

Modes and Mechanisms of Speciation

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Keywords

Speciation

Process where an inbreeding population diverges into different reproductively isolated groups.

Genomics

Discipline that applies DNA sequencing methods and bioinformatics to analyze the genetic structure of organisms.

Gene flow

The movement of alleles between different populations.

Reproductive isolation

Barriers of reproduction and gene exchange between different incipient species.

Symbiosis

Two or more dissimilar organisms living in close association.

■ In *The Origin of Species*, Charles Darwin described the formation of new species as the “mystery of mysteries”. More than 150 years after Darwin first posed the problem much progress has been made in discerning what creates the great diversity of life. Speciation is the evolutionary process where a group of inbreeding populations diverges into two or more reproductively isolated groups. In this chapter, we summarize the current understanding of speciation in sexually reproductive organisms. In particular, we describe how barriers to gene flow evolve and focus on the most important factors promoting speciation. Further, we integrate different perspectives by describing recent progress from many different model systems for the study of speciation. Building on this work, we emphasize new genomic approaches to the study of speciation and how advances in DNA sequencing methods will revolutionize our understanding of the genetic basis of the speciation process. We conclude by summarizing our current understanding of speciation and show that although much of Darwin’s mystery is solved, many important questions remain.

1

Introduction

It is estimated that there are about 8.7 million eukaryotic species on Earth [1], with some assessments being as high as 100 million [2]. But what exactly are species, and what evolutionary processes are responsible for generating this great diversity of life? Charles Darwin once described the formation of new species as the “mystery of mysteries” [3]. While speciation may be one of the least understood features of evolution [4], since Darwin’s time much progress has been made in discerning how speciation occurs. In particular, the mystery of how genetic barriers to gene flow can evolve to reproductively isolate formerly interbreeding populations to generate new sexual species is solved in large part, although many important questions still remain.

In this chapter, the current understanding of speciation in sexually reproducing organisms is summarized and some of the more important factors and mechanisms involved in speciation are highlighted. First, an investigation is made into how species are defined, after which an outline is provided of what constitutes the barriers to gene flow and the nature of traits causing reproductive isolation (hereafter abbreviated RI). Next, different modes of speciation (largely geographically defined) are examined and the different mechanisms that can underlie the evolution of RI and the formation of new species are elaborated on. Finally, future avenues of research are discussed, with attention focused on how technical advances in DNA sequencing can accelerate the understanding of the genetic basis for speciation. The reader is also referred to several excellent recent reviews and books on speciation that provide

further insights and addition information about the subject [5–13].

2

What Are Species?

Species are the fundamental units of biodiversity [6]. It therefore seems logical and important to have a working definition of what constitutes a species if the process of speciation is to be studied. Indeed, how could the origins of new species be understood if we had no idea how to define a species or what separates one species from another?

There is still ongoing debate about what a species is and whether Darwin himself defined species. While there are some who insist that, in *The Origin of Species* Darwin failed to define species, he did in fact describe species as groups of individuals that maintain observable discontinuities when and where they co-occur. In other words, species can be defined by gaps that exist in the distribution of the diversity of life. One of Darwin’s great contributions to biology and natural history was seeing that species were not static. Rather, the gaps are dynamic due to species being continually created and changing through time. Ultimately, if the gaps among species were traced further and further back into the past, they would disappear and converge into nested sets of shared common ancestors. Life is therefore a tree of diversity, with species being the various discernible branches (evolutionary lineages) hierarchically connected to one another through time by shared ancestry. Broadly speaking, species could be conceptualized as lineages that have different evolutionary trajectories branching from one another on the tree of life [14].

The existence of RI is now generally considered the critical criterion for recognizing different evolutionary lineages as species, epitomized by the conceptualization of sexual species as groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups [15]. This view was termed the Biological Species Concept (BSC) by Mayr [15, 16], and its roots can be traced back further to other biologists including Theodosius Dobzhansky [17], Eduard B. Poulton [18], Benjamin Walsh [19] and John Ray [20]. Thus, the BSC holds that species will not exchange genes often enough with other species through sexual reproduction to lose their distinction, if and when they were to co-occur. This criterion can be problematic for asexual organisms such as bacteria that, like sexual species, can form distinct units of biodiversity, but unlike sexual organisms, reproduce (divide) by binary fission. Nevertheless, the BSC is useful because it defines the central problem of studying speciation for sexual organisms as an understanding of how heritable barriers to gene flow evolve that separate formally interbreeding populations into reproductively isolated species.

So why is there still a problem defining species? Much of the reason stems from the difficulty in determining at what level of RI (reduced gene flow) populations should be considered varieties or races versus species. Obviously, elephants and mice represent “good species” that do not hybridize where they co-occur and, thus, do not exchange genes through sexual reproduction and remain distinct evolutionary lineages. Hence, there is no ambiguity when RI is complete. However, often taxa that many biologists would recognize as different species are not separated by absolute genetic barriers to gene flow: some

hybridization occurs and some regions of the genome may move (introgress) between populations, while others do not. As speciation is usually not a singular event in time but rather is a continuous process that unfolds over thousands, tens of thousands, hundreds of thousands, or millions of generations, where should the line be drawn along this continuum of population divergence and decreasing gene flow to designate species (Fig. 1)? Clearly, there may not be a demarcation that all agree on. As a result, some have argued that species represent arbitrary and artificial constructs of the human mind stemming from a need to define and organize entities into different categories [22].

Furthermore, what specifically is being referred to by gene flow? Is the metric of gene flow a measure of the overall level of hybridization, or a mean rate of genetic introgression for all genes in the genome between populations – is RI a property of the entire genome? Or, alternatively, is reference being made to those genes showing the lowest or highest degree of exchange between populations – is RI a genic characteristic of individual genes [23, 24]? During speciation, populations may show great heterogeneity in the porousness of different regions of the genome to introgression. It is therefore not always clear how a summary statistic such as the gross migration rate or frequency of hybridization effectively translates into species status. Of course, the same consideration applies to estimates for individual genes. Nevertheless, during speciation the effects of individual traits and genes on RI can become coupled, synergistically reducing gene flow, and elevating divergence more uniformly across the genome [25–31]. Thus, it is possible that marked increases in RI may be observed as lineages diverge [31]. If this were true, then it may be possible

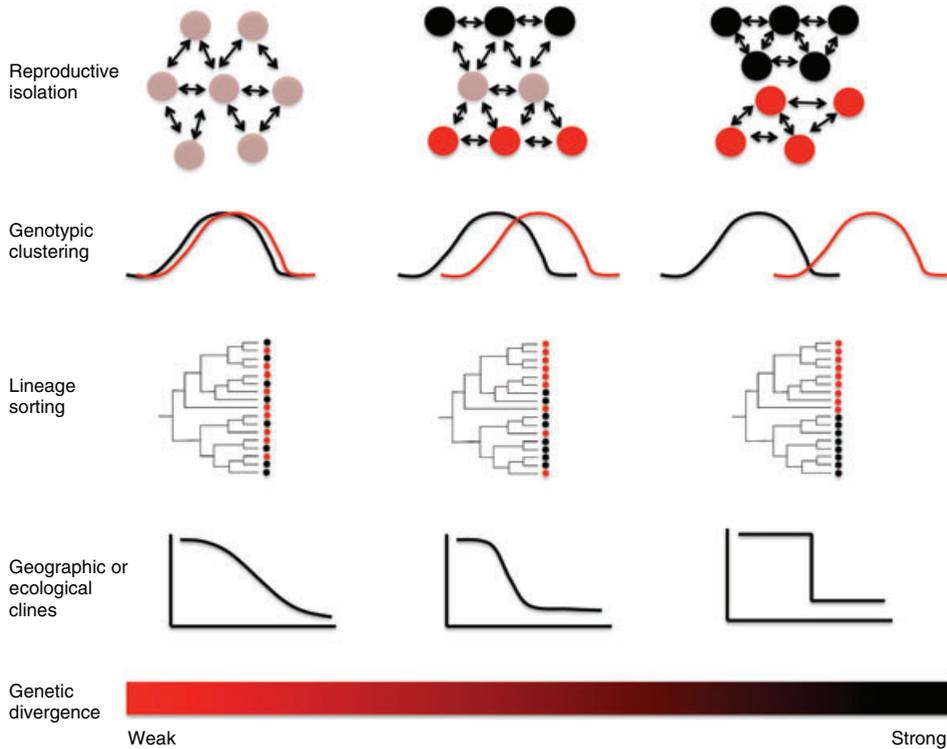


Fig. 1 Overview of different stages of population divergence along the speciation continuum from races or biotypes (on left) to completely isolated taxa (on right). Shown are how idealized populations of individuals are expected to cluster

according to their genotypes and what a genetic cline might look like between the populations if they were to have a parapatric distribution due to primary or secondary contact. Modified from Ref. [21].

