correlation between ecological distance and premating RI does not tell us that ecology drives RI; the reverse may also be true, or there may be no causal relationship at all. This limitation ultimately means that comparative studies of the speciation continuum cannot alone identify the factors that cause observable variation in the strength of RI. What they do provide is a means of conducting more general tests of hypotheses arising from theoretical or experimental work (Harvey and Pagel 1991; Funk et al. 2006). If used in this way, the continuum can advance our understanding of speciation by providing a framework for synthesizing the results of disparate 'microevolutionary' studies toward a more general 'macroevolutionary' understanding of how RI evolves.

BUILDING A SPECIATION CONTINUUM

The approach outlined above requires that we have access to estimates of RI for many pairs of taxa, along with other variables needed to test hypotheses. This brings us to another criticism that arose from the survey: that the speciation continuum is too difficult to apply practically. There is no denying that the estimation
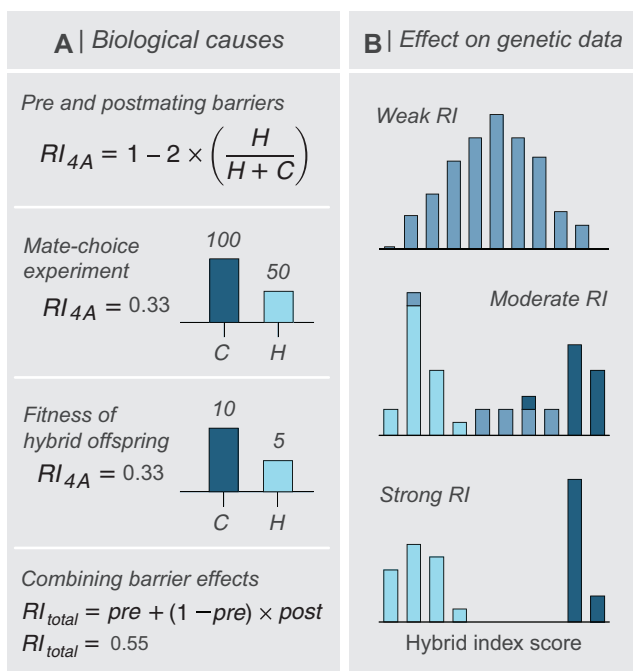
of RI is difficult: direct estimates of total RI require a barrage of field and laboratory studies, which is challenging for many organisms and impossible for others (Coyne and Orr 2004; Sobel and Chen 2014) (Box 2). Furthermore, there is a general concern that experimental estimates of barriers, which are usually made over a single generation in unnatural conditions, may give a poor indication of how they actually contribute to RI in nature (Butlin et al. 2012; Turelli et al. 2014; Irwin 2020; Perini et al. 2020). In some cases, estimates of RI can be obtained through the study of hybrid zones (Box 2), but these also make simplifying assumptions and only give information about the total strength of RI and no information about the specific barriers that cause it (e.g., prezygotic vs. postzygotic) (Box 2) (Butlin et al. 2012). Despite these difficulties, many researchers have risen to the challenge of estimating RI for the organisms that they study, meaning that a lot of the hard work is already being done.

Box 2: Measuring reproductive isolation

Empirical studies along the speciation continuum require that RI is measured in a way that is comparable across multiple population pairs. The mathematical framework proposed by Sobel and Chen (2014) is well-suited for this task, because the resulting estimates of RI are (i) linearly related to the probability of gene flow between populations and (ii) are equivalent across different isolating barriers. In practice, the methodology involves collecting appropriate observational and/or experimental data to estimate the effect of each potential barrier that might cause RI during the lifecycle of the organism (Fig. 6). Barrier-specific estimates of RI are calculated using equations tailored for different forms of isolation. Total isolation can then be estimated by combining individual barrier effects in accordance with their position in the chain of potential isolating barriers (Coyne and Orr 2004; Sobel and Chen 2014).

The difficulty of studying all potential barriers that could cause RI means that the above approach is laborious for most organisms and impossible for others. This has caused researchers to consider other ways of estimating RI from genetic data. The most compelling methods make use of data from hybrid zones, where we can see the effects of isolating barriers play out in the real world (Hewitt 1988). One very simple approach involves comparing the distribution of the hybrid index (HI) score, which is calculated for each individual from multiple unlinked diagnostic loci as $n_i/(n_i + n_j)$, where n_i is the number of alleles of ancestry i summed over loci and $n_i + n_j$ is the total number of sequenced alleles (Jiggins and Mallet 2000). If the total RI between populations is weak, the association of alleles among loci (LD) will decay to produce a unimodal distribution of HI scores. If RI is more

substantial, the production and viability of hybrids may be reduced, which is predicted to result in a flattened or trimodal distribution of the HI. In cases where RI is nearly complete, a bimodal distribution of HI scores is expected, with low levels of introgression expected to impact the shape of the two parental modes. More sophisticated methods for inferring the strength of RI from hybrid zones include geographic cline analysis (Barton and Gale 1993; Gay et al. 2008) and population genetic models that identify and infer the strength of barriers from genomic data (Aeschbacher et al. 2017; Ringbauer et al. 2018).

