Article:

Butlin, R.K. orcid.org/0000-0003-4736-0954, Servedio, M.R., Smadja, C.M. et al. (12 more authors) (2021) Homage to Felsenstein 1981, or why are there so few/many species? Evolution, 75 (5). pp. 978-988. ISSN 0014-3820

https://doi.org/10.1111/evo.14235

doi:10.1111/evo.14235

Homage to Felsenstein 1981, or why are there so few/many species?

Roger K. Butlin,[1,2,*] Maria R. Servedio,[3,*] Carole M. Smadja,[4,*] Claudia Bank,[5] Nicholas H. Barton,[6] Samuel M. Flaxman,[7] Tatiana Giraud,[8] Robin Hopkins,[9] Erica L. Larson,[10] Martine E. Maan,[11] Joana Meier,[12] Richard Merrill,[13] Mohamed A. F. Noor,[14] Daniel Ortiz-Barrientos,[15] and Anna Qvarnström[16]

[1]Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK and Department of Marine Sciences, University of Gothenburg, Gothenburg, Sweden

[2]E-mail: r.k.butlin@sheffield.ac.uk

[3]Department of Biology, University of North Carolina, Chapel Hill, NC 27599

[4]Institut des Sciences de l'Evolution de Montpellier, (ISEM—Université de Montpellier, CNRS, IRD), Montpellier, France

[5]Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, 3012 Bern, Switzerland and, Gulbenkian Science Institute, Oeiras, Portugal

[6]IST Austria, Klosterneuburg, Austria

[7]Department of Ecology and Evolutionary Biology, University of Colorado Boulder, Boulder, CO 80309

[8]Ecologie Systématique et Evolution, CNRS, Université Paris-Saclay, AgroParisTech, Orsay, France

[9]Department of Organismic and Evolutionary Biology, Arnold Arboretum, Harvard University, Boston, MA 02131

[10]Department of Biological Sciences, University of Denver, Denver, CO 80208

[11]Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, The Netherlands

[12]Department of Zoology, University of Cambridge, Cambridge, United Kingdom

[13]Division of Evolutionary Biology, Faculty of Biology, LMU Munich, Planegg-Martinsried 82152, Germany

[14]Biology Department, Duke University, Durham, NC 27708

[15]School of Biological Sciences, The University of Queensland, St Lucia, Queensland 4072, Australia

[16]Department of Ecology and Genetics, Uppsala University, Uppsala SE-751 05, Sweden

Received March 16, 2021
Accepted April 12, 2021

If there are no constraints on the process of speciation, then the number of species might be expected to match the number of available niches and this number might be indefinitely large. One possible constraint is the opportunity for allopatric divergence. In 1981, Felsenstein used a simple and elegant model to ask if there might also be genetic constraints. He showed that progress towards speciation could be described by the build-up of linkage disequilibrium among divergently selected loci and between these loci and those contributing to other forms of reproductive isolation. Therefore, speciation is opposed by recombination, because it tends to break down linkage disequilibria. Felsenstein then introduced a crucial distinction between "two-allele" models, which are subject to this effect, and "one-allele" models, which are free from the recombination constraint. These fundamentally important insights have been the foundation for both empirical and theoretical studies of speciation ever since.

KEY WORDS: Linkage disequilibrium, one-allele, recombination, reproductive isolation, speciation, two allele.

G. E. Hutchinson called his Presidential Address to the American Society of Naturalists, "Homage to Santa Rosalia, or why are there so many kinds of animals?" (Hutchinson 1959). He took, as

---

[*] Roger K. Butlin, Maria R. Servedio, and Carole M. Smadja wrote the Perspective, contributing equally. All authors provided commentaries.

a starting point for discussion, the two species of corixid bug that he found in a small pond below the sanctuary of Santa Rosalia on Monte Pellegrino in Sicily. Asking why there were two species, rather than 20 or 200, led him to consider how the total number of known animal species came to be so large. His answer was that there is an effectively unlimited number of niches available.

Hutchinson's conclusion begs an evolutionary question: is the evolutionary process likely to fill all available niches with distinct species? The dominant paradigm at the time suggested that speciation required a period of allopatry. Accepting this position, one might rephrase the question to ask whether patterns of dispersal and vicariance typically allowed sufficient opportunity for speciation. On the other hand, there was a growing literature on sympatric or parapatric speciation and this possibility highlighted a different direction of thought: are there genetic constraints on speciation? This is the question that led to "Skepticism towards Santa Rosalia, or why are there so few kinds of animals?" (Felsenstein 1981; dedicated to Sewall Wright in celebration of his 90th birthday). Joe Felsenstein outlined his purpose as follows:

> *"The purpose of this paper is to clarify the nature of the genetic forces involved in speciation, and to get some sense of the direction in which they act and their relative strengths."* (p.125)

In our view, he achieved this clarification with extraordinary insight and elegance. We consider his article one of the classics in the history of this journal and a foundational article in speciation biology. Therefore, it is an ideal candidate to be celebrated in this 75th anniversary year of the Society for the Study of Evolution.

The core of Felsenstein's article was a simple three-locus model. In this model, two loci, B and C, each have two alleles, one favored in each of two habitats (the BC haplotype has highest fitness in habitat 1 and the bc haplotype has higher fitness in habitat 2). A third locus, A, which also has two alleles, is not under direct selection but causes assortative mating (more A-A and a-a pairs than under random mating). There is some level of recombination between loci A and B, and between B and C, and there is migration between the two habitats. With this simple tool, Felsenstein demonstrated several key points:

1. That progress towards speciation requires, and can be measured by, the build-up of linkage disequilibrium (LD) between alleles underlying reproductive isolation (at the B and C loci for ecological, postzygotic isolation, and between these loci and the A locus for "behavioral," prezygotic isolation). Complete LD, that is, only ABC and abc haplotypes present, the former primarily in habitat 1 and the latter primarily in habitat 2, is the end-point of the speciation process.
2. That the requirement for LD implies that speciation is opposed by recombination; while selection builds associations recombination breaks them down, generating unfit Bc and bC haplotypes.
3. That the opposition by recombination depends on the extent of migration between habitats: in allopatry, that is, with no migration, recombination has no effect (BC is restricted to one habitat and bc to the other) but where there is migration there is a continuum of increasing opposition with increasing migration.
4. That the opposition by recombination can also depend on the mechanism underlying assortative mating. When assortment operates via the mechanism modelled with locus A, migration and recombination break down associations between the A and B/C loci and have to be overcome by strong selection. This is a "two-allele mechanism." However, Felsenstein introduced an alternative mode of assortment, called a "one-allele mechanism" (implicitly considered with another locus, D), "*where reproductive isolation is strengthened by substituting the same allele in the two nascent species*" (p.133) and where selection is, therefore, unopposed by recombination (see Fig. 1 for further explanation of the difference between two- and one-allele mechanisms).
5. Therefore, the constraints imposed by recombination and the genetic architecture of reproductive isolating traits affect the likelihood of speciation such that some ecological niches may not be filled.