to compare metrics of RI between pairs of populations at different stages of divergence along the “continuum of speciation” from freely interbreeding populations, to races, to subspecies, to completely isolated taxa, and associate the transitional phase of accelerate RI to species formation [31]. However, such a scenario requires further testing to determine its generality and may be difficult to apply in a uniform manner to all groups, especially those lacking several populations at varying stages of divergence. The key point is that while conceptually appealing as a framework for understanding speciation, unless essentially absent

or absolute, RI considered alone from a practical standpoint can be difficult to interpret as a metric for delineating species.

In this spirit, a number of ideas have been proposed to operationally define species as distinct genotypic clusters of individuals, as groups of related individuals sharing a common genealogy, and as distinct phylogenetic clades whose members can be distinguished by their possessing a uniquely evolutionarily derived trait(s) (termed autapomorphies) [23, 32–34]. These various definitions can be thought of as representing different stages of divergence for lineages along the speciation continuum on

the tree of life, from varieties to completely reproductively isolated taxa (genotypic clusters < shared genealogy < clades) (see Fig. 1) [35]. Moreover, when applied to sexually reproducing organisms, genotypic clusters, shared genealogy and the presence of autapomorphies are all the result of heritable barriers to gene flow evolving between sexually reproducing populations. Consequently, at their core, all the definitions are conceptually linked to the BSC and, thus, they all share potentially the same difficulty of imposing a biologically arbitrary divide on the continuous process of speciation. For example, when are genotypic clusters distinct enough to be considered species versus representing intraspecific polymorphisms? With gene flow, there will be some individuals of parental and/or mixed ancestry in both populations. So where should the divide be set? Is it sufficient that genotypic clusters just be observed at local "sympatric" (i.e., complete geographic overlap of populations) sites to consider two populations as species, or must all local populations cluster distinctly from one another across the entire range of geographic overlap to designate species status? Certainly geographic variation within taxa (e.g., clines or drift in local populations) could potentially play havoc with the global clustering of populations across the landscape. Similar questions also arise with respect to the genealogical definition, unless gene flow has ceased for a period of time between populations. Moreover, with a heterogeneous pattern of divergence, longstanding polymorphism (genetic variation) and/or a degree of gene flow, alleles within an individual can have differing genealogies, with some variants more closely related to genes in the alternate population. What then is the genealogy of these individuals? Is the decision made by majority rule? Finally, the presence of

an autapomorphy under a phylogenetic or cladistic species concept would suggest no gene flow and complete RI (although it is possible that very strong selection on a specific trait could produce the appearance of near fixation with gene flow), which would put us back in the same quandary that was faced before with the BSC. Only taxa that are completely isolated could be easily identified as species.

The take-home message with respect to the question of defining species is therefore that it is easy to become quickly lost and not see the forest through the trees. All paths eventually lead back, however, to the crucial problem of speciation highlighted by the BSC for sexually reproducing organisms: discerning the nature and origins of heritable barriers to gene flow that evolve to separate populations. As a result, there may not be universal agreement on when two populations (lineages) have diverged to the point of being considered different species (Fig. 1). However, this does not necessarily mean that species are not real and natural entities. As populations diverge, "gaps" will evolve that distinguish taxa as differentiated genotypic clusters, groups of individuals sharing a common genealogy, or phylogenetic clades possessing uniquely derived traits. However, drawing a hard line demarking species is problematic and, in this regard, named species may be considered somewhat arbitrary human constructs of convenience. Nevertheless, it is clear that the central issue of speciation is the same no matter what one's favorite definition of a species: to understand speciation requires identifying the traits and factors causing RI among populations of sexually reproducing organisms and their genetic bases and historical origins. This pursuit is at the heart of the BSC and unites all the various definitions of species. Similar considerations may actually apply

to most asexual organisms (e.g., bacteria can exchange parts of their genomes during conjugation). However, the issues of whether asexual species exist, what constitutes an asexual species, and how they may form are beyond the scope of this review.

3

Classifying Barriers to Gene Flow

After pondering what sexual species are and, in the process, defining the seminal issue driving speciation research, the different ways in which populations can be reproductively isolated from one another can now be discussed. Two pioneers in this area were Poulton [36] and Dobzhansky [37], who recognized the importance of two major divisions of RI constituting prezygotic and postzygotic barriers to gene flow (see Table 1).

3.1

Prezygotic Isolation

Prezygotic isolation refers to RI that occurs prior to zygote formation [8, 38]. It can

occur before mating by restricting individuals from different populations from copulating with each other. It can also occur after mating by impeding the union of gametes (fertilization) to form a zygote. Because prezygotic isolation acts earlier in the life cycle of sexually reproducing organisms than postzygotic barriers, it can have a relatively greater effect on reducing gene flow between populations [7, 39]. For example, consider two factors – one prezygotic and the other postzygotic – that each cause a 50% reduction in gene flow. If two taxa come into contact, the first prezygotic factor reduces the net rate of effective gene flow between the taxa by 50%. However, the second postzygotic factor only reduces the net rate of gene flow by 25% ($= 0.5 \times 0.5$), because the prezygotic factor already acted to restrict genetic exchange between the taxa by half. In addition, prezygotic isolation (in the form of mate choice) can directly affect who mates with whom. Thus, prezygotic isolation can generate assortative mating for traits under divergent selection, increasing the effectiveness of selection in differentiating populations and reducing gene flow beyond the direct effects of prezygotic isolation alone. Finally,

Tab. 1 Outline of heritable barriers to gene flow between population causing RI.

| | | | |
|------------|-------------|----------------------|---|
| Premating | Prezygotic | Geographic isolation | Geographic barriers prevent gene flow |
| | | Behavioral isolation | “Sexual isolation” different mate signals prevent populations from mating |
| | | Ecological isolation | Adaptation to different habitats and/or different activity or breeding time can inhibit gene flow |
| Postmating | Postzygotic | Mechanical isolation | Different genitalia structures impede successful transfer of sperm despite attempted mating (copulation) |
| | | Gametic isolation | Transferred gametes cannot effect fertilization |
| | | Extrinsic | Ecological inviability: hybrids fall between parental ecological niches Behavioral sterility: hybrids are less attractive (and successful) in obtaining mates |
| | | Intrinsic | Hybrid inviability: hybrids suffer developmental problems Hybrid sterility: hybrids suffer reproductive problems and produce fewer number or no functional gametes |

Modified from Ref. [38].

by preventing the formation of zygotes, prezygotic isolation can potentially impact the entire genome. This may or may not be the case for postzygotic isolation when hybrids form and are partly viable and fertile.

There are several different types of prezygotic isolation, as highlighted below.

3.1.1 Temporal isolation

Differences in the time of tissue availability of plants (e.g., flowering time) or in the mating time of animals are ways that individuals in different populations can become seasonally or temporally isolated from one another. Examples of this type of “temporal” or “allochronic” prezygotic isolation include the apple maggot fly, *Rhagoletis pomonella*, where apple and hawthorn host races of the fly are isolated by a difference in the fruiting times of their respective host plants [40, 41], members of the *Enchenopa binotata* species complex of treehoppers that are adapted to differences in when the phloem of their host trees flow [42, 43], periodical cicada that differ in their emergence times (14 years versus 17 years), and the pine processionary moth, *Thaumetopoea pityocampa*, that has summer and winter populations that are behaviorally and genetically differentiated [44].