A | *Biological causes*

Pre and postmating barriers

$$RI_{4A} = 1 - 2 \times \left(\frac{H}{H + C} \right)$$

Mate-choice experiment

$RI_{4A} = 0.33$

100

50

C   H

Fitness of hybrid offspring

$RI_{4A} = 0.33$

10

5

C   H

Combining barrier effects

$RI_{total} = pre + (1 - pre) \times post$

$RI_{total} = 0.55$

B | *Effect on genetic data*

Weak RI

Moderate RI

Strong RI

Hybrid index score

Figure 6. Measuring reproductive isolation. Information about the strength of RI can be estimated by studying its biological causes or its effect on genetic data. **(A)** The index RI_4 (Sobel and Chen 2014) can be calculated to estimate the individual strength of premating and postmating barriers based on the outcomes of heterospecific (H) and conspecific (C) trials of a behavioral experiment or cross. In this example, only half as many heterospecific mating trials yield a mating, and hybrid offspring have reduced fitness (0.5) compared with conspecific crosses. This translates to an estimate of RI = 0.33 for each barrier. Combining the barrier effects gives an estimate of total RI less than the sum of the individual barriers because the premating barrier acts before the postmating barrier, meaning that the relative contribution of the postmating barrier is smaller. **(B)** Patterns of variation in hybrid zones can also give insight into the strength of total RI between two taxa. Here, three distributions of hybrid index scores from real hybrid zones show patterns that are consistent with weak, moderate, and strong

RI (top: a unimodal hybrid zone between *Bombina bombina* and *B. variegata*; middle: a trimodal hybrid zone between *Allonemobius socius* and *A. fasciatus*; bottom: a bimodal hybrid zone between *Heliconius himera* and *H. erato*. B is adapted from Jiggins and Mallet 2000).

The bad news for continuum studies is that it appears that some of this effort is going to waste. While conducting our literature review, we examined most of the papers that have estimated RI using the mathematical framework proposed by Sobel and Chen (2014). Although the estimates of RI generated in these studies are directly comparable, meaning that they are ideal for comparative analyses, there is currently no standardized way for archiving the results in a way that maximizes their utility for our field. Some studies only present estimates of RI graphically, which makes it difficult to extract numerical estimates for use in comparative analyses. In other cases, the values for individual components of RI are calculated (i.e., to estimate total RI) but not provided, limiting our ability to study specific types of barriers. Of course, estimates could always be recalculated from raw data (when available), but this is complicated and time-consuming when many barriers have been measured, so any unnecessary double-handling of data should be avoided. We therefore urge researchers to consider providing these data (and other other relevant information) in a way that maximizes their accessibility and value beyond specific study goals. This may ultimately be facilitated by a dedicated database for curating estimates of RI and other explanatory variables for use in comparative meta-analyses.

The value of working together to build this kind of resource is highlighted by the series of comparative studies of RI in the genus *Drosophila* (recently reviewed by Matute and Cooper 2021). First consisting of 119 pairs of taxa assembled by Coyne and Orr (1989), this dataset has been expanded over the last three decades to include 630 pairs of taxa along with estimates of genetic divergence and other factors such as their range size and amount of range overlap (Yukilevich 2012). As the dataset has grown, it has provided more robust support for earlier findings (Coyne and Orr 1997) and has allowed tests of new, more complex hypotheses to be conducted as they have arisen (e.g., Yukilevich 2012; Nosil 2013). We can only imagine the long-term impact that a much larger, taxonomically broader dataset would have on our general understanding of the speciation process.

LOOKING FORWARD

Thinking about the future of our field, we anticipate that the value of continuum-based studies will only increase moving forward. We simply see no other way to synthesize the findings of many individual case studies of speciation toward a more general understanding of how RI evolves. Further, we cannot think of any

questions in speciation research that would not somehow benefit from continuum-based analyses. Rather than attempting to provide an exhaustive list of questions, we want to highlight just a few knowledge gaps and methodological advances that may help to stimulate future work.

Focusing first on comparative studies, we think that there is value in expanding the taxonomic focus, which is currently biased toward a few model systems (e.g., *Drosophila*). This will help us understand how taxonomically consistent patterns of speciation are across the tree of life. When focusing on more specific groups of organisms (e.g., birds or plants), studies considering the importance of different forms of isolation could focus more on specific types of barriers rather than broad categories. For example, instead of focusing only on pre- and postzygotic isolation, a continuum-based study of flowering plants could also test hypotheses about how the relative importance of flowering time, pollinator isolation, seed set, hybrid sterility, and other barriers changes with the overall strength of RI.