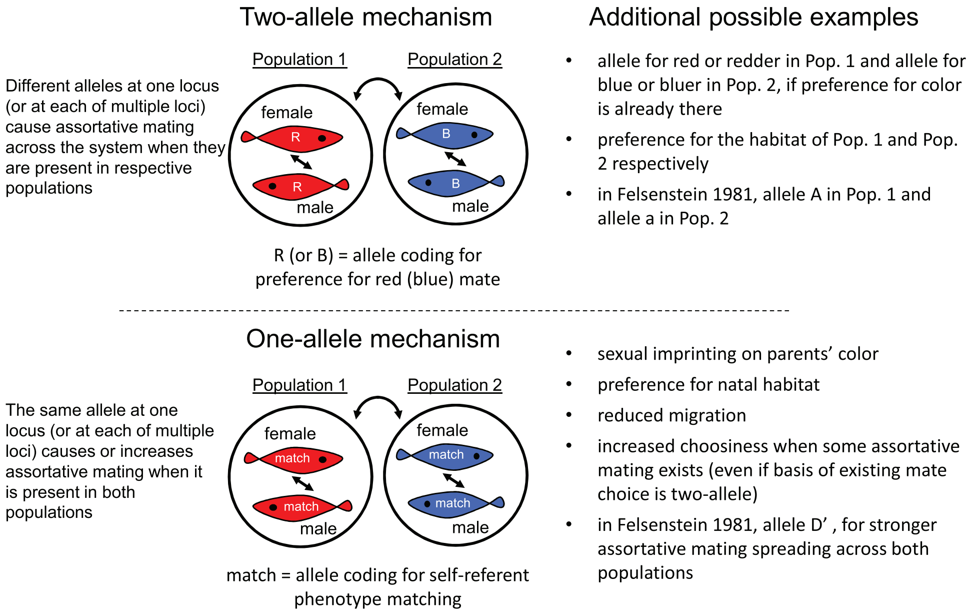
We note that Felsenstein acknowledged the fully compatible conclusions reached previously by others, especially Caisse and Antonovics (1978). He quoted them (p.134):

> *"They explicitly raise the question of why speciation does not always occur, and conclude that 'the conditions leading to isolation are far more stringent than those permitting genetic divergence'."*

As is often the case, the importance of Felsenstein's article was not its priority but rather the way it brought together and clarified key concepts. Felsenstein (1981) has been cited 678 times (Web of Science; 968 Google Scholar; 15 Feb. 2021). Citations peaked between 1995 and 2005 but continue (>20 in each of the last 4 years in Web of Science).

The Impact of Felsenstein (1981)

To explore the impact of Felsenstein's article, we (RKB, MRS & CMS) asked 12 active speciation biologists, at a range of career stages, to write short reflections. Together with our own personal responses, these commentaries are provided in full in Supporting information Appendix 1. They provide a sample that illustrates the impact and influence of Felsenstein's contributions on the development of speciation research and its current direction. In this Perspective, we aim to provide an overview of the major



Different alleles at one locus (or at each of multiple loci) cause assortative mating across the system when they are present in respective populations

R (or B) = allele coding for preference for red (blue) mate

Additional possible examples

- allele for red or redder in Pop. 1 and allele for blue or bluer in Pop. 2, if preference for color is already there
- preference for the habitat of Pop. 1 and Pop. 2 respectively
- in Felsenstein 1981, allele A in Pop. 1 and allele a in Pop. 2

The same allele at one locus (or at each of multiple loci) causes or increases assortative mating when it is present in both populations

match = allele coding for self-referent phenotype matching

- sexual imprinting on parents' color
- preference for natal habitat
- reduced migration
- increased choosiness when some assortative mating exists (even if basis of existing mate choice is two-allele)
- in Felsenstein 1981, allele D′ , for stronger assortative mating spreading across both populations

Figure 1. Illustration of the difference between one- and two-allele mechanisms underlying assortative mating. The example is drawn for two populations with spatial separation and gene flow (the curved arrow), but these populations could be sympatric. The top panel describes a two-allele mechanism and the bottom panel describes a one-allele mechanism. Under each type of mechanism, an example is given of the action of the alleles that have spread to cause assortative mating (assortative mating is represented by the double-headed arrows between the males and females). Two-allele mechanism: the allele(s) enhancing assortative mating between red individuals are different from the allele(s) enhancing the assortative mating between blue individuals. Any number of loci can be involved in assortative mating by a two-allele mechanism provided that assortative mating is enhanced by spread of an alternate allele in each population, in this case generating a color preference difference between populations. Thus, mating preferences, for example for red or blue, could be polygenic. One-allele mechanism: any allele that enhances assortative mating by spreading across the entire system of both populations would constitute a one-allele mechanism. Here, an allele for choosing mates on the basis of matching with their own color spreads in both populations. Again, matching could have a polygenic basis but, in a one allele mechanism no difference evolves between populations: both have an increased tendency to choose matched mates. Additional examples of possible two- or one-allele mechanisms are listed to the right.

contributions of the article. Although we draw on the commentaries (and cite them to connect our review to the individual contributions), the views expressed here are not necessarily shared by all contributors.

Ortiz-Barrientos describes Felsenstein (1981) as the "ABC" of speciation, a neat way to summarize the general view that this article lays crucial foundations. Many authors mentioned each of the five key points outlined above. The need for build-up of LD (1) was highlighted by Barton (as "coupling"; Supporting information Appendix 1.2), Flaxman, Giraud, Noor, and Smadja (Supporting information Appendix 1.4, 1.5, 1.11, 1.15). Elucidating the central role of recombination (2) was mentioned as a major contribution by almost all authors, often with specific reference to the process of reinforcement (Bank, Butlin, Noor, Ortiz-Barrientos, Qvarnström, Servedio, Smadja; Supporting information Appendix 1.1, 1.3, 1.11, 1.12, 1.13, 1.14, 1.15). For some authors, this led to consideration of the suppression of recombination and of other impacts of recombination (Butlin, Noor, Ortiz-Barrientos, Qvarnström; Supporting information Appendix 1.3, 1.11, 1.12, 1.13). The allopatry/sympatry debate (3) is less prominent today but Felsenstein's contribution was mentioned by Barton, Giraud, Hopkins, Larson, and Meier (Supporting information Appendix 1.2, 1.4, 1.6, 1.7, 1.9), in some cases related to the issue of ecological versus genetic constraints on speciation (5). For many contributors, the one-allele, two-allele distinction (4) was a critical revelation, although perhaps a misunderstood and insufficiently appreciated insight. It was the main focus for Bank, Flaxman, Maan, and Servedio (Supporting information Appendix 1.1, 1.4, 1.8, 1.14). Interestingly, an alternative potential component of speciation that avoids opposition by recombination, the "single-variation" (Rice and Hostert 1993), "magic trait" (Gavrilets 2004; Servedio et al. 2011), or "multiple-effect trait" (Smadja and Butlin 2011) model, was mentioned by Giraud, Maan, Merrill, and Smadja (Supporting information Appendix 1.5, 1.8, 1.10, 1.15).

