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Towards the completion of speciation: the evolution of reproductive isolation beyond the first barriers

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Speciation, that is, the evolution of reproductive barriers eventually leading to complete isolation, is a crucial process generating biodiversity. Recent work has contributed much to our understanding of how reproductive barriers begin to evolve, and how they are maintained in the face of gene flow. However, little is known about the transition from partial to strong reproductive isolation (RI) and the completion of speciation. We argue that the evolution of strong RI is likely to involve different processes, or new interactions among processes, compared with the evolution of the first reproductive barriers. Transition to strong RI may be brought about by changing external conditions, for example, following secondary contact. However, the increasing levels of RI themselves create opportunities for new barriers to evolve and, and interaction or coupling among barriers. These changing processes may depend on genomic architecture and leave detectable signals in the genome. We outline outstanding questions and suggest more theoretical and empirical work, considering both patterns and processes associated with strong RI, is needed to understand how speciation is completed.

This article is part of the theme issue 'Towards the completion of speciation: the evolution of reproductive isolation beyond the first barriers'.

1. Is the evolution of strong reproductive isolation different from the evolution of the first barriers to gene flow?

Speciation is the evolution of reproductive isolation (RI) through the accumulation of barriers to gene exchange. Opinions vary on when the speciation process ends: some require RI to be complete [1], while others allow some possibility for gene exchange (notably [2, pp. 33–35]); some require RI to be irreversible while others do not [2, pp. 37–38]. Divergence continues to accumulate after completion of RI. This is important for the evolution of biological diversity, as continued ecological divergence can facilitate coexistence in sympatry. Continued divergence can remove the possibility of speciation reversal when redundant barriers accumulate [3]. However, continuing divergence is not strictly part of the speciation process and in this theme issue we do not consider continuing divergence after the completion of RI.

Occasionally strong RI can appear rapidly, even in a single generation, in the case of ploidy change [4], chromosomal rearrangements (as reviewed by de Vos *et al*. [5] in this issue) and perhaps following hybridization [6,7]. However, more often the accumulation of barriers to gene flow is an extended process in which RI evolves slowly. Multiple different barriers evolve and are coupled [8], typically over tens of thousands to millions of generations [2, Table 12.1]. This suggests the idea of a 'speciation continuum', in which divergent populations can be placed

Introduction

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according to their current level of RI. The continuum is not unidirectional nor unidimensional, but rather a trajectory in multivariate space (see also three-dimensional speciation cube in [9]). During the speciation process, there may not be a monotonic increase in RI and the rate of accumulation of barriers to gene flow can vary widely (e.g. [10]). Therefore, the speciation continuum is not a temporal progression: RI might stall at an intermediate level [11], it might decrease following a change in the environment that disrupts barriers to gene flow (e.g. [12]), or it might fluctuate over time.

In this theme issue, we focus on the part of the speciation continuum where some barriers to gene flow have already evolved, as we are primarily concerned with the evolution of RI towards the completion of speciation. We contrast this with parts of the speciation process during which RI starts to accumulate and barriers to gene flow are being established against a background of weak or no divergence. Because RI rarely increases steadily with time, the contrast between parts of the continuum reflects the level of RI, not time since the start of a speciation event. Much attention in recent speciation research has focused on processes acting in parts of the continuum where RI is low, especially local adaptation. These studies are important but may not allow inferences about the speciation process closer to completion. First, they do not increase our understanding of the mechanisms and processes that can lead to the evolution of strong barriers. Second, the mix of processes contributing to speciation may change with increasing levels of RI, and feedback between barriers and processes may occur. A key question then is, in what way are the contributing barriers, processes and genomic patterns different between the different parts of the speciation continuum?

Ideally, to address questions on the evolution of strong RI, one would sample population pairs across the continuum and test whether the increase in RI is consistent with a specific process (e.g. a 'snowballing' of hybrid incompatibilities [13]), or whether patterns associated with a certain process increase along the continuum (e.g. signatures of reinforcement). Even when it is not possible to analyse comparable population pairs across the speciation continuum within taxa, we can learn from individual case studies of taxon pairs near the completion of speciation. These studies will contribute to an understanding of the reproductive barriers and genomic patterns that characterize the evolution of strong RI (see Stankowski *et al.* [14] and Yamasaki *et al.* [15] in this issue) and, when combined, can give important hints about the underlying processes. Theoretical studies and simulation approaches are necessary to complement empirical work and will allow a more direct understanding of processes facilitating strong RI (see Blanckaert *et al.* [16], Payne & Polechova [17] and Bisschop et al. [18] in this issue). Finally, as pointed out by Barton ([19], this theme issue), sometimes completion of speciation may not actually be favoured. This theme issue combines theoretical and empirical work on the progression to strong RI and considers the patterns and processes associated with high levels of isolation from various angles. We hope it will contribute to a more comprehensive view and a deeper understanding of the speciation process.