Comparative studies could also benefit enormously from speciation genomic approaches. For instance, there are now a multitude of demographic inference methods that can be used to broadly identify the history under which species have evolved. Therefore, it is possible to identify speciation events that have likely evolved under the same scenario (e.g., secondary contact vs. primary divergence; Fraïsse et al. 2020), which may help us to understand and control for the effects of demography in comparative studies. Extensions to demographic inference also show promise for identifying the proportion of the genome resistant to gene flow (Roux et al. 2016) and jointly approximating the number of barrier loci and the strength of barriers in a comparative context (Aeschbacher et al. 2017; Fraïsse et al. 2020). We are especially excited about the impact that new analytical tools for speciation genomics will have on continuum studies. Rather than simply examining how patterns of genome-wide variation change along the speciation continuum (e.g., π, F_{ST}, and d_{xy}), it may soon be possible to understand how the genomic distribution and effect size of barrier loci varies with the overall strength of RI (Box 3). For more thoughts on the future of comparative studies of speciation, see Matute and Cooper (2021).

In addition to comparative studies, there is a need for more experimental and simulation-based studies of the speciation continuum. Because the same speciation event can be sampled at many points during the process, experimental and simulation studies are the only direct way by which we can understand how different variables influence the trajectories that populations take as they move along the continuum. For example, how does variation in parameters such as the level of migration, strength and nature of selection, population size, divergence history, and level of standing variation influence the composition and strength of barriers over time? Replicated experiments (in organisms like yeast, bdelloid rotifers, and *Drosophila*) and simulations, seeded from the same ancestral population and evolving under the same conditions, can also provide unique insight into the predictability of several aspects of the speciation process. For example, do different replicate populations accumulate RI at the same rate and via the same types of barriers? Are there critical factors that determine whether speciation trajectories are more or less predictable? For more thoughts on the future of experimental speciation, see a recent perspective by White et al. (2020).

Box 3: How does RI play out at the level of the genome?

We have a relatively poor understanding of how isolating barriers acting at the organismal level cause RI at the genomic level. When total RI is 0, there are no barrier loci in the genome. However, any value of RI > 0 could be due to one or a few large effect loci, many small-effect barrier loci scattered throughout the genome, or, more likely, some combination of these alternatives (Butlin and Stankowski 2020). To get an idea of the genome-wide causes and effects of barriers, we need a reliable way to measure effective migration (m_e) that can be applied to small, defined genomic regions. In a comparative framework, we could then examine how variation in the genome-wide distribution of m_e varies with the total strength of RI, estimated at the organismal level. Empirical studies of this question have been limited by a lack of suitable analysis methods. For example, commonly used statistics for identifying barrier loci, including F_{ST} and d_{XY}, are measures of allele frequency differentiation and genetic distance, not m_e. Thus, they measure the combined impact of all processes that have shaped patterns of within- and between-population genetic variation, some of which have nothing to do with speciation per se (Whitlock and Mccauley 1999).

Newer methods offer some promise for overcoming this issue. The f_d statistic, an extension of Patterson's D, uses discordant ABBA and BABA site patterns to estimate f, the proportion of introgression for genomic windows (Martin et al. 2015). f_d is proportional to m_e, robust to genome-wide variation in diversity and effective population size, and is relatively simple to calculate provided that a four-taxon topology is available (Patterson et al. 2012; Martin et al. 2015). An especially promising approach for estimating m_e involves dividing the genome into blocks and fitting separate demographic models to the joint site frequency spectra to estimate the migration rate locally within the genome (Lohse et al. 2015, 2016) (Fig. 7). One issue with this approach, which is common to all methods for identifying barrier loci, is that defining blocks of sequence is essentially arbitrary. However, recent