An additional, and important, recurring theme from our contributors is the value of using simple models to reveal fundamental principles (Bank, Barton, Servedio, Smadja; Supporting information Appendix 1.1, 1.2, 1.14, 1.15, but see different reactions from Maan and Merrill; Supporting information Appendix 1.8, 1.10). In Felsenstein's words (p. 127), "*We are interested in this model, not as a particularly realistic model of speciation but as a means of investigating the direction in which various evolutionary forces are operating*." Some found this simplicity valuable in teaching about speciation (Giraud, Servedio, Smadja; Supporting information Appendix 1.5, 1.14, 1.15), while others find that the distance from reality can be problematic (Butlin, Maan; Supporting information Appendix 1.3, 1.8).

Below, we further explore some of these insights, and their ongoing impact on speciation research.

Speciation as the Build-up of Linkage Disequilibrium

Felsenstein's primary goal was to clarify the nature of the genetic forces involved in speciation and their relative strengths. To help his readers to understand and intuit the results of his model, he took special care to begin by explaining the general requirement for speciation: the build-up of LD (gametic phase disequilibrium) between alleles underlying reproductive isolation. The first level of LD he mentioned is between the B and C loci under natural selection, which is interpreted as partial postzygotic isolation between the two entities (the BC and bc types being more frequent than the maladapted Bc and bC types). Given this incomplete postzygotic isolation, another level of LD, this time between these loci under selection and the A locus, is necessary for assortative mating to evolve and for further progress in the direction of speciation to be made.

> *"In this model, natural selection produces linkage disequilibrium between B and C. With this disequilibrium established, natural selection will act so as to increase the magnitude of any disequilibrium between A and the B-C complex"* (p.128-129).

He emphasized that "*it is only when there is initial linkage disequilibrium that we see interaction between the assortative mating and the natural selection*," and then interpreted the further build-up of this association *"as progress in the direction of speciation"* (p. 126). Felsenstein also clearly described why the build-up of LD between ecological and assortment loci in his model is adaptive: since this association *"reduces the rate of production of the maladapted* [...] *types"* (Bc or bC types that have a lower average fitness than non-recombinant individuals BC or bc), it *"increases mean fitness"* (p.126).

As most contributors highlighted, Felsenstein was able through these clear descriptions to convey many important ideas. Maybe the most important is that progress towards speciation requires, and can be measured by, the build-up of LD between alleles underlying reproductive isolation. Barton (Supporting information Appendix 1.2) refers to "coupling" to describe this build-up of LD, a term that he introduced in 1983 in the context of hybrid zones, and that is now commonly used to describe the build-up of these associations among barrier effects (Flaxman, Merrill, Smadja; Supporting information Appendix 1.4, 1.10, 1.15, see also Butlin and Smadja 2018). For many contributors, this emphasis on LD helped them as early-career scientists to appreciate how linkage disequilibria can result from selection (Noor; Supporting information Appendix 1.11) and the general role of LD in speciation (Barton, Flaxman, Giraud, Noor, Smadja; Supporting information Appendix 1.2, 1.4, 1.5, 1.11, 1.15). The realization of the importance of LD motivated its estimation in hybrid zones (Gompert et al. 2017) and as Larson (Supporting information Appendix 1.7) says, "in most of the hybrid zones we study (those that persist) there is sufficient LD among genes contributing to reproductive barriers that selection prevails, and species boundaries are maintained." More recently, theoretical developments provided insights into the expected dynamics of genomic differentiation and LD in the course of the speciation process (e.g., Flaxman et al. 2013; Nosil et al. 2017; Schilling et al. 2018) and these predictions have been tested in empirical systems showing a continuum of divergence (Riesch et al. 2017; Schilling et al. 2018). However, great challenges remain to explore these patterns in a breadth of additional systems and to gain insight into the potential generality of coupling as a measure of speciation in action.

The main focus of Felsenstein's article on the build-up of LD between loci under selection and the locus causing assortative mating also helped to clarify for many the mechanisms behind the process of reinforcement (Butlin, Noor, Ortiz-Barrientos, Qvarnström, Servedio, Smadja; Supporting information Appendix 1.3, 1.11, 1.12, 1.13, 1.14, 1.15). Felsenstein's model was not the only theoretical treatment of reinforcement available at the time (Butlin 1987) but this "simple" three-locus model illuminated its key ingredients: as Butlin (Supporting information Appendix 1.3) says, "it put a finger on the nub of the problem: reinforcement depends on indirect selection on mate choice and so on association between mating and ecological traits or genes" and it clearly explained why the evolution of assortative mating in this context is adaptive (see discussions on adaptive coupling in Barton and de Cara 2009; Butlin and Smadja 2018). Felsenstein also framed this point within the context of cline theory:

> *"When $m < 0.5$, BC is the most frequent genotype in subpopulation I, and bc the most frequent genotype in subpopulation II. If there is a linkage disequilibrium of these loci with A, this is reflected in a higher frequency of one allele (say A) in subpopulation I, and a higher frequency of the other in subpopulation II. In effect, there are clines in all three loci. Slatkin (1975) has noted that when two loci have clines in the same*

> *region, there will be linkage disequilibrium between the two loci, and it will tend to steepen both clines and increase the adaptation of individuals to their environment. The cline in the A locus is favoured in the present context because it reinforces this disequilibrium"*. (p.128)

As mentioned by Flaxman, Maan, and Smadja (Supporting information Appendix 1.4, 1.8, 1.15), the model also illustrated how indirect selection on assortative mating arises from LD between ecological and assortment loci. Giraud (Supporting information Appendix 1.6) emphasizes that "when mating occurs at random regarding the ecological niche where selection occurred, it is very difficult to build LD between genes of adaptation, and also with genes of assortative mating," echoing Felsenstein's comment on the need for initial LD for reinforcement to take place. Many of the contributors—as well as others in the field (reviewed in Servedio and Noor 2003)—found motivation in this classic article to pursue research on reinforcement, both on theoretical (Bank, Barton, Butlin, Noor, Servedio; Supporting information Appendix 1.1, 1.2, 1.3, 1.11, 1.14) and empirical (Butlin, Hopkins, Noor, Ortiz-Barrientos, Qvarnström, Smadja; Supporting information Appendix 1.3, 1.6, 1.11, 1.12, 1.13, 1.15) fronts.

Barton (Supporting information Appendix 1.2) notes that, "though Felsenstein described his model as a coupling between two loci under divergent selection, and a third that causes assortative mating, the essential process is much more general." In 2009, Barton and de Cara generalized Felsenstein's model to follow associations among any number of incompatibilities, which may include both assortment and hybrid inviability, and showed that this process, of coupling between incompatibilities, can go beyond the classical view of reinforcement: strong isolation can evolve through the coupling of any kind of incompatibility, whether prezygotic or postzygotic. This led Butlin and Smadja (2018) to propose an extended view of reinforcement. Moreover, the more complex the phenotypic architecture of reproductive isolation is, the more levels of trait associations are required, which led many authors to consider how the nature of prezygotic barriers (signal-preference, matching rule, one-allele) and the modes of selection on these traits influence the build-up of LD and therefore the likelihood of speciation (e.g., Servedio 2009; Maan and Seehausen 2011; Servedio et al. 2011; Smadja and Butlin 2011; Kopp et al. 2018).