3.1.2 Mating isolation

Individuals from different populations may behaviorally prefer to mate in or on their natal home turf [45, 46]. Such differences in habitat choice can translate directly to mate choice, and establish systems of positive assortative mating in which individuals tend to mate with other individuals of like preferences and traits. Thus, differences in where organisms prefer to mate can also result in prezygotic RI and be important catalysts for speciation. Examples of habitat or host choice include the *R. pomonella*

species complex (Fig. 2), where different species such as the two host races of *R. pomonella*, the blueberry maggot *Rhagoletis mendax*, the snowberry fly *Rhagoletis zephyria* and the undescribed flowering dogwood fly have diverged, in part, due to preferring to mate on different host plants [41, 47, 48]. Other cases of divergence associated with habitat choice involve pea aphids [49], walking sticks in the genus *Timema* [50, 51] (Fig. 2), and three-spine sticklebacks [52] (Fig. 2).

3.1.3 Habitat isolation

Prezygotic isolation can also stem not only from habitat choice but may also be due to differential performance or adaptation to alternate habitats. For example, migrants from different populations may not survive well in non-natal habitats, and thus not mate and be prezygotically isolated from resident individuals [8]. Adaptation to different environments may also change the behavior and appearance of organisms in different habitats that can result in individuals avoiding mating with one another [8].

3.1.4 Behavioral isolation

Behavioral differences in mate preferences can also reduce crossbreeding between populations. This can occur through “sexual isolation” in which individuals from different populations prefer different signals or courtship displays to mate. Such differences have been proposed to evolve by a number of different means of sexual selection including runaway, chase away (sexual conflict), good genes and sensory drive (for reviews, see Refs [27, 53]). Several examples exist in which differences in mating cues result in prezygotic isolation. In birds [54], amphibians [55, 56] and insects [57] there are many cases in which females do not respond to the acoustic



Fig. 2 Model organisms of speciation. (a) The apple maggot fly, *Rhagoletis pomonella* (© Hannes Schuler); (b) the three-spined stickleback, *Gasterosteus aculeatus* (© Ingo Seiel); (c) the annual phlox, *Phlox drummondii* (© Gertrud Kanu); (d) butterfly

of the genus *Heliconius* (© Montse Poch); (e) the cichlid *Pundamilia nyererei* (© Greg and Lee Ann Stevens); (f) a *Timema* stick insect (© Moritz Muschik).

signals produced by heterospecific males [58]. Chemical signals where males do not respond to pheromones produced by females of different species represent chemosensory prezygotic barriers. For instance, chemoreception plays a major role in mate detection in different moth species, and this may lead to behavioral isolation [59]. Finally, visual signals where females respond to a conspecific color or pattern in males play a significant role in behavioral isolation in butterflies and birds. Species of the neotropical butterfly genus *Heliconius* differ in wing pattern, and males prefer to mate with females with the same wing pattern [60, 61].

3.1.5 Pollination in plants

In contrast to animals, behavioral prezygotic isolation in plants is often caused by differences in the animals or insects pollinating the plants. Pollinators often vary in their preferences for visiting flowers of different size, shape, and color.

As a consequence of these behavioral differences, pollinators can affect the evolution of floral morphology and the movement of pollen (gene flow) among plants, which potentially leads to speciation [62]. Phylogenetic studies have suggested that plant speciation is not uncommonly accompanied by changes in the pollination system [63]. Genetic studies have shown that changes of a few genes can influence floral differences and affect pollinator activity [64], whereas RI due to hybrid sterility can have a more complex genetic basis [65–67]. It is therefore possible that shifts in traits affecting pollination and pollination systems leading to RI have the potential to occur more rapidly or readily than other forms of isolation in plants. It should be noted, however, that additional forms of prezygotic isolation in plants associated with pollen tube growth and egg/sperm compatibility also have the potential to evolve rapidly and be major

factors underlying RI and speciation in plants [68].

3.1.6 Mechanical isolation

Mechanical isolation due to incompatibilities between the reproductive structures of organisms is another form of prezygotic isolation. In animals, different species can evolve different-shaped sex organs that do not fit properly between males and females. Dufour [69] was the first to describe such a “lock-and-key” mechanism where the male genitalia (the key) in one taxa evolves in a species-specific manner due to sexual selection that eventually does not fit with the organ (the lock) of interspecific females. One of the best-studied examples is the sympatrically overlapping Japanese carabid beetles *Carabus maiyasanus* and *Carabus iwakianus* that show low hybridization because of mismatching genitals between males and females of different species [70]. In plants, mechanical isolation can occur if structures of the flowers hinder the capture of pollen from other species [38]. Moreover, an analogous form of RI to mechanical isolation can occur if key features of pollinators, such as the beak of a bird versus the proboscis of a moth, vary in a manner such that they differentially fit in the flowers to different plants and, thereby, affect pollen transfer.

3.1.7 Gametic isolation

Gametic isolation (postmating prezygotic isolation) can occur if gametes of different species fail to unite. Coyne and Orr [38] defined two different types of gametic isolation:

- *Noncompetitive isolation*: This is due to the poor transfer of sperm between different taxa, the inviability of gametes in the reproductive tract of females of the opposite taxon, poor movement of sperm

or cross-attraction between gametes, failure of fertilization at gametic contact, or a reduced rate of oviposition.

- *Competitive isolation*: This results when sperm of different taxa are present in the reproductive tract of a female at the same time and conspecific sperm are favored over and fertilize a disproportion number of eggs than heterospecific sperm. In the absence of conspecific sperm, heterospecific sperm alone may not show a dramatic reduction in fertility compared to conspecific sperm. Sperm competition has been documented in ground crickets, where multiple mating of a female with a conspecific and a heterospecific male resulted in the fertilization of the majority of the eggs by the conspecific male and between conspecific pollen grains in the stigma of plants [71].

The take-home message with respect to prezygotic isolation is that there are many different ways, both prior to and after mating, that the gametes of different taxa can be prevented from fusing to form zygotes. Given that prezygotic RI impedes gene flow before postzygotic RI in the life cycle, it can generate assortative mating for traits under divergent selection, and can also potentially affect the whole genome. Thus prezygotic RI may often be an important component to speciation, particularly in cases of speciation with gene flow.

3.2

Postzygotic Isolation

Postzygotic isolation occurs when zygotes (offspring) produced from “hybrid” matings are inviable or sterile. Postzygotic RI can be due to negative interactions of hybrids with external environmental conditions (extrinsic), or due to internal

genomic incompatibilities independent of the environment (intrinsic) [38].

3.2.1 Intrinsic postzygotic isolation

Intrinsic genomic hybrid incompatibility was first postulated by Bateson in 1909 [72], and elaborated upon and empirically studied by Dobzhansky [37] and Muller [73] in different *Drosophila* species. Under the Bateson–Dobzhansky–Muller (D-M) model, populations initially become geographically isolated and independently evolve different mutations. Following subsequent secondary contact, when hybrids form, they will possess mutations from the two populations that, when brought together for the first time in individuals of mixed ancestry, can interact negatively with one another and cause sterility or inviability. RI is considered intrinsic because the developmental irregularities occur and are consistent regardless of environmental conditions [38], which is not the case for extrinsic isolation.

One important characteristic of intrinsic RI is that, under certain circumstances depending upon the nature of interactions in developmental and physiological pathways, the number of loci contributing to D-M genomic incompatibilities can exponentially increase (“snowball”) through time [74]. If individual incompatibilities have roughly equivalent effects on fitness, then the increase in contributing loci can result in a corresponding nonlinear increase in the rate that intrinsic RI evolves between populations.