advances in inferring genome-wide genealogies from large numbers of samples offer considerable promise in this regard as the genome can be efficiently partitioned into blocks based on ancestry (Kelleher et al. 2019).
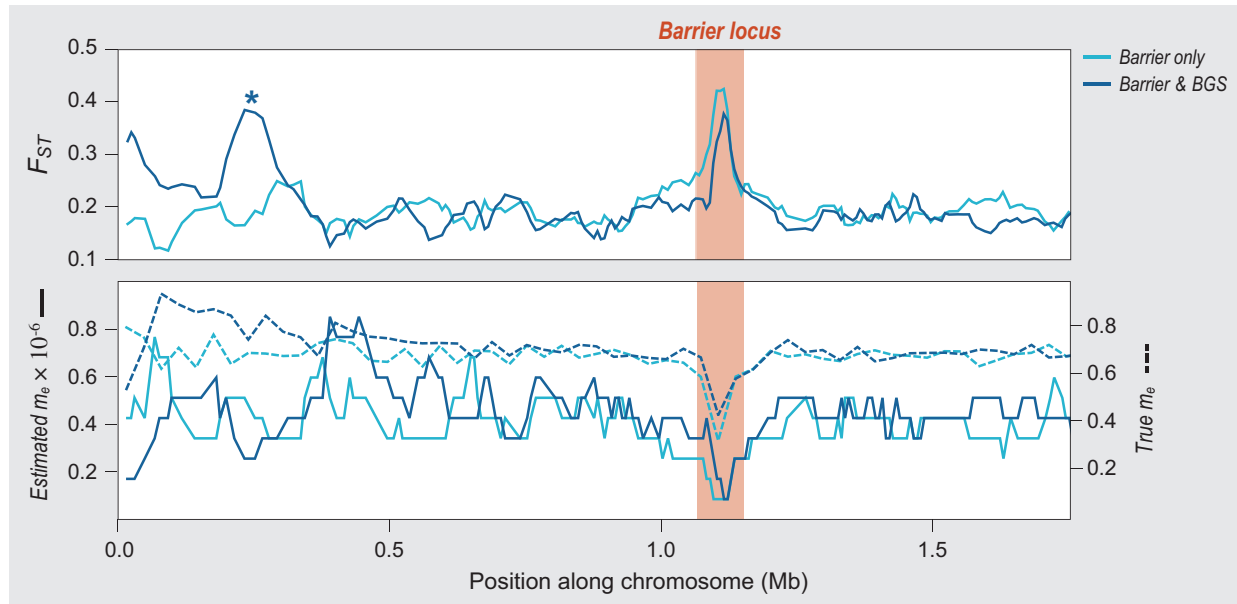
Figure 7. Old and new methods for detecting barrier loci in the genome applied to simulated data. Top panel: patterns of allele frequency differentiation (measured by F_{ST}) between two populations separated by a barrier to gene flow, with and without background selection (BGS). In the simulation without BGS, a single peak of F_{ST} reveals the location of a predefined barrier locus. In the second simulation, a spurious F_{ST} peak (marked by the asterisk) arises due to the effect of BGS. Bottom panel: estimates of m_e inferred using the new analysis tool gIMble (Laetsch et al. in prep.) which takes a model-based approach to detecting barrier loci through the block-wise analysis of the Joint site frequency spectrum. For both simulations, gIMble infers a pattern of m_e across the chromosome that is highly correlated with the true m_e, with a strong reduction in m_e at the barrier locus. Moreover, the inference of m_e is not strongly impacted by the potentially confounding effect of BGS as in the F_{ST} scan. Note that the values for the true m_e and estimated m_e are measured on different scales: the true m_e is measured as the proportion of cross-species coalescence events that are more recent than the population split (dashed lines), whereas the estimate of m_e is measured as the per lineage (and per generation) probability of migration (solid lines). Simulations were conditioned on the divergence and gene flow history of *Heliconius melpomene* and *H. cydno* and the recombination map and annotation of these species for the first 1.75 Mb of chromosome 18 (Davey et al. 2017). BGS targets were restricted to genic regions; the barrier locus was simulated at the position of the wing patterning gene *optix*. Simulations and analysis were conducted and kindly provided by Gertjan Bisschop and Konrad Lohse.

Conclusion

Speciation researchers are no strangers to the power of language and concepts in shaping and sometimes even misleading our thinking (Harrison 2012). New terms rooted in old ideas can easily gain traction without being clearly defined or critically evaluated in terms of their strengths and limitations. The speciation continuum concept has clearly fallen victim to this, as it has become embedded in the literature over a short period without a clear consensus of what it represents, or what it can tell us. With the hope that it will help direct the efforts of our field, we have proposed what we think is a clear, logical definition of the speciation continuum. In arriving at the definition, we have come to the conclusion that it is unhelpful and sometimes illogical to see the continuum as a pathway toward a destination, a map of progress,

a series of stages, an indication of the degree of genetic, phenotypic, or ecological divergence, or as a temporal reconstruction. However, when defined as a continuum of RI, we think that the concept becomes far more than just a convenient narrative portraying how speciation might unfold. Rather, we see it as a conceptual and analytical framework that will inform theory and act as a foundation for comparative and experimental studies of speciation. Provided that we work together and keep realistic expectations, we think that the concept will play an increasingly important role in understanding the continuum of reproductive isolation that surrounds us.

AUTHOR CONTRIBUTIONS

Both authors conceptualized and wrote the manuscript.

LITERATURE CITED

Aeschbacher, S., J. P. Selby, J. H. Willis, and G. Coop. 2017. Population-genomic inference of the strength and timing of selection against gene flow. Proc. Natl. Acad. Sci. USA 114:7061–7066.

Barraclough, T. G. 2019. The evolutionary biology of species. Oxford Univ. Press, Oxford, U.K.