The emphasis that Felsenstein placed on LD as the key to speciation is valuable in itself. However, crucially, it also enabled him to place the mechanisms that favor or prevent the build-up of LD at the core of his article: "*the association of isolating mechanisms increases mean fitness. This in itself does not guarantee that the association will be brought about, and we are particularly interested in cases in which it will not"* (p.126).

The Negative Role of Recombination

The requirement for LD emphasized in Felsenstein's model helped to identify the main evolutionary forces at play when gene flow occurs between the diverging entities:

> *"An understanding of the role of recombination between A and the other loci is* […] *important. It tends to break down the association between the prezygotic and postzygotic isolating mechanisms, so that it is always eroding the degree of progress toward speciation. In this light, it should be clear why restricting recombination between A and B makes speciation easier. There is a continual conflict between selection, which increases the association between isolating mechanisms, and recombination, which reduces it. Restricting this recombination can only improve chances for speciation. We have now identified the evolutionary force responsible for favouring speciation–natural selection–and the force opposing it – recombination."* (p.129)

The realisation of the negative role of recombination in speciation is certainly one of the major contributions of this article and is highlighted by most contributors as such. Flaxman points out that "perhaps foremost, Felsenstein's (1981) work illuminated the fundamental and complex roles of recombination in modulating stable polymorphisms, LD, the coupling of multi-locus clines, and the coupling of pre- and postzygotic barriers" and Noor says that "describing the impact of Felsenstein (1981) on studies of speciation and on my career, I would highlight the word 'recombination*'*." More generally, many authors mention how Felsenstein's article elegantly highlighted the tension between selection favoring assortative mating and recombination eliminating the LD necessary for it to evolve (Butlin, Hopkins, Larson, Merrill, Noor; Supporting information Appendix 1.3, 1.6, 1.7, 1.10, 1.11). Butlin (Supporting information Appendix 1.3) adds that "this message was present in the other models, but buried relatively deeply." This key message became even more important as genomic data revealed the importance of gene flow in the history of divergence of many species (Butlin, Hopkins, Meier, Qvarnström; Supporting information Appendix 1.3, 1.6, 1.9, 1.13).

Felsenstein emphasized the importance of identifying the mechanisms restricting recombination or alleviating the tension between selection and recombination to understand how reproductive isolation can evolve in the presence of gene flow, which has been a continuing goal in speciation research. Interestingly, he offered testable predictions on the possible mechanisms that could restrict this deleterious effect of recombination. The one-allele mechanism he explored in his article is one of the solutions to the problem of recombination (see next section). But he also made predictions in the more general case of two-allele models, noting that his results may have some "*implications for linkage relations between genes affecting the isolating mechanism and the genes which affect adaptation to the different* *environments*," and predicting that "*we may find that there has been some tendency for two-allele reproductive isolating mechanisms to have arisen at loci linked to loci which affect the substantive adaptations*" (p.135). Although coupling among barrier loci does not require physical linkage, genomic architectures that eliminate or decrease recombination (from regions of reduced recombination, close physical linkage or pleiotropy) are expected to facilitate coupling and, hence, speciation (Ortiz-Barrientos et al. 2016; Butlin and Smadja 2018). This prediction directly influenced research on the genetics of speciation over the past decades.

Many researchers became interested in the role of recombination modifiers and genomic regions of reduced recombination on the fate of speciation. Trickett and Butlin (1994) added an inversion (known for its recombination suppression effects) to Felsenstein's initial model and found that the inversion could spread and increase the final level of reproductive isolation. Ortiz-Barrientos and Noor did not find support for a colocation between genes underlying enhanced mating discrimination between two *Drosophila* species and known inversions (Ortiz-Barrientos et al. 2004), but this association has been found in various systems and the interest in the role of inversions, and more generally chromosomal rearrangements, in speciation has grown since 1981 both at the theoretical and empirical levels (e.g., Rieseberg 2001; Kirkpatrick and Barton 2006; Feder et al. 2014; Faria et al. 2019). In some systems, other genomic regions sheltered from recombination turned out to be associated with barrier loci: as Qvarnström (Supporting information Appendix 1.13) recalls, genes underlying species-specific traits and a preference for them in *Ficedula* flycatchers were found on a sex chromosome (Saether et al. 2007). Beyond these regions of reduced recombination, tight physical linkage among barrier loci is also predicted by Felsenstein's and more recent models (Yeaman and Whitlock 2011; Yeaman 2013). Merrill (Supporting information Appendix 1.10) points out how Felsenstein's prediction influenced his research on *Heliconius* butterflies: "as predicted by Felsenstein [we revealed] an isolating mechanism (divergent mating preferences) linked to loci which affect the substantive adaptations (warning pattern) (Merrill et al. 2019)." Meier (Supporting information Appendix 1.9) also mentions Felsenstein's article as influential for her research on cichlids and *Heliconius* butterflies, where tight linkage was found between coadapted alleles (Marques et al. 2019; Meier et al. 2020), although she acknowledges that "testing this prediction is challenging because tightly linked loci appear as a single divergent region and often contain genes of unknown functions." Merrill also notes that the polygenic nature of some barrier traits could make interpretations about the role of tight linkage more difficult.

An alternative route to speciation that avoids opposition by recombination is mentioned by Giraud, Maan, Merrill, and Smadja (Supporting information Appendix 1.4, 1.8, 1.10, 1.15) but was not discussed by Felsenstein. This is the case where one of the loci under divergent selection (B or C) also contributes to assortment. It can happen in two ways (Smadja and Butlin 2011): either alleles at the selected locus can have pleiotropic effects on both a selected trait and an assortment trait, or a single trait can have effects on both local adaptation and assortment. Both options have been widely discussed, although they are not always separated. They relate to the nature of the assortment process (Kopp et al. 2018) and there are important distinctions to be made depending on the roles of the "magic" or "multiple effect" traits in that process (Servedio et al. 2011; Smadja and Butlin 2011). For example, a single trait under divergent selection and contributing to a matching rule form of assortment might increase the probability of speciation more than a trait contributing only as a signal. The case that Giraud (Supporting information Appendix 1.5) describes is an important one, emphasizing that assortment is about much more than mating behavior.