2. Are there stages in the speciation process and what causes them?

General evolutionary processes that are known to contribute to speciation include differential adaptation driven by divergent selection, divergence in mating traits due to sexual selection and mutation order processes resulting in incompatibilities driven by either selection or drift, the mix of effects being dependent on spatial context [2]. These processes can lead to the evolution of extrinsic barriers (divergent adaptation) as well as intrinsic barriers and assortment. Importantly, these processes can take place in any part of a speciation trajectory. When some RI is already present, they may strengthen existing barriers that evolved earlier, lead to the emergence of new barriers or increase RI by coupling of barriers (i.e. by causing the effects of multiple barriers to work together in opposing gene flow; [8]).

There is a long-standing idea that the continuum might be divided into more or less discrete 'stages' or 'phases' (here we use the former). This idea dates back to Darwin and Wallace but has received renewed attention recently (see Lowry [20] for a history of the debate over 'stages of speciation'). Hendry *et al.* [21] and Mérot *et al.* [22] illustrate the heuristic value of this view when applied to empirical cases. If separate stages exist, they might reflect changing patterns and processes along the speciation continuum; therefore, identifying and understanding what causes stages will directly relate to the questions posed by this theme issue.

Changes in the strength of RI, the rate at which RI accumulates or the forces driving the speciation process could all be used to define stages. The stages might also be imposed externally. The classic example is divergence in allopatry followed by secondary contact and hybridization, which is expected to introduce new potential mechanisms for the evolution of reproductive isolation, notably reinforcement. Another example of stages associated with external factors is described in this issue by Muschick *et al.* [23]. They show that shifts between closely related host plants generate little RI (in terms of host preference), while shifts between more distantly related hosts occur rarely, but lead to greater RI in *Timema* stick insects. Host switches could therefore help populations move to a stage with stronger RI.

Dieckmann *et al.* [9] argued for the necessity of recognizing stages in generating a coherent overview of speciation precisely because of the changes to the mix of individual processes involved. Others have argued that the initiation of RI, its accumulation and its completion are likely to involve different mixes of processes, and different impacts of the spatial setting and of the genetic architecture [24]. Focusingon genetic architecture, and on speciation with gene flow, Wu [25] and Feder *et al.* [26,27] considered the existence of stages. They suggested the initial stage is dominated by direct selection on individual barrier loci leading to localized genomic barriers, which then extend around directly selected loci owing to physical linkage, and finally to a more genome-wide barrier effect leading towards complete RI. Stages with higher RI have a stronger contribution of indirect selection via linkage disequilibrium (LD). Similar transitions between regimes dominated by direct selection versus LD have been described in earlier work [28]. Feder *et al.* [27] suggested that the build-up of LD might generate a sharp transition between these regimes under divergence with gene flow. If so, there may be very distinct stages in some speciation trajectories.

Under which conditions we expect distinct stages remains an open question (box 1: Question 1). In addition, in order to better understand speciation, it is not sufficient to identify stages (if they exist). Rather, we need to ask whether, how and why the processes contributing to speciation change along the speciation continuum.

Box 1. Outstanding questions about the progression towards complete RI during speciation.