Another feature of intrinsic RI is that it may evolve at different rates between sexes. Haldane’s rule [75] states that when hybrids of one sex are disproportionately sterile or inviable, it will most often be the heterogametic sex (i.e., the sex of a species in which the sex chromosomes are not the same). In the heterogametic sex,

the effects of recessive mutations differing between species can be exposed. Haldane’s rule will result when in a hemizygous state in the heterogametic sex (i.e., when an individual has only one copy of a sex-linked gene instead of the usual two copies in diploids), these mutations have greater negative consequences on fitness (more than twice) than in a heterozygous state in the homogametic sex. There is generally widespread adherence to Haldane’s rule in different mammal, bird, and insect species [38], regardless of which sex is heterogametic.

D-M incompatibilities are not the only causes of intrinsic hybrid sterility. Meiotic drive that occurs when one gene or haplotype is inherited by more than half of the progeny, generating a segregation ratio differing Mendelian expectations can also cause intrinsic hybrid sterility [76]. In this case, different selfish meiotic drive systems and their “suppressors” can independently evolve in allopatric populations. When brought together in hybrids the different suppressor systems may not act properly, and the drive systems can become active, resulting in reduced gamete production in hybrids and leading to their sterility. Similar considerations can also apply to transposable elements, where active elements in hybrids can result in both intrinsic sterility and inviability [9].

3.2.2 Extrinsic postzygotic isolation

In contrast to intrinsic isolation, extrinsic postzygotic isolation can evolve both in allopatry and in cases of speciation with gene flow (see below). Another distinction is that extrinsic postzygotic isolation is ecologically context-dependent. Thus, hybrids may not suffer any inherent loss of fitness when reared, for example, under benign conditions in the laboratory, but they fare poorly in Nature [77]. Thus, extrinsic RI is

not due to inherent negative interactions among genes *per se*, but results from the way in which these genes interact with the environment to cause hybrids to have reduced fitness in the wild. In general, it appears that extrinsic postzygotic isolation often evolves before intrinsic isolation [78], suggesting that divergent ecological adaptation may often play a critical role in initiating speciation.

One study examining the role that extrinsic postzygotic RI plays in population divergence involves populations of the stickleback fish, *Gasterosteus aculeatus* (Fig. 2), which is endemic to lakes in British Columbia. Here, the fish have evolved repeatedly and in parallel into two different ecomorphs: one living in the limnetic zone (open water area lakes), and the other in the benthic zone (deeper level). Although crosses between the two morphs result in viable offspring in the laboratory, hybrids perform poorly in Nature and grow more slowly than the parental morphs [79–81]. Hybrids have phenotypes generally less well-suited for utilizing benthic and limnetic niches than their parents and, thus, suffer decreased fitness and viability [79, 81]. A similar example comes from ecologically divergent, host-associated populations of *Neochlamisus bebbianae* leaf beetles, where F_1 and backcross hybrids grow slower on both host plants than do the parental forms [82, 83].

The take-home message with respect to postzygotic isolation is that, like prezygotic RI, there are many different ways that following zygote formation, hybrid offspring can suffer reduced fitness and fail to survive, produce viable gametes, and/or mate. Given that prezygotic RI is not expected to be complete early in speciation, postzygotic RI is also likely to evolve and be an important contributor to reducing gene flow during the speciation process for most taxa.

Indeed, in cases of allopatric (geographic) speciation (see below), when there is no chance for hybridization between diverging populations, there is no reason to assume that postzygotic isolation should not evolve at the same rate as prezygotic RI [38].

4

Modes of Speciation

Modes of speciation are usually defined on the basis of the geographic distribution of populations during the speciation process (i.e., the degree and pattern of spatial overlap of diverging populations as RI evolves). Major geographic modes include allopatric (complete physical separation of populations), parapatric (partial geographic overlap of populations) and sympatric speciation (complete geographic overlap of populations). Parapatric speciation can be further divided into instances of primary versus secondary contact. In cases of primary contact, the diverging populations were always connected by a region of geographic overlap where gene flow was occurring as they evolved into different species. In contrast, in secondary contact, populations were initially geographically separated and diverged in allopatry before expanding their range(s) to geographically overlap and hybridize. Distinguishing between these two possibilities is important because it bears on the question of whether speciation is possible without the complete geographic isolation of populations at some stage of the process (see Refs [4, 38, 84–86] for further discussions of different geographic modes of speciation and controversy surrounding them).

In addition to geography, different modes of divergence can also be defined based on the mechanism(s) causing divergence. For example, in ecological speciation, RI results

from populations differentially adapting to different environments or habitats due to divergence selection. Adaptations advantageous in one habitat may be detrimental in others, resulting in the reduced viability or fertility of migrants and hybrids causing fitness tradeoffs [4, 5, 8]. In contrast, in mutation order speciation, genes under directional selection to similar ecological conditions or experiencing drift can differentiate between two geographically isolated populations [5, 87]. When brought back together for the first time, these loci can negatively interact and cause postzygotic RI and D-M incompatibilities. Various other chromosomal modes of speciation have also been proposed in which differences in ploidy levels (the number of complete sets of chromosomes individuals possess) or structural features of chromosomes (e.g., inversions, translocations, or chromosomal fusions or fissions) disrupt proper meiosis in hybrids and generate RI [88].

4.1

Allopatric Speciation

The important role that geographic isolation can play in facilitating speciation was first emphasized by Poulton [18] and underscored by Mayr [15, 16]. Speciation could be thought of as a “tug of war” between evolutionary processes causing populations to diverge from one another, and migration and gene flow causing them to homogenize. Whichever force is stronger will win out. When populations are allopatric and geographically isolated, then the homogenizing effects of gene flow can be removed from the speciation equation due to the lack of migration between populations. Essentially, a state of complete prezygotic RI exists between allopatric populations, allowing them to evolve independently. Subsequently, any genetic change occurring

between the populations, whether due to genetic drift or directional and divergent selection, can potentially generate RI by any of the means discussed above (D-M incompatibilities, ecological fitness tradeoffs, sexual selection, polyploidy, translocations, etc.). Indeed, certain types of RI, like those due to intrinsic D-M incompatibilities, are more likely to evolve via a geographic than non-geographic mode. The reason for this is that mutations causing genomic incompatibilities which result in reduced fitness will normally be eliminated by selection in populations. However, in allopatric populations these mutations may not cause incompatibilities and could, in fact, be favored by natural selection. It is only when the mutations that have accumulated independently in isolates are brought together for the first time in hybrids that they interact negatively and inadvertently causing sterility and inviability. Thus, geographic isolation may be a common precursor to speciation for many organisms, especially those that primarily display strong intrinsic RI.

One indication of the importance of geography in population divergence and speciation is the observation of reproductive isolation-by distance (RIBD) [89, 90]. In RIBD, the degree to which pairs of populations are genetically differentiated and reproductively isolated from one another is correlated with the geographic distance separating them. Thus, increased geographic separation – and, by implication, reduced migration and homogenization due to gene flow – equates with increased divergence and RI between populations. Ring species are classic examples of RIBD: Populations are distributed in a spatially arrayed circle such that, starting at the middle of the ring ever more-differentiated populations can be found moving in opposite directions around the circumference

until they eventually meet as fully isolated species when they come into contact at its end [15, 91, 92]. Possible examples of ring species include the greenish warbler (*Phylloscopus trochiloides*) encircling the inhospitable Tibetan plateau [91, 93], and the *Ensatina eschscholtzii* salamanders encircling the inhospitable central valley of California [94].

4.1.1 Peripatric speciation

Peripatric speciation is a type of allopatric divergence where small satellite populations become geographically isolated on the fringes of the distribution of what usually is a larger central population [38]. Often, these peripheral populations pass through a genetic bottleneck and experience founder effects when they colonize areas at the boundaries and beyond of the species range [15]. They can also experience strong selection due to differing environmental conditions at the edge of the range, as well as be subject to extensive genetic drift due to their smaller effective population sizes [95]. All of these factors can contribute to rapid genetic change in peripatric populations that may result in the origin of a new species. The colonization of oceanic islands or archipelagoes may present conditions potentially conducive to peripatric speciation. One possible example involves the radiation of a myriad of different *Drosophila* species that are endemic to different islands in the Hawaiian chain [96].