As noted by many contributors, progress has been made in the characterization of the genetic basis and architecture of reproductive isolation and the flood of genomic data provides unprecedented opportunities, but great challenges remain in particular to reconcile Felsenstein's framework with patterns emerging from population genomics. Characterizing the genetic basis of reproductive isolation remains challenging in particular for multiple and complex traits (Merrill; Supporting information Appendix 1.10), and the recent empirical focus on detecting barrier loci from genomic differentiation risks missing a major contribution from one-allele processes (Bank, Butlin, Meier; Supporting information Appendix 1.1, 1.3, 1.9). Regions of low recombination, although of interest for speciation, can bias estimates of genomic differentiation and selection (Nachman and Payseur 2012; Ravinet et al. 2017; Wolf and Ellegren 2017; Lotterhos 2019), and the realization of the ubiquitous variation of recombination landscapes among individuals, populations, and taxa (Stapley et al. 2017) makes it even more challenging to associate recombination patterns and barrier loci (Ortiz-Barrientos et al. 2016). Noor and Ortiz Barrientos (Supporting information Appendix 1.11, 1.12) reflect this wider interest in recombination by saying that one of Felsenstein's impacts has been to place the evolution of recombination rates itself as a central part of their research. In Felsenstein's haploid model, recombination between the B and C loci is needed to generate the unfit Bc and bC types and so the selection pressure for increased assortment. Low recombination between these loci can impede speciation. However, Ortiz-Barrientos (Supporting information Appendix 1.12) notes that modifiers that suppress recombination will be favored by selection if linked to these loci, a process that is very like reinforcement. On the other hand, increased recombination is favored during adaptation to new environments, and so perhaps in early stages of speciation (Ortiz-Barrientos et al. 2016). Clearly, there are many complexities in the role of recombination that still need to be disentangled.

Qvarnström (Supporting information Appendix 1.13) nicely summarizes some of these challenges by saying that "40 years after the publication of Felsenstein's 1981 article, the genomic basis—at the resolution of allelic variation—underlying the identified species-specific adaptations and isolating phenotypes in the flycatchers still remains unknown. Thus, revealing how the evolution of these traits has interacted with variation in recombination rates across the genome remains a major future challenge." This is probably true for most biological systems.

One- and Two-Allele Models

While Felsenstein's (1981) observations about the negative role of recombination in speciation are often cited in isolation, he presented this argument as integrally linked to the distinction between one- and two-allele models. This is especially clear in the Summary section of his article, where he also presents a concise explanation of these different mechanisms:

> "*This model suggests that there is an important distinction between two kinds of speciation. One involves speciation by substitution of the same alleles in the two nascent species [one-allele models], the other by substitution of different alleles [two-allele models]. Only in the latter case does recombination act as a force retarding or blocking speciation*." (p.136)

In Figure 1, we include a (nonexhaustive) list of potential examples of one- and two-allele mechanisms. They can vary widely in their specific characteristics.

Virtually all of our commentators reflected on the influential nature of Felsenstein's insight in categorizing these types of mechanisms, and practically all mathematical models of speciation can be fitted into one of these categories. Several authors also noted, however, that the distinction between one- and two-allele models is complex, and is often misunderstood in the literature. Bank (Supporting information Appendix 1.1) comments, for example, that it is easy to "mix up [the one- vs. two-allele distinction] with the distinction of matching rules and preference/trait mechanisms of assortative mating (Kopp et al. 2018)." We have also seen the one-/two-allele distinction confused in the literature with the concept of "magic traits." Flaxman (Supporting information Appendix 1.4) notes that the distinction is "generally underappreciated, in part because it is ignored or not widely understood." Butlin (Supporting information Appendix 1.3) notes that the focus of the terminology on "alleles" rather than on divergent versus unidirectional selection may be one source of confusion underlying this concept.

Part of the complexity underlying the distinction between one- and two-allele models stems from the fact, pointed out by Bank, Noor, and Servedio (Supporting information Appendix 1.1, 1.11, 1.14), that all models must have an underlying two-allele component in order for divergence to be possible. Servedio (Supporting information Appendix 1.14) additionally points out that although models that assume initial divergence of a two-allele component and examine the spread of a one-allele component will find that speciation occurs easily, and models that do the opposite will find it to be harder, both cases will look identical to an observer examining the situation post-hoc. It may, therefore, be hard for a researcher to know which component ultimately triggered isolation. Felsenstein himself commented that "*there is nothing to prevent both kinds of processes from going on at the same time*," and indeed many models do contain both components evolving simultaneously. Bank et al. (2012) additionally developed a model that included a "1.5 allele-mechanism" (where an allele involved in premating isolation can be neutral in one of the populations), illustrating that the one-allele two-allele distinction cannot be universally applied, even within simple models. The picture becomes more complex in natural systems. Merrill (Supporting information Appendix 1.10), for example, points out that between *Heliconius cydno* and *Heliconius melpomene* there are at least three loci that contribute to divergent mating preferences and a "highly polygenic species boundary (Martin et al. 2019)" that complicates questions of what factors are causing or preventing divergence. While the one-allele versus two-allele distinction does apply to polygenic traits (despite the terminology of "alleles"), this example illustrates that many natural cases are far from being as straightforward as an initial understanding of these model-types implies.

What, then is the evidence for one- and two-allele models in natural systems? In a section titled "*Predictions*," Felsenstein suggested that cases fitting both one- and two-allele models should be seen in allopatry, but that two-allele models should be less common in sympatry. However, he also stated, "*I find it easier to imagine genetic variation of the two-allele sort than of the one-allele sort*" (p.135). This is still a matter for speculation, as even 40 years later there is not enough information on these types of mechanisms to rigorously evaluate how commonly they occur, let alone how often they are implicated in the speciation process. Some, at least, of our commentators disagree with Felsenstein, suggesting that one-allele mechanisms may be more common than he anticipated, particularly when isolation arises through modified migration, adaptive habitat selection, or behavioral imprinting (Flaxman Maan, Noor, Qvarnström, Smadja; Supporting information Appendix 1.4, 1.8, 1.11, 1.13, 1.15). As pointed out by several contributors, such as Bank, Butlin, and Meier (Supporting information Appendix 1.1, 1,3, 1.9), one-allele components may be particularly difficult to identify using genetic techniques because they do not differ between species. Ortiz-Barrientos et al. (2004), however, were able to use high-resolution genetic mapping to identify a one-allele mechanism that strengthened isolation between *Drosophila pseudoobscura* and *Drosophila persimilis*. Behavioral one-allele mechanisms may not only be common, as suggested by our contributors above, but may also be more feasible to identify than other types of one-allele mechanism, at least in principle. Mechanisms, such as sexual imprinting, leading to phenotype matching have been shown to play a role in maintaining species or population differentiation in, for example, cichlids (Verzijden and ten Cate 2007) and poison frogs (Yang et al. 2019), and sexual imprinting is common in general in birds (ten Cate and Vos 1999; Campbell and Hauber 2009). Interestingly, Maan (Supporting information Appendix 1.8) notes that in many cases these mechanisms will predate divergent selection, thus, placing them in the category that Servedio (Supporting information Appendix 1.14) pointed out will be more difficult for speciation; an ancestral one-allele mechanism (in this case, e.g., imprinting) with a novel two-allele mechanism (e.g., a trait that must diverge, but not sweep, despite gene flow).