Barton, N., and B. O. Bengtsson. 1986. The barrier to genetic exchange between hybridising populations. Heredity 57:357–376.

Barton, N. H. 1983. Multilocus clines. Evolution 37:454–471.

———. 2000. Genetic hitchhiking. Philos. Trans. R. Soc. Lond. B Biol. Sci. 355:1553–1562.

———. 2020. On the completion of speciation. Philos. Trans. R. Soc. B Biol. Sci. 375:20190530.

Barton, N. H., and M. A. R. De Cara. 2009. The evolution of strong reproductive isolation. Evolution 63:1171–1190.

Barton, N. H., and K. S. Gale. 1993. Genetic analysis of hybrid zones. Pp. 13–45 in R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, Oxford, U.K.

Burri, R., A. Nater, T. Kawakami, C. F. Mugal, P. I. Olason, L. Smeds, A. Suh, L. Dutoit, S. Bureš, L. Z. Garamszegi, et al. 2015. Linked selection and recombination rate variation drive the evolution of the genomic landscape of differentiation across the speciation continuum of Ficedula flycatchers. Genome Res. 25:1656–1665.

Butlin, R., A. Debelle, C. Kerth, R. R. Snook, L. W. Beukeboom, R. F. C. Castillo, W. Diao, M. E. Maan, S. Paolucci, F. J. Weissing, et al. 2012. and What do we need to know about speciation? Trends Ecol. Evol. 27:27–39.

Butlin, R. K., and S. Stankowski. 2020. Is it time to abandon the biological species concept? No. Natl. Sci. Rev. 7:1400–1401.

Butlin, R. K., J. Galindo, and J. W. Grahame. 2008. Sympatric, parapatric or allopatric: the most important way to classify speciation? Philos. Trans. R. Soc. Lond. B Biol. Sci. 363:2997–3007.

Cooney, C. R., J. A. Bright, E. J. R. Capp, A. M. Chira, E. C. Hughes, C. J. A. Moody, L. O. Nouri, Z. K. Varley, and G. H. Thomas. 2017. Corrigendum: mega-evolutionary dynamics of the adaptive radiation of birds. Nature 552:430.

Coyne, J. A., and H. A. Orr. 1989. Patterns of speciation in Drosophila. Evolution 43:362–381.

———. 1997. "Patterns of speciation in Drosophila" revisited. Evolution 51:295–303.

———. 2004. Speciation. Sinauer Associates, Sunderland, MA.

Coughlan, J. M., and D. R. Matute. 2020. The importance of intrinsic postzygotic barriers throughout the speciation process. Philos. Trans. R. Soc. Lond. B Biol. Sci. 375:20190533.

Cruickshank, T. E., and M. W. Hahn. 2014. Reanalysis suggests that genomic islands of speciation are due to reduced diversity, not reduced gene flow. Mol. Ecol. 23:3133–3157.

Davey, J. W., S. L. Barker, P. M. Rastas, A. Pinharanda, S. H. Martin, R. Durbin, W. O. McMillan, R. M. Merrill, and C. D. Jiggins. 2017. No evidence for maintenance of a sympatric Heliconius species barrier by chromosomal inversions. Evol. Lett. 1:138–154.

Dieckmann, U., M. Doebeli, J. A. Metz, and D. Tautz. 2004. Adaptive speciation. Cambridge Univ. Press, Cambridge, U.K.

Drès, M., and J. Mallet. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. Philos. Trans. R. Soc. Lond. B Biol. Sci. 357:471–492.

Feder, J. L., S. P. Egan, and P. Nosil. 2012. The genomics of speciation-with-gene-flow. Trends Genet. 28:342–350.

Flaxman, S. M., J. L. Feder, and P. Nosil. 2013. Genetic hitchhiking and the dynamic buildup of genomic divergence during speciation with gene flow. Evolution 67:2577–2591.

Fraïsse, C., I. Popovic, J. Romiguier, E. Loire, A. Simon, N. Galtier, L. Duret, N. Bierne, X. Vekemans, and C. Roux, 2020. DILS: demographic inferences with linked selection by using ABC. Molecular Ecology Resources. https://doi.org/10.1111/1755-0998.13323.

Funk, D. J., P. Nosil, and W. J. Etges. 2006. Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. Proc. Natl. Acad. Sci. USA 103:3209–3213.

Gay, L., P.-A. Crochet, D. A. Bell, and T. Lenormand. 2008. Comparing clines on molecular and phenotypic traits in hybrid zones: a window on tension zone models. Evolution 62:2789–2806.

Gould, S. J. 1990. Wonderful life: the burgess shale and the nature of history. WW Norton & Company, New York.

Gould, S. J., and M. H. Nitecki. 1988. Evolutionary progress. Univ. of Chicago Press, Chicago.