4.2

Sympatric Speciation

While allopatry is a widely accepted and common mode of speciation [38], sympatric speciation in the absence of complete geographic isolation and in the face of continuous gene flow is more controversial. Indeed, Mayr [16, 97] considered allopatry

as a prerequisite for speciation in animals. However, with the increasing study of the role of ecology and sexual selection in speciation over the past few decades [98–102], sympatric speciation has gained greater acceptance among evolutionary biologists as being possible and occurring at least part of the time in Nature [46, 103].

In contrast to allopatric speciation, where speciation is initiated in the absence of gene flow, sympatric speciation occurs between geographically overlapping populations in the face of continuous gene flow [16]. A central tenet of many sympatric speciation models is that strong fitness tradeoffs exist, resulting in host or habitat specialist populations evolving that are reproductively isolated from other populations by ecological barriers to gene flow [45, 46]. Sympatric speciation is generally thought to be difficult since gene flow will prevent the evolution of specialization and RI [16, 38]. However, both theoretical models [104–106] and empirical studies [47, 100, 101, 107] have shown that, under certain circumstances, speciation with ongoing gene flow can occur. “Magic traits” in which the same traits or loci under divergent ecological selection also are involved in mate choice and positive assortative mating can also facilitate speciation with gene flow [105], as can frequency-dependent selection on resource use generated by competition (adaptive dynamics) [108, 109].

One of the best-studied examples of sympatric speciation is the apple maggot *R. pomonella*, a major economic pest of apples in North America [47, 100, 110, 111]. The native ancestral host for *R. pomonella* is hawthorn (*Crataegus* sp.). After introduction of the domesticated apple *Malus domestica* to North America about 400 years ago, a portion of the

hawthorn-infesting population shifted to and started to attack sympatrically occurring apples [47, 112]. Within less than 150 years this led to the formation of ecologically and genetically differentiated apple host race of the fly [47, 100, 111]. A number of other closely related and morphologically similar sibling species are sympatric with *R. pomonella* and attack different host plants native to North America. These considerations led Bush [113] to propose that the whole *R. pomonella* sibling species group arose via sympatric host shifts. In this case, fly taxa attacking flowering dogwood, blueberries, snowberries and silky dogwood plants are in varying stages of ecological divergence along the speciation continuum ranging from host races to morphologically distinct species [113–116].

4.3

Parapatric Speciation

Populations are partially geographically isolated during parapatric speciation (Fig. 2). Parapatric speciation can occur when populations adapt to different ecological habitats or accumulate different mutations by selection or drift in different portions of their ranges, but still at least partially overlap in their distributions such that gene flow is ongoing. Thus, the overall rate of gene flow is higher than in allopatric speciation, but lower compared to sympatric speciation. Although, in principle, parapatric speciation may be common it is perhaps the most difficult mode to verify [38]. Historical data of past species ranges are often missing, making it difficult to determine if currently overlapping taxa represent cases of parapatric divergence versus secondary contact of taxa that already completed the speciation process in allopatry prior to their present overlap. Certain cichlid fish in Africa may represent examples of

parapatric divergence via the evolution of different opsin genes that enable them to see at different water depths [117].

4.4

A Plurality of Modes

The strict geographically based division of speciation into purely allopatric, sympatric or parapatric modes may not always reflect the reality of speciation [110, 118]. During speciation, the geographic distribution of populations may change over time, and RI may evolve at times when populations were alternately in allopatry, parapatry, and sympatry. Indeed, the genetic bases for speciation may often have originated at a different place and time than the proximate selection pressures causing population divergence. For instance, secondary contact of geographically isolated *R. pomonella* populations in the past appears to have led to the introgression of raw genetic material that affected diapause traits and contributed to the genetic shift of host races in sympatry [110, 119]. Similar examples of “transporter” speciation involving standing genetic variation “recycled” to contribute to independent adaptive divergence have been documented for stickleback fishes [120] and *Heliconius* butterfly hybridization [121]. Moreover, different types of RI may have evolved and contributed to population divergence at different stages of the speciation process. Thus, Dieckmann *et al.* [122] have classified different “routes” to speciation, involving varying combinations of factors, modes and mechanisms of population divergence through time. In addition, several authors (e.g., Refs [123, 124]) have advocated for a reclassification of RI models from the current framework dominated by geographic context to one that includes mechanisms of speciation. The take-home message is that one size

does not fit all, and that speciation may often involve a plurality of different modes and mechanisms through time and space.

5

Evolutionary Mechanisms Causing Population Divergence

As discussed above, a number of factors and mechanisms interact to cause populations to evolve and become reproductively isolated from one another and eventually speciate. Some of these processes are examined in more depth in the following sections.

5.1

Natural Selection and Ecology

Natural selection may be the most important single factor responsible for the origin of new species. Divergent ecological selection can generate extrinsic RI and facilitate speciation between populations in allopatry, parapatry, or sympatry. In effect, traits adapting individuals to one habitat result in reduce fitness in the alternative habitat (tradeoffs) such that migrants and individuals of mixed hybrid ancestry have lower survivorship, fertility and ability to attract mates than parental types. Divergent ecological selection pressures can include abiotic and biotic factors such as food resources or climatic conditions, as well as interspecies interactions such as diseases, competition, and behavioral interference [5, 123].

Divergently selected genes can also inadvertently cause D-M incompatibilities in hybrids. This indirect effect of divergent selection in generating intrinsic RI will generally be more common in cases of allopatry than parapatry or sympatry, at least early in the speciation with gene flow

process when hybridization is common. The reason for this is that alleles and new mutations that cause intrinsic reductions in fitness in hybrids in all habitats will usually be quickly eliminated from populations. However, under certain circumstances, as populations progress further along in speciation and effective gene flow rates become significantly reduced, it is possible for new mutations that cause both fitness tradeoffs and intrinsic genomic incompatibilities between populations to establish in sympatry or parapatry, as long as the negative pleiotropic consequences of the mutations are comparatively less than the survival advantage they confer in favorable habitats [125].

Allopatric populations can also evolve intrinsic RI when adapting to the same environmental conditions. Here, allopatric populations can independently accumulate different sets of beneficial mutations that are universally favored across all populations. However, these mutations may pleiotropically cause genomic D-M incompatibilities in hybrids. Thus while beneficial, they may not spread between populations following secondary contact, remain diverged, and contribute to RI, leading to speciation (termed “mutation order” speciation [5, 87]). In sympatry, it is difficult for intrinsic RI to evolve due to adaptation to the same ecological conditions. Any new universally favored mutation will quickly spread between populations, and any allele(s) causing intrinsic reductions in fitness will be selectively removed from populations. Similarly, in the overlapping portion of the range of parapatric populations it is also difficult for D-M incompatibilities to evolve via adaptation to similar ecology. However, they can still evolve if the mutations contributing to intrinsic RI arise in the non-overlapping portions of the population ranges.

Empirical evidence for a role of selection in allopatric speciation can be seen in the molecular patterns of evolution displayed by several putative “speciation genes” that have been associated with D-M incompatibilities. In these cases, statistically high levels of replacement substitutions in coding regions of loci, particularly in relation to levels of standing non-synonymous polymorphism, imply past episodes of positive natural selection [126, 127]. However, not all genes under selection and contributing to intrinsic RI are speciation genes. Strictly speaking, only genes that diverged and increased RI prior to taxa speciating (or becoming completely isolated) are truly “speciation genes” [87]. Differences that evolved afterward represent post-speciation divergence. As a result, verifying speciation genes and drawing inferences

about them can be complicated. Not only are mapping and functional studies needed to identify candidate loci, but also details of the natural history of populations must be discerned to establish the evolutionary chronology of RI. As discussed above, several potential speciation genes affecting intrinsic postzygotic RI have been characterized, many in *Drosophila* (Table 2). For the most part, however, the epistatic partners interacting with these genes to cause D-M incompatibilities and the nature of these interactions still largely remain to be resolved [138].