Extrinsic Versus Intrinsic Constraints on Speciation

Felsenstein summarized his core conclusion by saying: "*We have now identified the evolutionary force responsible for favoring speciation— natural selection—and the force opposing it—recombination*" (p.129). By setting the debate in the context of Hutchinson's (1959) essay on species diversity, Felsenstein placed the focus clearly on ecology as the source of the divergent selection that drives speciation. In the absence of genetic constraints, he envisaged something akin to Hubbell's (2001) neutral model: "*an 'island biogeography' model of speciation*" where there would be "*a balance between speciation and the extinction of small species*" and he imagined "*that the number of species in nature is far smaller, and their size far larger, than such a model would predict*" (p.124). This argument depends on the fact that genetic constraints are only relevant with gene flow. If there are genetic constraints, such that speciation with gene flow is less likely than allopatric speciation, then diversity is expected to depend on the opportunity for allopatric divergence as well as the strength of the genetic constraint. Thus, the issues of ecological versus genetic determinants of species diversity and of the likelihood of speciation with gene flow are intertwined.

In his commentary, Barton (Supporting information Appendix 1.2) reflects this argument (quoting Felsenstein, p.124): "There are indefinitely many ecological niches, and only mild restriction to gene flow is needed to allow adaptation to these niches, and so '*one might come away* […] *with the disturbing impression that* [speciation] *is all but inevitable*.'" However, there is a critical difference here because Barton is suggesting that divergent selection can drive speciation in the presence of substantial gene flow, in contrast to the emphasis on strict allopatry that was prevalent in 1981. Arguably, Felsenstein (1981) started an important move in this direction. At a time when much of speciation thinking was caught up in a polarized allopatry-sympatry debate (e.g., Bush 1994), he considered a continuum of migration from $m = 0$ to $m = 0.5$ and emphasized that his critical one-/two-allele distinction cut across this migration continuum. Qvarnström (Supporting information Appendix 1.13) sees Felsenstein's article as a "tipping point" towards thinking about this continuum and away from "heated debate" about distinct alternatives. This debate was not always conducted at an objective, scientific level: Maan (Supporting information Appendix 1.8) notes that "proponents of certain mechanisms [were characterized as] naïve believers, while opponents [were] down-to-earth realists." Hopefully, we can do better now. The trend that Felsenstein helped to start has led to the view that completely allopatric speciation is rare because the evolution of reproductive isolation is typically played out over a long time in a changeable world (Abbott et al. 2013). Within this framework, it is more constructive to focus on mechanisms than on disputes about modes of speciation. The spatial and temporal extent of gene flow certainly matters for speciation but there is no sharp allopatry-sympatry divide.

That said, the allopatry-sympatry debate was slow to subside and remnants of it remain. It can be easier to categories than to think about the gene flow continuum. Giraud (Supporting information Appendix 1.5) describes Felsenstein's model as a "simple model of sympatric speciation," but she also makes the interesting point that fungi inhabiting different hosts are still in sympatry because only their genotypes control their separation, and so their probability of interbreeding (reflecting ideas about phytophagous insects; Bush 1994). Hopkins (Supporting information Appendix 1.6) suggests that Felsenstein's article was at the start of "a wave of research arguing gene flow also rarely, if ever, occurs during the evolution of reproductive isolation." This implies that his argument for genetic constraints on speciation was, at least initially, interpreted as support for the allopatric speciation orthodoxy (he was also cited in this spirit in Bush 1994) whereas we tend to see the opposite, particularly because the one-allele mechanism partially frees the evolution of reproductive isolation from the recombination constraint. Larson's (Supporting information Appendix 1.7) summary that, "sympatric speciation will be rare unless one-allele genetic variation is common," is closer to Felsenstein's conclusion, although it would be better to say "speciation with gene flow" than "sympatric speciation" and Barton's point (Supporting information Appendix 1.2) is that the constraint is only strong when there is little obstacle to gene flow.

Meier (Supporting information Appendix 1.9) argues that Felsenstein's article, "highlights how genetics and ecology need to be combined to understand speciation and patterns of species richness" and this closely reflects Felsenstein's own conclusion:

> *"Only when we can bring genetic and ecological constraints on speciation into a common framework will we begin to have a satisfactory overview of the speciation process. Only then will geneticists be able to join ecologists in paying homage to Santa Rosalia."* (p.136)

Are we any closer now to this common framework? Meier's own work on cichlid species flocks in African rift valley lakes supports the idea that both ecological opportunity and relaxation of genetic constraints are important for rapid diversification (e.g., Meier et al. 2017; 2019). However, the major genetic constraint discussed in this case is the availability of suitable variation, which is provided by hybridization. This is critical for the rapid accumulation of diversity, because mutation is too slow, but maybe not for the evolution of reproductive isolation in individual speciation events. Indeed, reproductive isolation is not completed in many cichlid taxa before further diversification occurs. More closely related to Felsenstein's recombination constraint is the role of *opsin* gene variation, underpinning traits that are involved in both adaptation to different niches and mate choice (Seehausen et al. 2008).

These issues in cichlids reflect a more general problem in the connection between the speciation process and patterns of diversity. Rabosky and Matute (2013) found a surprising lack of correlation across taxa between the rate at which reproductive isolation evolves and the rate of speciation estimated by phylogenetic methods. This decoupling suggests that divergent populations may often collapse back into single units if reproductive isolation is not complete or newly evolved species may frequently go extinct before they contribute to diversity on a macroecological scale. In the latter case, the genetic constraints envisaged by Felsenstein may be critical for the speciation process but they may be much less relevant to explaining patterns of species diversity.

Interestingly, Germain et al. (2021) take Hutchinson's "Homage to Santa Rosalia" article as their starting point in considering the "Origin of Coexisting Species" but they do not cite Felsenstein (1981). They argue that some modes of speciation are more likely than others to generate species that can coexist and so persist. Sadly, there is little of the integration of genetic with ecological constraints that Felsenstein called for. Perhaps, the decoupling observed by Rabosky and Matute (2013) means that this integration is not needed but coexistence requires strong reproductive isolation as well as low niche overlap (as Germain et al. acknowledge, and see Irwin and Schluter 2021) and this surely means that genetic constraints on the evolution of isolation remain significant. Qvarnström (Supporting information Appendix 1.13) makes the related point that "speciation events may in general be more likely when they can endure periods of gene flow." We would prefer to see more efforts to bring ecological and genetic approaches to speciation together, before reaching the conclusion that these two factors can be treated separately. In thinking about these processes, a lack of speciation should be viewed as just as interesting an outcome as "successful" speciation, avoiding the implicit bias that Maan (Supporting information Appendix 1.8) sees creeping into the language we use.

Value of Simple and Well-Presented Models

What is it about this article that caused it to have such an impact on so many researchers? Its insights are profound, but, as many of our contributors made clear, its value is also in its essence as a simple and well-presented model. As Noor (Supporting information Appendix 1.11) points out, prior models on speciation and local adaptation had presented many of the individual components of the arguments that Felsenstein developed. Yet he follows that "Felsenstein (1981) presented a simple, elegant, clear synthesis with added insights." Simplicity and clarity are its hallmarks. Felsenstein commented on his goal to distil the speciation process when he said, "*…it is the simplest model I can find which exhibits many of the genetic effects which will be found in more complex, more realistic models of speciation*" (p.125). Barton (Supporting information Appendix 1.2) comments that in addition to "appreciat[ing] its fresh perspective," "I also appreciated its style—using a model as simple as possible, and using clear logic to make a fundamental point." Meier (Supporting information Appendix 1.9) found this approach gave her, as a student, a general appreciation of theoretical work, stating that "I found it fascinating how elegantly simple models lead to clear predictions."