Gourbière, S., and J. Mallet. 2010. Are species real? The shape of the species boundary with exponential failure, reinforcement, and the "missing snowball". Evolution 64:1–24.

Grahame, J. W., C. S. Wilding, and R. K. Butlin. 2006. Adaptation to a steep environmental gradient and an associated barrier to gene exchange in Littorina saxatilis. Evolution 60:268–278.

Harrison, R. G. 2012. The language of speciation. Evolution 66:3643–3657.

Harvey, P. H., and M. D. Pagel. 1991. The comparative method in evolutionary biology. Oxford Univ. Press, Oxford, U.K.

Hendry, A. P. 2009. Ecological speciation! Or the lack thereof? Can. J. Fish. Aquat. Sci. 66:1383–1398.

Hendry, A. P., D. I. Bolnick, D. Berner, and C. L. Peichel. 2009. Along the speciation continuum in sticklebacks. J. Fish Biol. 75:2000–2036.

Hewitt, G. M. 1988. Hybrid zones-natural laboratories for evolutionary studies. Trends Ecol. Evol. 3:158–167.

Hey, J. 2001. The mind of the species problem. Trends Ecol. Evol. 16:326–329.

Hudson, R. R. 1990. Gene genealogies and the coalescent process. Pp. 1–42 in D. J. Futuyma and. J. Antonovics, eds. Oxford surveys in evolutionary biology. Vol. 7. Oxford Univ. Press, Oxford, U.K.

Irwin, D. E. 2020. Assortative mating in hybrid zones is remarkably ineffective in promoting speciation. Am. Nat. 195:E150–E167.

Jiggins, C. D., and J. Mallet. 2000. Bimodal hybrid zones and speciation. Trends Ecol. Evol. 15:250–255.

Johnson, E. A., and K. Miyanishi. 2008. Testing the assumptions of chronosequences in succession. Ecol. Lett. 11:419–431.

Kelleher, J., Y. Wong, A. W. Wohns, C. Fadil, P. K. Albers, and G. McVean. 2019. Inferring whole-genome histories in large population datasets. Nat. Genet. 51:1330–1338.

Kulmuni, J., R. K. Butlin, K. Lucek, V. Savolainen, and A. M. Westram. 2020. Towards the completion of speciation: the evolution of further reproductive isolation once the first barriers are in place. Philos. Trans. R. Soc. B Bio. Sci. 286:20190528.

Kimura, M. 1962. On the probability of fixation of mutant genes in a population. Genetics 47:713–719.

Kimura, M., and T. Ohta. 1969. The average number of generations until extinction of an individual mutant gene in a finite population. Genetics 63:701–709.

Laetsch, D. R., G. Bisschop, S. Aeschbacher, S. H. Martin, D. Setter, and K. Lohse. Demographically explicit scans for barriers to gene flow using genome-wide IM blockwise likelihood estimation: gIMble. Unpublished manuscript.

Lohse, K., M. Clarke, M. G. Ritchie, and W. J. Etges. 2015. Genome-wide tests for introgression between cactophilic Drosophila implicate a role of inversions during speciation. Evolution 69:1178–1190.

Lohse, K., M. Chmelik, S. H. Martin, and N. H. Barton. 2016. Efficient strategies for calculating blockwise likelihoods under the coalescent. Genetics 202:775–786.

Lowry, D. B. 2012. Ecotypes and the controversy over stages in the formation of new species. Biol. J. Linn. Soc. Lond. 106:241–257.

Lowry, D. B., and B. A. Gould. 2016. Speciation continuum. Elsevier, Amsterdam, The Netherlands.

Lyell, C. 1830. Principles of geology. Vol. 1. Univ. of Chicago Press, Chicago.

Mallet. 2009. Alfred Russel Wallace and the Darwinian Species Concept: His Paper on the Swallowtail Butterflies (Papilionidae) of 1865. Gayana 73: 42-54

Marques, D. A., K. Lucek, J. I. Meier, S. Mwaiko, C. E. Wagner, L. Excoffier, and O. Seehausen. 2016. Genomics of rapid incipient speciation in sympatric threespine stickleback. PLoS Genet. 12:e1005887.

Martin, Simon H., Kanchon K. Dasmahapatra, Nicola J. Nadeau, Camilo Salazar, James R. Walters, Fraser Simpson, Mark Blaxter, Andrea Manica, James Mallet, and Chris D. Jiggins. 2013. "Genome-wide evidence for speciation with gene flow in Heliconius butterflies. Genome Research 23:1817–1828.

Martin, S. H., J. W. Davey, and C. D. Jiggins. 2015. Evaluating the use of ABBA–BABA statistics to locate introgressed loci. Mol. Biol. Evol. 32:244–257.

Matute, D. R., and B. S. Cooper. 2021. Comparative studies on speciation: 30 years since Coyne and Orr. Evolution. https://doi.org/10.1111/evo.14181.