Evidence for a role of divergent natural selection in speciation is most easily documented in cases of speciation with gene flow. Here, the logic is that genes and traits not under selection or not closely physically linked to such genes will be homogenized

Tab. 2 Selected overview of “speciation genes” causing intrinsic RI hybrid inviability and sterility.

| | Gene | Organism | Details | References |
|--------------------|--|---|---|----------------|
| Hybrid inviability | <i>cytc</i> | <i>Tigriopus californicus</i> | A mitochondrial gene that interacts with nuclear genes | [128, 129] |
| | <i>Lhr</i> , <i>Hmr</i> | <i>D. simulans</i> , <i>D. melanogaster</i> | Two functionally divergent genes that cause hybrid lethality | [130] |
| | <i>NB-LRR</i> <i>Nup69</i> , <i>Nup160</i> | <i>Arabidopsis thaliana</i> <i>D. simulans</i> , <i>D. melanogaster</i> | Disease resistance gene Interaction of different nuclear pore complex proteins | [131] [132] |
| Hybrid sterility | <i>Aep2</i> | <i>S. bayanus</i> , <i>S. cerevisiae</i> | Incompatibility of mitochondrial and nuclear genome | [133] |
| | <i>OdsH</i> | <i>D. simulans</i> , <i>D. mauritiana</i> | Homeobox gene that causes enhancement of sperm production | [134, 135] |
| | <i>Overdrive</i> | <i>D. pseudoobscura</i> | Causes RI and segregation distortion in F ₁ hybrids | [136] |
| | <i>Prdm9</i> | <i>Mus musculus</i> | Encodes a histone H3 methyltransferase | [137] |

Modified from Ref. [87].

by gene flow. Hence, genome scans of genetic markers between populations can reveal loci showing exceptional divergence (outlier loci) that can be inferred to be under divergent selection [5]. Such studies have identified putative “islands of speciation” containing genes under divergent selection contributing to RI in the genomes of several organisms including mosquitoes, *Timema* walking sticks, *Heliconius* butterflies, flycatchers, whitefish, and sticklebacks [139–144]. A stronger case for divergent selection can be made if migration and gene flow is occurring between populations, as it is possible that populations evolved RI in the absence of gene flow. Under certain conditions, this could result in patterns of genomic divergence similar to those predicted in sympatric and parapatric speciation [145, 146]. In addition, divergent selection may be differentiation populations if the markers in genomic regions displaying significant divergence are associated with different traits or environmental/ecological conditions. The latter can be accomplished through genetic mapping studies involving test crosses of individuals with differing phenotypes, through genome wide association studies (GWASs) genotyping individuals sampled from natural populations that vary in their phenotypes, and through selection experiments in which key environmental variables are manipulated in the laboratory or field (i.e., by transplant experiments moving individuals between habitats) to test for a genetic response in predicted directions at the speciation islands. Such a strategy has been used to confirm the action of divergent ecological selection for *Timema* walking sticks [144, 147] and host races of *Rhagoletis* fruit flies attacking apple and hawthorn host plants [148]. Several additional studies have described the role that natural selection and ecology play in

the speciation of different plant taxa (e.g., *Helianthus*, *Mimulus*, *Phlox*, reviewed in Ref. [149]; see Fig. 2), amphibians (e.g., *Bombina* [150]), fishes (e.g., *Poecilia* [151]; see Fig. 2), and insects (e.g., *Heliconius* [121]).

5.2

Sexual Selection

In species in which individuals differ in characters that influence mating preference and success, sexual isolation can be a factor causing prezygotic RI [53]. Darwin [152] observed that species that have elaborated sexual characters tend to be more species-rich than those that do not and, thus, suggested that the evolution of sexual characters may cause sexual selection and be involved in speciation.

Sexual selection can occur between individuals of the same sex, or involve interactions between individuals of different sexes [153]. Intrasexual selection involves competition between individuals within a given sex (usually males) to gain reproductive access to the opposite sex (usually females). Intrasexual selection can lead to the evolution of sexual dimorphism (phenotypic differences between males and females) that can involve traits associated with physical combat, such as antlers in deer or horns in beetles [153]. In contrast, intersexual selection occurs when individuals (usually males) compete to be chosen by the opposite sex (usually females). Intersexual selection can lead to the evolution of sexual characters such as the elongated tail of the red-collared widowbird, *Euplectes ardens* [154] or the span between eyes of the stalk-eyed fly, *Cyrtodiopsis dalmanni* [155].

Following up on Darwin's lead, recent comparative studies have confirmed that sexually selected taxa are generally more

species-rich [156], implying that assortative mating caused by divergent sexual selection may facilitate speciation [38]. In passerine birds, for example, clades with more sexually dichromatic taxa contained more species [157]. Sexual selection also appears to be an important factor in the radiation of cichlid fishes, especially in the lakes of the African Rift Valley [102] (Fig. 2).

Although sexual selection can be an important contributor to RI and speciation [158], major questions remain concerning the mechanism(s) responsible for its evolution. One central issue is whether sexual selection primarily originates due to mating preference differences alone or is the consequence of ecological selection. In the former instance, individuals may choose to mate with the opposite sex based on traits that initially have no inherent fitness effects on their bearers in and of themselves. This represents sexual selection in the classic Fisherian “run away” sense, where peacock females, for example, may prefer to mate with males possessing a large tail feather simply because it catches their fancy and their sons will, thus, tend to have large tail feathers too and gain a mating advantage [159]. (Note: “chase away” selection can also occur in which males evolve certain traits that confer increased fertility but have detrimental consequences for females, resulting in a battle between the sexes in which females evolve measures to counteract the effects of the male traits [160].) In contrast to the classic Fisher model, sexual selection could also be dependent on traits under ecological selection. In this case, adaptation to different environments or habitats can lead to divergence in morphological characters that influence mate choice [161]. This could be due to sexual selection for mates advertising their possession of “good genes” conferring higher fitness to

a particular environment [162], or due to sensory drive in which males evolve exaggerated displays taking advantage of an adaptive change in perception associated with higher survivorship in a habitat. For example, if seeing a particular color is advantageous, males showing this color in abundance could gain a mating advantage [163]. In the case of stickleback fish and African cichlids, traits involved in ecological niche specialization also contribute to positive assortative mating [164, 165]. Empirical and theoretical studies suggest that sexual selection may therefore often go hand-in-hand with ecological differentiation, forming the basis for “magic traits” facilitating speciation [12, 105]. However, differences in mating traits can also form prezygotic isolation without ecological differentiation. Moreover, a recent theoretical study showed that sexual selection alone enables the overlapping coexistence of ecologically equivalent species [166]. Determining whether sexual selection alone plays a direct role in speciation and the process responsible for sexual selection (runaway, chase away, good genes, sensory drive, etc.) remain areas requiring much further study to verify [158].

5.3

Hybrid, Polyploid, and Homoploid Speciation

Hybridization and polyploidization are two – sometimes interrelated – processes that can facilitate speciation. In hybrid speciation, interbreeding between two differentiated populations generates new genotypic and phenotypic variation that serves as the basis for creating new forms reproductively isolated from parental populations. In polyploid speciation, changes in the number of whole sets of chromosomes in the genome in derived versus parental populations can cause problems

in development or in meiosis in hybrids, generating RI. Polyploid speciation appears to be much more common in plants [167] than in animals [168].

Hybrid speciation can occur by either an allopolyploid or homoploid route [169]. In allopolyploid speciation, hybridization between different taxa is a trigger for polyploidization [170, 171]. The new hybrid polyploid, having a different chromosomal constitution, is immediately reproductively isolated from the parental populations. Subsequent ecological adaptation of the allopolyploid populations to novel environmental conditions is often required for the new species to persist and not be displaced by parental forms. It is also possible, however, for polyploidy to occur without hybridization conspecifically in a process termed autopolyploidy and generate RI leading to speciation [172, 173].