1. What underlying processes create heterogeneity and the appearance of stages in the speciation continuum (e.g., as discussed by Muschick *et al*. [23] in this theme issue)? Do distinct stages occur in some speciation trajectories and not others?
2. Are there systematic differences in the types of barriers present at different points in the speciation continuum [2]? The classical expectation is for postzygotic barriers (local adaptation or intrinsic incompatibility) to evolve first, followed by prezygotic barriers (in a trajectory where allopatric divergence is followed by secondary contact; see e.g. North *et al*. [29] in this theme issue) but alternative scenarios have been proposed, as discussed e.g. by Coughlan & Matute [30] in this theme issue. For example, learned behavioural barriers could provide the reduction in gene flow needed for the establishment of ecological differentiation (e.g. Rometsch *et al*. [31] in this theme issue). Are changes in barrier types along the speciation continuum driven mainly by external conditions (such as secondary contact) or by opportunities created by the first barriers to appear (e.g. Tinghitella *et al*. [32] in this theme issue)? Do barriers evolve independently or in concert?
3. Are some speciation trajectories characterized by tipping points, or bi-stable states, resulting in sharp transitions from slow to rapid accumulation of RI? If so, does rapid accumulation lead directly to completion of speciation or is it followed by a slower stage involving other processes (such as reinforcement)? What is the evidence for tipping points and what mechanisms contribute to progressing through tipping points?
4. Under what circumstances do divergence and genomic hitchhiking contribute to the accumulation of RI? When in the speciation continuum do they contribute, and can they be used to distinguish between different stages of speciation? What is the empirical evidence for these processes and how can the existing evidence be extended and improved? Does moving from two-deme models to more realistic models of continuous space lead to different predictions for the role of divergence and genomic hitchhiking?
5. The snowball effect depends on assumptions of epistatic interactions in the genome (see Satokangas *et al*. [33] in this theme issue). Does accumulation of incompatibilities accelerate in snowball fashion under biologically relevant assumptions of epistasis? Are complex incompatibilities involving multiple loci more stable in the face of gene flow (see Blanckaert *et al*. [16] in this theme issue) and therefore more important for the completion of speciation compared to two-locus incompatibilities? How common are incompatibilities whose expression depends on the environment compared with purely intrinsic incompatibilities?
6. Any change in the genetic composition of a population alters the opportunities and constraints on future evolution. How much does this kind of feedback contribute to divergence between populations and the accumulation of RI? Does this occur mainly by stochastic 'mutation order' processes, co-evolution of gene networks, and new ecological opportunities opened up by early adaptive substitutions, or by processes such as sexual or genomic conflict or sexual selection that are partly independent of the environment?
7. Reinforcement is a classical process in cases where some RI already exists, but it remains unclear how often, and how much it contributes to speciation (see North *et al*. [29] in this theme issue). Does reinforcement complete RI following secondary contact? If so, is this only when the initial isolation is already strong? How common is reinforcement in speciation trajectories with continuous or frequent gene flow? How widely does reinforcement in the broad sense (adaptive coupling with barrier enhancement, [8]) operate across different types of barrier effects?

3. The dynamics of reproductive isolation accumulation are likely to change with increasing reproductive isolation

In principle, barriers resulting from the basic processes described above could simply accumulate and steadily increase reproductive isolation. Species would just be divergent populations 'writ large', as suggested for *Rhagoletis* flies [34,35]. Nosil *et al*. [10] argue that, even with gene flow, strong selection on individual loci might generate this kind of steady accumulation of RI (in contrast with weaker selection on multiple loci which can create sharp transitions, see below).

Such a steady accumulation of RI seems unlikely in most scenarios, owing to the emergence of new processes along the speciation continuum (discussed below). These may lead to feedback or interaction between different barriers and processes, making these emergent properties important factors in the evolution of strong RI (box 1: Question 2). Even without changing processes and mechanisms, there are reasons to expect differences between parts of the speciation continuum. For example, if some types of barriers evolve systematically more slowly (e.g. because they require large numbers of new mutations), they are likely to appear later in the speciation process. Even if this is not the case and different barriers emerge completely at random, one could expect the relative contribution of postzygotic barriers to RI to decrease over the course of speciation. This is simply because it becomes more likely that the emergence of barriers acting earlier in the life cycle decreases the impact of late-acting barriers on total isolation. This argument is general [36]; it does not rely on reinforcement of prezygotic barriers.

Although possible, and perhaps useful as a null model, speciation trajectories that are not characterized by feedback mechanisms or interplay between different barriers as RI increases are probably rare in nature. This is because the introduction of barriers to gene flow has many effects: LD is increased, particularly between loci underlying RI, effective gene flow is reduced, and there is stronger selection against hybrids, and a larger potential for negative epistatic interactions between alleles from different populations [13]. In addition, once some initial barriers have established, coupling between pre- and postzygotic barriers [37], intrinsic and extrinsic barriers [38] or between different barrier loci [28] can occur. All these features can modify the trajectory

of speciation, potentially leading to different barrier types, effect sizes and genomic patterns at higher levels of RI compared with low RI. Add to this the likelihood of externally imposed changes in geographical distribution and demography, and uniform speciation trajectories become very improbable (e.g. cessation of gene flow by strong RI in cichlids requires a combination of premating prezygotic isolation supplemented with intrinsic and extrinsic postzygotic barriers whose strengths and importance vary among different adaptive radiations; see Rometsch *et al.* [31] in this theme issue). Some changes will not occur in every speciation trajectory (e.g. addition of reinforcement) while others might be inevitable (e.g. accumulation of incompatibilities in allopatry; [13]).