Mayr, E. 1942. Systematics and the origin of species from the viewpoint of a zoologist. Columbia University Press, New York.

Merot, C., C. Salazar, R. M. Merrill, C. D. Jiggins, and M. Joron. 2017. What shapes the continuum of reproductive isolation? Lessons from Heliconius butterflies. Proc. R. Soc. B Biol. Sci. 284:20170335.

Merrill, R. M., Z. Gompert, L. M. Dembeck, M. R. Kronforst, W. O. McMillan, and C. D. Jiggins. 2011. Mate preference across the speciation continuum in a clade of mimetic butterflies. Evolution 65:1489–1500.

Moyle, L. C., and T. Nakazato. 2010. Hybrid incompatibility "snowballs" between Solanum species. Science 329:1521–1523.

Moyle, L. C., M. S. Olson, and P. Tiffin. 2004. Patterns of reproductive isolation in three angiosperm genera. Evolution 58:1195–1208.

Nordborg, M., and P. Donnelly. 1997. The coalescent process with selfing. Genetics 146:1185–1195.

Nosil, P. 2012. Ecological speciation. Oxford Univ. Press, Oxford, U.K.

———. 2013. Degree of sympatry affects reinforcement in Drosophila. Evolution 67:868–872.

Nosil, P., and J. L. Feder. 2012. Genomic divergence during speciation: causes and consequences. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 367: 332–342.

Nosil, P., L. J. Harmon, and O. Seehausen. 2009. Ecological explanations for (incomplete) speciation. Trends Ecol. Evol. 24:145–156.

Orr, H. A., and M. Turelli. 2001. The evolution of postzygotic isolation: accumulating Dobzhansky-Muller incompatibilities. Evolution 55:1085–1094.

Patterson, N., P. Moorjani, Y. Luo, S. Mallick, N. Rohland, Y. Zhan, T. Genschoreck, T. Webster, and D. Reich. 2012. Ancient admixture in human history. Genetics 192:1065–1093.

Perini, S., M. Rafajlović, A. M. Westram, K. Johannesson, and R. K. Butlin. 2020. Assortative mating, sexual selection, and their consequences for gene flow in Littorina. Evolution 74: 1482–1497.

Phadnis, N., E. P. Baker, J. C. Cooper, K. A. Frizzell, E. Hsieh, A. F. A. de la Cruz, J. Shendure, J. O. Kitzman, and H. S. Malik. 2015. An essential cell cycle regulation gene causes hybrid inviability in Drosophila. Science 350:1552–1555.

Phillips, B. L., S. J. E. Baird, and C. Moritz. 2004. When vicars meet: a narrow contact zone between morphologically cryptic phylogeographic lineages of the rainforest skink, *Carlia rubrigularis*. Evolution 58:1536–1548.

Phillips, J. D. 2015. The robustness of chronosequences. Ecol. Modell. 298:16–23.

Presgraves, D. C. 2002. Patterns of postzygotic isolation in Lepidoptera. Evolution 56:1168–1183.

Price, T. D., and M. M. Bouvier. 2002. The evolution of F1 postzygotic incompatibilities in birds. Evolution 56:2083–2089.

Rabosky, D. L., F. Santini, J. Eastman, S. A. Smith, B. Sidlauskas, J. Chang, and M. E. Alfaro. 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. Nat. Commun. 4:1958.

Räsänen, K., and A. P. Hendry. 2008. Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. Ecol. Lett. 11:624–636.

Ravinet, M., R. Faria, R. K. Butlin, J. Galindo, N. Bierne, M. Rafajlović, M. A. F. Noor, B. Mehlig, and A. M. Westram. 2017. Interpreting the genomic landscape of speciation: a road map for finding barriers to gene flow. J. Evol. Biol. 30:1450–1477.

Ravinet, M., K. Yoshida, S. Shigenobu, A. Toyoda, A. Fujiyama, and J. Kitano. 2018. The genomic landscape at a late stage of stickleback speciation: high genomic divergence interspersed by small localized regions of introgression. PLoS Genet. 14:e1007358.

Richards, R. A. 2010. The species problem: a philosophical analysis. Cambridge Univ. Press, Cambridge, U.K.

Ringbauer, H., A. Kolesnikov, D. L. Field, and N. H. Barton. 2018. Estimating barriers to gene flow from distorted isolation-by-distance patterns. Genetics 208:1231–1245.

Roesti, M., S. Gavrilets, A. P. Hendry, W. Salzburger, and D. Berner. 2014. The genomic signature of parallel adaptation from shared genetic variation. Molecular Ecology, 23: 3944–3956.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145:1219–1228.

Roux, C., C. Fraïsse, J. Romiguier, Y. Anciaux, N. Galtier, and N. Bierne. 2016. Shedding light on the grey zone of speciation along a continuum of genomic divergence. PLoS Biol. 14:e2000234.