The power of this reductionist modelling framework has been influential across academic generations (as mentioned by Noor; Supporting information Appendix 1.11). Giraud (Supporting information Appendix 1.5) summarizes the effect of Felsenstein's article on her as follows: "… it was because of Felsenstein's article (Felsenstein 1981), and also other models on speciation (Maynard Smith 1966; Rice 1984), that I really felt the power and interest of theoretical modelling. I have therefore ever since used Felsenstein's simple model (Felsenstein 1981) in teaching each year to make the issue of speciation understandable to my students, but also to illustrate why we need theoretical models in evolutionary biology."

Several contributors, like Giraud (Supporting information Appendix 1.5), have used Felsenstein's model as a teaching tool, generally (but not always), finding value in its relative simplicity. Smadja (Supporting information Appendix 1.15) covers it regularly in a class to Masters students and finds that "every year, I am amazed by the students' reactions to this elegantly simple but influential model and how relevant it is as a pedagogical tool." Butlin (Supporting information Appendix 1.3) finds that it is difficult for undergraduate students, although Servedio (Supporting information Appendix 1.14) has had success with it at that level, albeit in a modelling class where students are already steeped in abstraction.

Regardless of whether they use it themselves in the classroom, one of the most consistent messages from our contributors was that they were introduced to Felsenstein as a graduate student (or in one case, Larson, as an undergraduate; Supporting information Appendix 1.7), and that it contributed to their intellectual path into the genetics of speciation. Both for empiricists and for theoreticians it has provided lasting clarity and understanding about interactions between the evolutionary forces involved in speciation with gene flow, as well as inspiration for both empirical and theoretical speciation studies. In this way, its influence on the current generation of speciation biologists cannot be overstated.

Conclusions

Felsenstein's (1981) article has profoundly influenced the work of both theoreticians and empiricists studying speciation over the last 40 years. The reasons for its impact lie in the use of a simple model to clarify fundamentally important processes and distinctions. Flaxman (Supporting information Appendix 1.4) puts it nicely: "Speciation genetics may have no Golden Rule of its own, but Felsenstein (1981) distilled his own and previous works into pithy insights that are about as close as we can get to hard and fast rules about SWGF [speciation with gene flow]."

How will future generations use this classic work? We hope that they will take Barton's (Supporting information Appendix 1.2) advice and "occasionally look up from the flood of simulation and sequence data that engulfs us, to remember the big questions that Felsenstein addressed in his classic paper." It seems that Meier (Supporting information Appendix 1.9) recognizes this need because she says, "Big data has led to a shift from hypothesis-driven to more data-driven science. Despite having started at the beginning of this shift, I hope articles like Felsenstein (1981) will soon become more central again in speciation research. Combining theory and big data will likely advance our understanding the fastest." We also hope that ecological and genetic approaches to speciation and diversity can also be brought together in the way that Felsenstein envisaged, leading towards a complete answer to the question, "Why are there so few/many species?"

ACKNOWLEDGMENTS

RKB was funded by the Natural Environment Research Council (NE/P012272/1 & NE/P001610/1), the European Research Council (693030 BARRIERS), and the Swedish Research Council (VR) (2018-03695). MRS was funded by the National Science Foundation (Grant No. DEB1939290).

This is contribution number ISEM 2021–071 of the Institut des Sciences de l'Evolution de Montpellier.

LITERATURE CITED

Abbott, R., D. Albach, S. Ansell, J. W. Arntzen, S. J. E. Baird, N. Bierne, J. Boughman, A. Brelsford, C. A. Buerkle, R. Buggs, et al. 2013. Hybridization and speciation. J. Evol. Biol. 26:229–246.

Bank, C., R. Burger, and J. Hermisson. 2012. The limits to parapatric speciation: Dobzhansky-Muller incompatibilities in a continent-island model. Genetics 191:845–863.

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

Bush, G. L. 1994. Sympatric speciation in animals: new wine in old bottles. Trends Ecol. Evol. 9:285–288.

Butlin, R. K. 1987. Species, speciation, and reinforcement. Am. Nat. 130:461–464.

Butlin, R. K., and C. M. Smadja. 2018. Coupling, reinforcement, and speciation. Am. Nat. 191:155–172.

Caisse, M., and J. Antonovics. 1978. Evolution in closely adjacent plant populations. IX. Evolution of reproductive isolation in clinal populations. Heredity 40:371–384.

Campbell, D. L. M., and M. E. Hauber. 2009. Spatial and behavioural measures of social discrimination by captive male zebra finches: implications of sexual and species differences for recognition research. Behav. Processes 80:90–98.

Faria, R., K. Johannesson, R. K. Butlin, and A. M. Westram. 2019. Evolving inversions. Trends Ecol. Evol. 34:239–248.

Feder, J. L., P. Nosil, and S. M. Flaxman. 2014. Assessing when chromosomal rearrangements affect the dynamics of speciation: implications from computer simulations. Front. Genet. 5:295.

Felsenstein, J. 1981. Skepticism towards Santa Rosalia, or why are there so few kinds of animals? Evolution 35:124–138.

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.

Gavrilets, S. 2004. Fitness landscapes and the origin of species. Princeton Univ. Press, Princeton, NJ.

Germain, R. M., S. P. Hart, M. M. Turcotte, S. P. Otto, J. Sakarchi, J. Rolland, T. Usui, A. L. Angert, D. Schluter, R. D. Bassar, et al. 2021. On the origin of coexisting species. Trends Ecol. Evol. 36:284–293.

Gompert, Z., E. G. Mandeville, and C. A. Buerkle. 2017. Analysis of population genomic data from hybrid zones. Ann. Rev. Ecol. Evol. Systematic. 48: 207—229.

Hubbell, S. P. 2001. The unified neutral theory of biodiversity and biogeography. Princeton Univ. Press, Princeton, NJ.

Hutchinson, G. E. 1959. Homage to *Santa Rosalia* or why are there so many kinds of animals. Am. Nat. 93:145–159.

Irwin, D., and D. Schluter. 2021. Hybridization and the coexistence of species. bioRxiv. http://doi.org.10.2021.04.04.438369.

Kirkpatrick, M., and N. Barton. 2006. Chromosome inversions, local adaptation and speciation. Genetics 173:419–434.

Kopp, M., M. R. Servedio, T. C. Mendelson, R. J. Safran, R. L. Rodríguez, M. E. Hauber, E. C. Scordato, L. B. Symes, C. N. Balakrishnan, D. M. Zonana, et al. 2018. Mechanisms of assortative mating in speciation with gene flow: connecting theory and empirical research. Am. Nat. 191:1–20.