Below, we discuss feedback processes and mechanisms that lead to coupling of barrier effects, especially reinforcement, that can only occur once some RI already exists. These are likely to be the dominant reasons for changes in the processes operating in different parts of the speciation continuum.

4. Feedback, interactions and the snowball effect

Processes that drive divergence (e.g. divergent selection, sexual selection, drift) and increase RI may vary along the speciation continuum, e.g. because LD increases, effective gene flow decreases (unless in complete allopatry) and the total number of barrier loci increases with higher RI. Both in allopatry and in settings with gene flow these changes can lead to positive feedback, altering the rate at which RI increases. Positive feedback, where some initial barrier allows the spreading of the barrier effect or increases opportunities for other barriers to establish, might generate rapid transitions from weak to strong overall isolation and so justify the identification of distinct stages in the speciation process (box1: Question 3). Feedback within and between processes can be hard to isolate in natural populations but separation can be achieved in models.

One type of feedback acting in the presence of gene flow is divergence hitchhiking, where a divergently selected locus provides a barrier locally in the genome that allows accumulation of differentiation at linked sites, while gene flow continues to homogenize allele frequencies elsewhere in the genome [39]. Feedback occurs if the localized barrier facilitates the spread of other barrier alleles, allowing the size of the 'genomic island of differentiation' and the total RI to increase. The efficacy of this process depends on the extent of LD and so the chance that new mutations will benefit from the reduction in gene flow. Its effectiveness has been debated [40–42]. Arguably, divergence hitchhiking is mostly relevant when total RI is still low, because with strong RI the density of selected loci becomes high enough to cause LD even between distant and physically unlinked loci, generating a general barrier to gene flow. 'Genome hitchhiking' (a genome-wide reduction in the effective migration rate) could then be considered characteristic of situations closer to complete RI [43] (box 1: Question 4).

The interaction between selection and recombination can generate a sharp discontinuity in the strength of the genome-wide barrier to gene flow (e.g. *Populus* trees in Shang *et al.* [44] and sticklebacks in Yamasaki *et al.* [15] in this issue). In the context of secondary contact, Barton [28] showed that a strong genome-wide barrier is expected when the ratio of selection to recombination (known as the 'coupling coefficient') exceeds a critical value owing to accumulation of incompatibilities (see also [37]). Above this critical value, secondary contact is much more likely to lead to speciation, for example, because the associations needed for reinforcement to be effective are much more likely to be maintained. An analogous transition is seen in the models of speciation with continuous gene flow by Flaxman *et al.* [45]: where the genomic architecture allows LD (i.e. in models where sets of genes are transmitted from parent to offspring, with or without linkage, rather than sampled randomly from the population) and where selection per locus is weak relative to migration ($s \ll m$), reproductive isolation accumulates slowly until the genomic density of selected loci reaches a critical point where LD can build up. LD increases the effective selection on each locus and this generates positive feedback leading to a rapid increase in RI. However, this result is based on the assumption of locally adaptive mutations under weak selection, and modifications to the model might change whether and how rapidly the feedback process occurs. For example, the occurrence of globally beneficial mutations is excluded from Flaxman *et al.* [45] and other similar models of local adaptation. When they occur, their sweeps interfere with the evolution of local adaptation, reducing the differentiation between populations and delaying the evolution of strong RI (as shown by Bisschop *et al.* [18] in this issue).

Nosil *et al.* [10, p.1] suggested that behaviours analogous to those seen in the Flaxman *et al.* [45] models may be more general: 'divergence process[es] involving small changes can suddenly speed up at a 'tipping point' in speciation, at least in theory'. How widespread these effects are, in theory or in reality, is currently an open question (box 1: Questions 3 and 4). There is some empirical evidence consistent with tipping points [10]. The 'grey zone' of speciation studied by Roux *et al.* [46] shows a transition zone between 0.5 and 2% net synonymous divergence during which the probability of gene flow decreases sharply, which could be interpreted as consistent with the tipping point idea. On the other hand, the identified 'grey zone' is quite wide and contains multiple semi-isolated taxa.