Russell, S. T. 2003. Evolution of intrinsic post-zygotic reproductive isolation in fish. Ann. Zool. Fennici 40:321–329.

Seehausen, O. 2009. Progressive levels of trait divergence along a 'speciation transect' in the Lake Victoria cichlid fish Pundamilia. Pp. 155–176 *in* R. K. Butlin, J. R. Bridle, and D. Schluter, eds. Speciation and patterns of diversity. Cambridge Univ. Press, Cambridge, U.K.

Seehausen, O., R. K. Butlin, I. Keller, C. E. Wagner, J. W. Boughman, P. A. Hohenlohe, C. L. Peichel, G.-P. Saetre, C. Bank, A. Brännström, et al. 2014. Genomics and the origin of species. Nat. Rev. Genet. 15: 176–192.

Servedio, M. R., and J. Hermisson. 2019. The evolution of partial reproductive isolation as an adaptive optimum. Evolution 74:4–14.

Shaw, K. L., and S. P. Mullen. 2014. Speciation continuum. J. Hered. 105:741–742.

Smadja, C. M., and R. K. Butlin. 2011. A framework for comparing processes of speciation in the presence of gene flow. Mol. Ecol. 20:5123–5140.

Sobel, J. M. 2014. Ecogeographic isolation and speciation in the genus Mimulus. Am. Nat. 184:565–579.

Sobel, J. M., and G. F. Chen. 2014. Unification of methods for estimating the strength of reproductive isolation. Evolution 68:1511–1522.

Sobel, J. M., G. F. Chen, L. R. Watt, and D. W. Schemske. 2010. The biology of speciation. Evolution 64:295–315.

Stelkens, R. B., K. A. Young, and O. Seehausen. 2010. The accumulation of reproductive incompatibilities in African cichlid fish. Evolution 64:617–633.

Stelkens, R. B., C. Schmid, and O. Seehausen. 2015. Hybrid breakdown in cichlid fish. PLoS One 10:e0127207.

Stankowski, S., M. A. Chase, A. M. Fuiten, M. F. Rodrigues, P. L. Ralph, and M. A. Streisfeld. 2019. Widespread selection and gene flow shape the genomic landscape during a radiation of monkeyflowers. PLoS Biol. 17:e3000391.

Stankowski, S., and M. Ravinet. In Press. Quantifying the use of species concepts. Current Biology.

Supple, M. A., R. Papa, H. M. Hines, W. O. McMillan, and B. A. Counterman. 2015. Divergence with gene flow across a speciation continuum of Heliconius butterflies. BMC Evolutionary Biology. 15: 1–12.

Tajima, F. 1989. The effect of change in population size on DNA polymorphism. Genetics 123:597–601.

Taylor, E. B., J. W. Boughman, M. Groenenboom, M. Sniatynski, D. Schluter, and J. L. Gow. 2006. Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (Gasterosteus aculeatus) species pair. Molecular Ecology. 15: 343–355.

Turelli, M., J. R. Lipkowitz, J. R., and Y. Brandvain. 2014. On the Coyne and Orr-igin of species: effects of intrinsic postzygotic isolation, ecological differentiation, X chromosome size, and sympatry on Drosophila speciation. Evolution 68:1176–1187.

Turner, T. L., M. W. Hahn, and S. V. Nuzhdin. 2005. Genomic islands of speciation in Anopheles gambiae. PLoS Biol. 3:e285.

Walker, L. R., D. A. Wardle, R. D. Bardgett, and B. D. Clarkson. 2010. The use of chronosequences in studies of ecological succession and soil development. J. Ecol. 98:725–736.

Wallace, A. R. 1865. I. On the phenomena of variation and geographical distribution as illustrated by the Papilionidæ of the Malayan region. Trans. Linn. Soc. Lond. 25:1–71.

Walsh, B. D. 1864. On phytophagic varieties and phytophagous species. Proc. Entomol. Soc. Phila. 3:403–430.

White, N. J., R. R. Snook, and I. Eyres. 2020. The past and future of experimental speciation. Trends Ecol. Evol. 35:10–21.

Whitlock, M. C., and D. E. Mccauley. 1999. Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. Heredity 82:117–125.

Wright, S. 1943. Isolation by distance. Genetics 28:114–138.

Wu, C.-I. 2001. The genic view of the process of speciation. J. Evol. Biol. 14:851–865.

Wu, C. I., and C. T. Ting. 2004. Genes and speciation. Nature Reviews Genetics. 5: 114–122.

Yukilevich, R. 2012. Asymmetrical patterns of speciation uniquely support reinforcement in Drosophila. Evolution 66:1430–1446.

Associate Editor: M. Kopp
Handling Editor: T. Chapman

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Written results from the speciation survey, summarized in Fig. 3, Q4.