Lotterhos, K. E. 2019. The effect of neutral recombination variation on genome scans for selection G3-Genes Genomes Genet. 9:4245.

Maan, M. E., and O. Seehausen. 2011. Ecology, sexual selection and speciation. Ecol. Lett. 14:591–602.

Marques, D. A., J. I. Meier, and O. Seehausen. 2019. A combinatorial view on speciation and adaptive radiation. Trends Ecol. Evol. 34:531–544.

Martin, S. H., J. W. Davey, C. Salazar, and C. D. Jiggins. 2019. Recombination rate variation shapes barriers to introgression across butterfly genomes. PLoS. Biol. 17:e2006288.

Maynard Smith, J. 1966. Sympatric speciation. Am. Nat. 100:637–650.

Meier, J. I., R. B. Stelkens, D. A. Joyce, S. Mwaiko, N. Phiri, U. K. Schliewen, O. M. Selz, C. E. Wagner, C. Katongo, and O. Seehausen. 2019. The coincidence of ecological opportunity with hybridization explains rapid adaptive radiation in Lake Mweru cichlid fishes. Nat. Commun. 10:5391.

Meier, J. I., D. A. Marques, S. Mwaiko, C. E. Wagner, L. Excoffier, and O. Seehausen. 2017. Ancient hybridization fuels rapid cichlid fish adaptive radiations. Nat. Commun. 8:14363.

Meier, J. I., P. A. Salazar, M. Kučka, R. W. Davies, A. Dréau, I. Aldás, O. Box Power, N. J. Nadeau, J. R. Bridle, C. P. Rolian, et al. 2020. Haplotype tagging reveals parallel formation of hybrid races in two butterfly species. bioRxiv. http://doi.org.10.2020.2005.2025.113688.

Merrill, R. M., P. Rastas, S. H. Martin, M. C. Melo, S. Barker, J. Davey, W. O. McMillan, and C. D. Jiggins. 2019. Genetic dissection of assortative mating behavior. PLoS. Biol. 17:e2005902.

Nachman, M. W., and B. A. Payseur. 2012. Recombination rate variation and speciation: theoretical predictions and empirical results from rabbits and mice. Philosophicl. Transact. Royal Soc. B-Biol. Sci. 367:409–421.

Nosil, P., J. L. Feder, S. M. Flaxman, and Z. Gompert. 2017. Tipping points in the dynamics of speciation. Nat. Ecol. Evol. 1:0001.

Ortiz-Barrientos, D., B. A. Counterman, and M. A. F. Noor. 2004. The genetics of speciation by reinforcement. PLoS. Biol. 2:2256–2263.

Ortiz-Barrientos, D., J. Engelstaedter, and L. H. Rieseberg. 2016. Recombination rate evolution and the origin of species. Trends Ecol. Evol. 31:226–236.

Rabosky, D. L., and D. R. Matute. 2013. Macroevolutionary speciation rates are decoupled from the evolution of intrinsic reproductive isolation in Drosophila and birds. Proc. Natl. Acad. Sci. U.S.A 110:15354–15359.

Ravinet, M., R. Faria, R. K. Butlin, J. Galindo, N. Bierne, M. Rafajlovic, 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.

Rice, W. R. 1984. Disruptive selection on habitat preference and the evolution of reproductive isolation: a simulation study. Evolution 38:1251–1260.

Rice, W. R., and E. E. Hostert. 1993. Laboratory experiments on speciation: what have we learned in 40 years. Evolution 47:1637–1653.

Riesch, R., M. Muschick, D. Lindtke, R. Villoutreix, A. A. Comeault, T. E. Farkas, K. Lucek, E. Hellen, V. Soria-Carrasco, S. R. Dennis, et al. 2017. Transitions between phases of genomic differentiation during stick-insect speciation. Nat. Ecol. Evol. 1:0082.

Rieseberg, L. H. 2001. Chromosomal rearrangements and speciation. Trends Ecol. Evol. 16:351–358.

Saether, S. A., G. P. Saetre, T. Borge, C. Wiley, N. Svedin, G. Andersson, T. Veen, J. Haavie, M. R. Servedio, S. Bures, et al. 2007. Sex chromosome-linked species recognition and evolution of reproductive isolation in flycatchers. Science 318:95–97.

Schilling, M. P., S. P. Mullen, M. Kronforst, R. J. Safran, P. Nosil, J. L. Feder, Z. Gompert, and S. M. Flaxman. 2018. Transitions from single- to multilocus processes during speciation with gene flow. Genes 9:274.

Seehausen, O., Y. Terai, I. S. Magalhaes, K. L. Carleton, H. D. J. Mrosso, R. Miyagi, I. van der Sluijs, M. V. Schneider, M. E. Maan, H. Tachida, et al. 2008. Speciation through sensory drive in cichlid fish. Nature 455:620–623.

Servedio, M. R. 2009. The role of linkage disequilibrium in the evolution of premating isolation. Heredity 102:51–56.

Servedio, M. R., and M. A. F. Noor. 2003. The role of reinforcement in speciation: theory and data. Ann. Rev. Ecol. Evol. Systematcs 34:339–364.

Servedio, M. R., G. S. Van Doorn, M. Kopp, A. M. Frame, and P. Nosil. 2011. Magic traits in speciation: ‘magic’ but not rare? Trends Ecol. Evol. 26:389–397.

Slatkin, M. 1975. Gene flow and selection in a two-locus system. Genetics 81:787–802.

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.

Stapley, J., P. G. D. Feulner, S. E. Johnston, A. W. Santure, and C. M. Smadja. 2017. Variation in recombination frequency and distribution across eukaryotes: patterns and processes. Philosophicl. Transact. Royl. Soc. B-Biol. Sci. 372:20160455.

ten Cate, C., and D. R. Vos. 1999. Sexual imprinting and evolutionary processes in birds: a reassessment. Pp. 1–31 *in* P. J. B. Slater, J. S. Rosenblat, C. T. Snowden, and T. J. Roper, eds. Advances in the study of behavior, Vol. 28. Academic Press, Waltham, MA.

Trickett, A. J., and R. K. Butlin. 1994. Recombination suppressors and the evolution of new species. Heredity 73:339–345.

Verzijden, M. N., and C. ten Cate. 2007. Early learning influences species assortative mating preferences in Lake Victoria cichlid fish. Biol. Lett. 3:134–136.

Wolf, J. B. W., and H. Ellegren. 2017. Making sense of genomic islands of differentiation in light of speciation. Nat. Rev. Genet. 18:87–100.

Yang, Y. S., M. R. Servedio, and C. L. Richards-Zawacki. 2019. Imprinting sets the stage for speciation. Nature 574:99–102.

Yeaman, S. 2013. Genomic rearrangements and the evolution of clusters of locally adaptive loci. Proc. Natl. Acad. Sci. U.S.A 110:E1743–E1751.

Yeaman, S., and M. C. Whitlock. 2011. The genetic architecture of adaptation under migration-selection balance. Evolution 65:1897–1911.

Associate Editor: T. Chapman
Handling Editor: T. Chapman.

Supporting Information

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

Supplementary material