In addition to hitchhiking, the accumulation of genetic incompatibilities can accelerate the evolution of RI, and so generate marked changes across the speciation continuum. Coughlan & Matute [30] argue in this issue that genetic incompatibilities may be important already in the beginning of the speciation process and discuss how they can enhance the evolution of strong reproductive isolation via reinforcement and the 'snowball effect'. This effect occurs because the number of genetic incompatibilities increases faster than linearly with time if each new mutation has the potential to be incompatible with any other mutation [13]. In Orr's model [13], the number of potential pairwise incompatibilities increases with the square of the number of genetic differences that have accumulated between two populations. This acceleration potentially operates in all spatial settings. Evidence for the snowball effect is mixed [47–51]. Importantly, the rate at which incompatibilities accumulate, and so the snowball effect, depends on the number and distribution of epistatic interactions in the genome. Models explicitly incorporating empirical gene interaction networks might lead to different predictions from standard population genetic models, as discussed in this issue by Satokangas *et al.* [33]. As more data have accumulated on the molecular basis of epistatic interactions within the genome, models of incompatibilities have incorporated more realistic assumptions.

Kalirad & Azevedo [52], and Dagilis *et al*. [53] showed that considering both positive and negative epistasis and accounting for differences in within-population and between-population epistasis predicts a snowball-like effect for hybrid fitness. In these models, loss of hybrid fitness can accelerate owing to changes in the magnitude and/or direction of average epistatic effects of mutations over time, rather than changes in the numbers of incompatibilities. On the other hand, Blanckaert & Hermisson [54] have shown that pre-existing incompatibilities do not always facilitate invasion of further barrier genes under gene flow, which can seriously disrupt the snowball effect. Clearly, open questions remain (box 1: Question 5).

A different feedback process occurs when each evolutionary change can open up unique new possibilities. As two populations diverge at functional loci, the set of new mutations that are positively selected in one population will start to diverge from the set that is positively selected in the other population. This is partly due to direct epistatic interactions between mutations and accumulating structural variation in the genome [55] and partly due to a changing ecological environment enabled by divergence. It is related to mutation order effects [56] in the sense that the events governing the first mutations to spread in each population set the populations off on different trajectories. This is another possible area for future research (box 1: Question 6). One could argue that reinforcement falls into this category: the spread of alleles that reduce hybrid fitness creates a selection pressure for assortative mating that was initially absent [57]. However, we treat reinforcement separately (see following section §5).

Remarkably, a strengthening of RI might occur even if existing barriers enable the spread of *the same* new mutation in both diverging populations, as discussed in this theme issue. In their model, Blanckaert *et al*. [16] show that an incompatibility between two derived alleles, with at least one of them being locally adapted, can generate a much stronger barrier if it is accompanied by the fixation of the same new allele at a third locus, if this allele displays a specific set of epistatic interactions.

5. Coupling and reinforcement

The barriers that form in the initial stages of speciation may not coincide, in the sense that they might separate different groups of populations in space or in some other dimension (such as time or niche). For example, local adaptation might distinguish groups of populations occupying different habitats while incompatible allelic combinations separate groups of populations with a common demographic history. Such spatially scattered barriers may often be hard to detect empirically, as they are often weak and affect only a small number of loci. RI can be enhanced by processes that bring these barrier effects together so that they separate coincident groups of populations ('coupling' *sensu* [8]). Spatial movement of clines attracting one another when they overlap [38], and cycles of population expansion and contraction [58] are examples of potential processes that can achieve coupling of barrier effects after the evolution of some RI, i.e. in the part of the speciation continuum where substantial barriers already exist. A similar effect might be caused by short periods of allopatry because divergence at loci contributing to local adaptation is limited by gene flow, particularly if locally adapted alleles also cause some incompatibility in hybrids.

In addition to coupling of existing barrier effects, the presence of some initial barriers may result in selection favouring the evolution of new barrier effects or the enhancement of existing effects. This form of coupling includes reinforcement as traditionally viewed (indirect selection favouring an increase in prezygotic isolation as a result of reduced fitness of hybrids), but also other processes where indirect selection contributes to the origin or strengthening of barriers, whether pre- or postzygotic [8]. All of these processes are most likely to operate when the initial reproductive isolation is already strong. They might be supplemented by direct selection pressures, such as the costs of wasted mating efforts or sexual selection induced by changes in female preference [59]. In this theme issue Tinghitella *et al*. [32] further introduce an ecological element, implicating different ecological conditions that change mating interactions and select for environment-dependent mate preferences. Payne & Polechová [17] argue in this issue that pre-existing niche preference speeds up further increase of assortment, thus facilitating reinforcement in the later stages of speciation.