Homoploid hybrid speciation does not involve ploidy changes. Instead, hybridization produces novel combinations of genes that can adapt hybrids to new habitats or biotic conditions that cause them to be reproductively isolated from parental populations [174, 175]. Divergent ecological adaptation is thought to be crucial in order for hybrid populations to avoid being swamped by gene flow from parental species or being competitively excluded [38]. A case study in tephritid flies showed that hybridization of *Rhagoletis mendax* (that infests blueberries) with *R. zephyria* (that infests snowberries) formed a new hybrid population that attacks *Lonicera* (honeysuckle) [176]. As a consequence of infesting and ecologically specializing on honeysuckle, the hybrid fly population can persist sympatrically with both parental populations. Many *Heliconius* butterflies may have been formed by hybridization [177], involving changes in just a few genes affecting mimicry [121]. Certain *Helianthus*

sunflowers also appear to have originated by hybridization and inhabit novel, often marginal, habitats to which they are divergently adapted [178]. In contrast to the butterflies, many genomic components from both parental taxa are present in hybrid sunflower species.

Hybrid and polyploid speciation are now generally accepted as occurring not uncommonly in Nature. The major question here concerns how frequently homoploid hybrid speciation occurs, although recent studies have suggested that it may be more common than previously thought [9, 174, 179–181].

5.4

Reinforcement

When hybridization generates offspring of lower fitness than parental matings, any trait or behavior that causes individuals to be more discriminant in their choice of partners and mate only with those of like kind can be favored. Such circumstances represent one of the few cases in which natural selection may directly favor the evolution of increased prezygotic isolation between populations to further the speciation process [182]. This process is termed “reinforcement,” and was first proposed by Alfred Russel Wallace [183] and later more extensively and accurately described by Theodosius Dobzhansky [184].

There are several important considerations with regard to reinforcement. First, populations must be hybridizing with at least some gene flow occurring for the evolution of increased prezygotic RI to constitute reinforcement [38]. If hybrids are completely inviable or sterile, then this represents “character displacement.” The difference may seem semantic, but if gene flow has ceased altogether between taxa,

then they have already speciated and the evolution of increased prezygotic isolation through character displacement is not contributing to the speciation process.

Second, early theoretical studies suggested that it was difficult for increased prezygotic isolation to evolve in the face of moderate to high gene flow [185], as it would be difficult for alleles causing assortative mating to be maintained in the correct linkage phase with those causing postzygotic RI. Recombination would break up favored combinations of genes that are responsible for mate choice, thereby affecting assortative mating and genes causing D-M incompatibilities. Similar arguments pertaining to the antagonism between selection and recombination were made concerning sympatric speciation [185]. However, the theory applies only to two-allele systems, where a polymorphism exists for individuals possessing one allele to cause assortative mating and individuals possessing the alternative allele to do likewise. In one-allele systems, in which a mutation causes assortative mating between individuals of like phenotypes, linkage and selection–recombination antagonism are not issues [186]. The same is true for magic traits, in which the trait responsible for divergent adaptation also directly affects mating and, thus, generates prezygotic RI [105]. For example, populations adapted to resources that are present at different times of the year, such as the apple and hawthorn host races of *R. pomonella*, can have different timing of life histories matching these resources. As a result, individuals using the alternate resources will be temporally offset from one another, and variations in life history timing will directly affect mate choice and result in allochronic mating isolation. Moreover, when individuals mate in preferred habitats, divergent ecological

selection will generate a degree of disequilibrium (the nonrandom association of alleles at different loci) between habitat choice and performance genes, even for physically unlinked loci [104]. The reason for this is that individuals which survive in a particular environment will tend to return to the same environment to mate (termed “habitat” or “host fidelity” [41]), creating a system of positive assortative mating for choice and performance genes that can mitigate the selection–recombination antagonism and allow speciation with gene flow to progress. Essentially, a feedback mechanism is established in which increased survival creates stronger selection pressures for increased reinforcement by favoring stronger habitat fidelity and sexual selection against hybridization [28, 104].

Third, reinforcement is also believed to be difficult in circumstances not only when gene flow was moderate to high but also when it was low. Here, selection pressures favoring assortative mating would be reduced because very few hybrid matings would occur. Moreover, in situations of parapatry, the migration of conspecifics from areas outside of the contact zone (where there would be no selection for assortative mating) could swamp out the evolution of any prezygotic RI in the area of geographic overlap and hybridization. However, unless there is significant cost to being overly choosy, increased prezygotic isolation would eventually evolve between postzygotically isolated populations, provided that the populations maintained their isolation and did not fuse. In addition, divergent sexual selection occurring in the allopatric ranges of the populations could also spill over to the region of contact. Here, sexual selection could help further reduce hybridization (for a review, see Ref. [182]).

A fourth important consideration with regard to reinforcement is that the concept was originally developed for scenarios of secondary contact, in which populations evolved postzygotic isolation in allopatry prior to re-establishing contact and hybridizing in sympatry or parapatry. However, as selection against hybrids is a requisite for sympatric speciation [109], reinforcement can also occur in instances of primary contact in sympatry or parapatry, provided that fitness tradeoffs have evolved between populations.

As discussed above, the role of reinforcement in speciation has been controversial [187]. However, during the past few decades theoretical studies [188], as well as empirical evidence in *Drosophila* fruit flies [189, 190], rainforest frogs [191], stickleback fishes [192], *Timema* walking sticks [193] (Fig. 2), the Texas wildflower *Phlox drummondii* [194] (Fig. 2), and birds [195], have bolstered the case for the process. Today, it is generally assumed that reinforcement can sometimes be an important contributor to speciation in sympatry, parapatry, and allopatry [4, 196].

5.5

The Role of Endosymbionts in Speciation

An unexpected and novel mechanism driving speciation comes from the microbial communities associated with sexually reproducing organisms. Endosymbiont bacteria are ubiquitous in animal hosts and have important consequences for their host's nutrition [197], behavior [198], and defense against natural enemies [199], including protection against viruses [200]. The diverse effects of microorganisms on their hosts led Wallin [201] to hypothesize that microorganisms may play a role in speciation. The development of modern genetics and gene mapping by Morgan

[202] and the pioneering work on the genetics of speciation by Dobzhansky [37] led to a focus on the phenotypic effects of mutations in the genome as being the ultimate source of RI. As a result, Wallin's idea sank into oblivion [203]. However, technical progress in profiling microbial symbionts during the past two decades have provided new insights into the impacts that endosymbionts have on the biology of their hosts and have rekindled interest in the question of the involvement of endosymbionts in speciation [204].

One common endosymbiont is *Wolbachia* [205], which infects a broad range of arthropod and nematode hosts [206]. *Wolbachia* are maternally inherited, and consequently can increase their transmission rate by skewing the reproduction of their hosts toward females through cytoplasmic incompatibility (CI [207]), male killing [208], feminization [209] and induced parthenogenesis [210]. Reciprocal CI caused by populations possessing different strains of *Wolbachia* has been a focus of interest because it can generate RI contributing to speciation [211]. When *Wolbachia*-infected males mate with uninfected females (unidirectional infection), no or few offspring are produced, and all other crosses are fertile (Fig. 3). As a result, once infection levels have passed a threshold, *Wolbachia* can sweep through a host population [212, 213]. When both partners are infected with different strains of *Wolbachia*, however, CI also occurs and matings are infertile, but now in both directions (Fig. 3). It is this aspect of CI, where populations have acquired different strains of the bacteria (bidirectional infection), that has peaked interest in the possible role of *Wolbachia* in speciation [211].

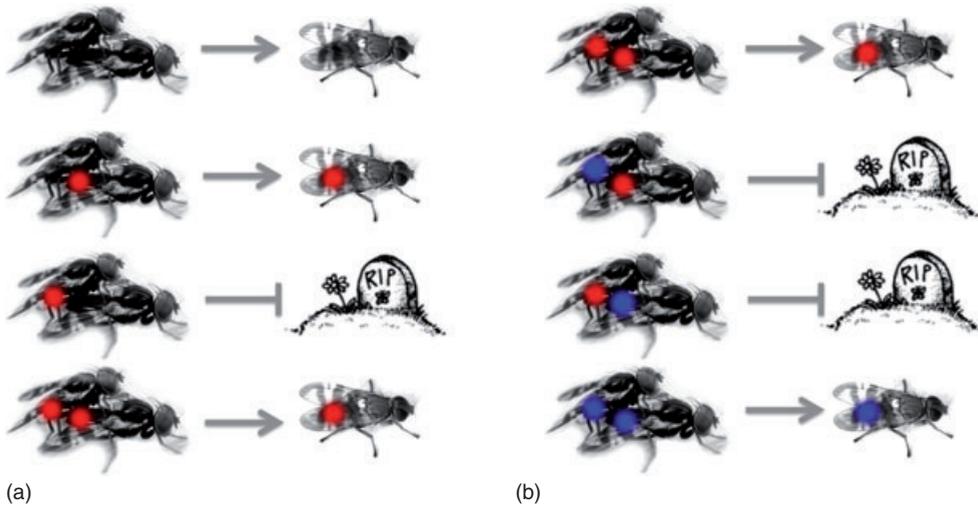


Fig. 3 Schematic representation of cytoplasmic incompatibility. (a) Unidirectional CI occurs if an infected male (red dot) mates with an uninfected female; (b) Bidirectional CI occurs if a male

infected with one *Wolbachia* strain (red dot) mates with a female infected with a different strain (blue dot), and vice versa.

One of the first examples that implied a role for *Wolbachia* in speciation involved parasitic wasps of the genus *Nasonia* [214]. *Nasonia giraulti* and *Nasonia longicornis* are infected with different strains of *Wolbachia*, and crosses between these two closely related sister species of wasps resulted in no offspring. However, after antibiotic curing, hybrids between the *Nasonia* species were completely viable and fertile, which suggested that *Wolbachia* had induced bidirectional CI caused RI in the early stages of wasp speciation [214]. Theoretical studies have confirmed that high levels of bidirectional CI can reduce gene flow and help facilitate speciation [215].

However, CI is not the only mechanism by which endosymbionts can cause RI. Interactions between beneficial bacterial communities and their hosts can influence host behavior and cause prezygotic RI. For example, *Drosophila melanogaster*

larvae reared on different diets acquired different symbionts and displayed strong mate discrimination [216]. However, the prezygotic RI was lost when the flies were treated with antibiotics, eliminating their symbionts [216]. Gene-microbe interactions may also cause postzygotic RI. The beneficial gut community of closely related *Nasonia* species is linked to the phylogeny of their hosts [217], and crosses between wasps possessing different gut bacteria have resulted in hybrid lethality dying during larval development. However, antibiotic curing of the gut bacteria showed near normal fitness in the hybrid larvae, implying that the gut microbiota was responsible for hybrid lethality [217].

The overall contribution that endosymbionts make to speciation is still controversial [38], and they may not often be the primary factor initiating or driving population divergence. However, recent results have suggested that endosymbionts

can be involved in speciation, and the RI they cause can make them potential agents facilitating the evolution of reinforcement [218]. Therefore, it seems reasonable to include considerations of symbionts and CI into classic models of nuclear-based speciation [204].

6

Next-Generation DNA Sequencing and the Genetics of Speciation

Advances in next-generation (Next-Gen) DNA sequencing are quickly changing the ability to determine the identity, nature, interactions, and genomic architecture of genes underlying RI and speciation. The ability to sequence the genomes of model and non-model genetic organisms is accelerating the analysis of the genetics of speciation. The new methods are providing an immense number of variable markers that help to answer many different biological questions such as mapping the location of speciation genes in greater detail in the genome, or finding differentiated genomic regions across the genome of different populations or species [219].

6.1

Next-Generation Sequencing and the Speciation Continuum

Next-generation DNA sequencing techniques are allowing genome scans to be performed between pairs of taxa at varying stages of divergence along the speciation continuum. In cases of primary or secondary contact, in which gene flow is occurring between populations, such genome scans can identify genes (or gene regions) showing significant differentiation between taxa above baseline neutral

expectation (outlier loci) that represent candidates for being (or containing) speciation genes contributing to RI. In addition, the pattern of differentiation across the genome can provide insights into the genetic architecture of speciation and possible mechanisms facilitating population divergence.

For example, it is becoming increasingly possible to assess whether genes contributing to RI generally have large or small effect sizes. That is, do just a few genes that have major effects on traits associated with RI underlie speciation, or are most traits responsible for RI polygenic and encoded by many loci of small effect [87]? Similarly, questions concerning whether selection acting directly on individual genes to cause population divergence (genic speciation), or processes involving genetic coupling and hitchhiking in which the combined effects of multiple loci together overcome gene flow to allow speciation to progress (genomic speciation [78]), can also be addressed.

Genome scans may also help resolve issues concerning the involvement of structural features of the genome (e.g., inversions that locally reduce recombination rates and enhance linkage disequilibrium) in facilitating population divergence [11, 220]. Moreover, the ability to survey entire genomes will allow a more comprehensive determination of whether taxa can be distinguished on the basis of: (i) forming distinct genetic clusters; (ii) sharing most common recent ancestry; and (iii) possessing diagnostically fixed autapomorphies. In other words, the taxonomic status of populations can be assessed in reference to different current genetic definitions of species. In addition, if the coupling of loci of smaller effect occurs during speciation to cumulatively enhance RI genome-wide, then a marked acceleration in the rate of

genomic divergence may be observed as populations reach this stage in speciation [29–31].

Finally, advances in next-generation sequencing enable the comparison of genomes of different species. Having an annotated genome for an organism in which the locations of protein-coding regions, regulatory regions affecting gene expression, and other transcribed sequences are known, can be useful for determining the identities of specific candidate genes or sequences causing RI. With this knowledge, transplant experiments and genetic introgression studies involving repeated backcrossing of hybrids to one or the other parental types can be conducted to confirm the involvement of candidate speciation genes in population divergence, and to study the nature of their interactions with other loci in causing RI [87].

6.2

Combining Genomic and Experimental Approaches

One of the classic examples of experimental evolution is the study of Rice and Salt [221], who designed a maze with extreme environments in which individuals of *D. melanogaster* could choose different habitats. These authors showed that, by crossing just individuals from the extreme habitats, selection on extreme habitats caused prezygotic isolation. Combining manipulative laboratory and field transplant experiments with Next-Gen DNA sequencing offers a particularly powerful means of characterizing the effects of divergent selection on population divergence and RI. One example involves a recent “evolve-and-sequence” study of walking sticks [144], where individuals of different populations were reared on

their natal and non-natal host plant environments in Nature and high-throughput sequenced to characterize the genomic architecture of early stages of speciation. Similarly, Chenoweth *et al.* [222] released 12 populations of *Drosophila serrata* in a novel environment and characterized the influence of sexual and natural selection on different regions of the genome.

7

Final Thoughts

In conclusion, students of speciation now live in an era of high-throughput genomics, enabling a greater push to understanding the genetic basis of speciation. However, the answers to the mystery of speciation still rest on combining what can be learned from DNA sequences with experiments, crosses in the laboratory, and studies of the natural history of organisms in the wild. Genomics provides one means (and a powerful one at that!) to help understand the natural history of the “how and why” of speciation. Genomics will quicken the pace of discovery, lead to a wealth of new information on the molecular and genetic bases of gene flow barriers, and will hasten the compilation of comparative data sets for meta-analyses to test for broad patterns and possible rules of speciation. However, this information loses significance when divorced from the proper ecological, historical, and phylogenetic context of speciation. In this chapter, a broad outline has been presented of the current concept of what species are and how they form. Yet, it must be highlighted that despite all of these technical advances, it is still necessary to climb back into Darwin’s “tree of life” with the enthusiasm of a naturalist to immerse oneself in life’s great diversity to make sense of the endless forms that

speciation continuously generates, and to fully appreciate and comprehend its consequences. Recently, there have been rapid gains in resolving the genomic basis and processes responsible for the origins of new species, and therefore the mystery of speciation has been somewhat dissipated for certain aspects of the process highlighted here. However, the mystery has only deepened for other aspects. For example, is there a role for non-Mendelian epigenetic factors in speciation, as well as for social and cultural variation (which were not covered here)? Clearly, there remains much to be done to comprehend the grandeur in Darwin's view of life.

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