The classical scenario for reinforcement is the enhancement of assortative mating following secondary contact. The spatial distributions of most taxa have fluctuated through time, with many populations temporarily persisting in geographical isolation from each other, for example, in distinct glacial refugia [60]. Speciation can then have two clear stages: during the period of allopatry populations accumulate intrinsic and/or extrinsic incompatibilities and these postzygotic barriers act upon secondary contact to drive an enhancement of isolation by reinforcement. Both modelling and empirical data now strongly support the possibility of reinforcement in this scenario, although the outcome depends on many factors [61]. Strong postzygotic isolation, multiple-effect traits [62], low costs of mate choice, matching mechanisms of assortative mating [63] and one-allele systems (where no divergence between populations is required to enhance assortment; [64,65]) all promote the evolution of assortative mating. Although the possibility of classical reinforcement is now well established, its overall contribution to speciation remains unclear (box 1: Question 7). There have been few attempts to measure its frequency [66]. Although the patterns of reinforcement are often observed at the phenotypic level, the genomic footprint of reinforcement is much less well understood and data are scarce [57,67] (box 1: Question 7). Focusing on copy-number variation detectable in paired-end sequencing data, North *et al*. [29] test for the expected patterns of genomic divergence under a reinforcement scenario and show that these common, but understudied fine-scale structural variants make for strong candidate targets of reinforcing selection. While some 'adaptive coupling' processes have been well studied (especially classical reinforcement), others have not (box 1: Question 7). For example, habitat choice is a potent source of assortment but it is a relatively understudied form of barrier [68,69]. However, these processes might also be self-limiting in the sense that the selection pressure for increased isolation derives from the production of hybrids and so declines as hybrids become rare, for whatever reason [70]. Self-limitation might also apply to other processes that mainly occur when RI is already relatively strong, for example fitness gains from approaching adaptive optima decline as the optima are approached, potentially leading to a stable balance between divergent selection and gene flow [11,71].

The evolution or enhancement of new barriers to gene flow as a result of costs associated with existing barriers (e.g. [72]) is a much more general feature of systems with high RI than suggested by the historical focus on the classical form of reinforcement [8]. These processes can only operate when populations are in contact because costs of hybridization are not expressed during periods of allopatry, and only to a limited degree in narrow hybrid zones. These mechanisms then require some minimum amount of interbreeding. However, they do not require divergence followed by secondary contact: they are also potentially a key part of speciation with continuous gene flow (e.g. [73] and Osborne *et al.* in this issue [74]). They are not limited to assortative mating, or even to enhancement of prezygotic isolation [8]. More work is needed on these other forms of coupling (box 1: Question 7).

6. Conclusion

We expect the parts of the speciation continuum where substantial RI is already in place to have distinctive features and contain a different mix of processes, including feedback between barriers, compared with initiation of RI. We suggest that more attention to these features and processes is important for a full understanding of speciation, and we present a list of open questions in box 1. Natural systems, but also analysis of anthropogenic and experimental hybridization events, can provide clues to answering open questions (e.g. Viard *et al.* in this issue [75]).

Empirically understanding how processes change along the speciation continuum is challenging, particularly because the necessary contrasts are not available in most taxa, and because many of the processes are notoriously difficult to study even for a single taxon pair (e.g. reinforcement). Progress will come from a combination of case studies of taxon pairs nearing complete isolation (see Stankowski *et al.* [14], Yamasaki *et al.* [15], Osborne *et al.* [74] and North *et al.* [29] in this theme issue), comparative approaches (e.g. de Vos *et al.* [5] and Meyers *et al.* [35] in this theme issue), theoretical studies highlighting what drives and hinders the evolution of strong RI (e.g. Blanckaert *et al.* [16], Payne & Polechová [17] and Bisschop *et al.* [18] in this issue), meta-analyses (as discussed in Rometsch *et al.* [31] and Shang *et al.* [44] in this issue), the identification of processes defining stages (see Muschick *et al.* [23] and Tinghitella *et al.* [32] in this issue) and combined approaches as outlined in this issue (Coughlan & Matute [30] and Satokangas *et al.* [33]). This theme issue is dedicated to Christian Lexer ([76], this theme issue).

Data accessibility. This article has no additional data.

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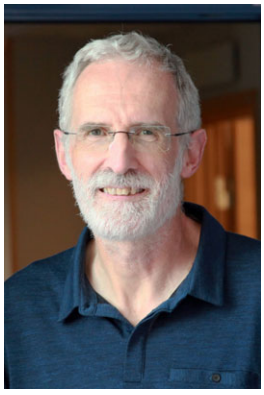

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