Feature Review

What do we need to know about speciation?

The Marie Curie SPECIATION Network*

Speciation has been a major focus of evolutionary biology research in recent years, with many important advances. However, some of the traditional organising principles of the subject area no longer provide a satisfactory framework, such as the classification of speciation mechanisms by geographical context into allopatric, parapatric and sympatry classes. Therefore, we have asked where speciation research should be directed in the coming years. Here, we present a distillation of questions about the mechanisms of speciation, the genetic basis of speciation and the relationship between speciation and diversity. Our list of topics is not exhaustive; rather we aim to promote discussion on research priorities and on the common themes that underlie disparate speciation processes.

The future focus of speciation research

Evolutionary biology seeks to explain two major features of the living world: the fit of organisms to their environment (i.e. adaptation; see Glossary) and organismal diversity. Biological diversity exists at many levels, but we focus on the discontinuous distribution of phenotypes and genotypes in sexually reproducing organisms into units that we call 'species' (Box 1). At this level, diversity is explained by the balance between extinction and speciation and, consequently, speciation is a central topic of evolutionary science. An enormous amount of research since *The Origin of Species* [1] has been performed on speciation, especially during the past 20 plus years [2,3]. Given recent advances, we contemplated where this research effort should be focused in the coming years. Our members* identified their key speciation questions (see the supplementary material online). What follows is a distillation of the resulting list

Glossary

Adaptation: evolutionary process by which populations become better suited to their own environment through genetic change.

Allopolyploidy: the formation of a new species through hybridisation of different species, resulting in an increase in ploidy level.

Biological species concept: the most widely used species concept, which defines species in terms of interbreeding. Mayr proposed that 'species are groups of interbreeding natural populations that are reproductively isolated from other such groups'.

Character displacement: greater trait divergence between species in localities where the two species co-occur than in locations where only one species occurs, caused by either ecological or reproductive interactions between species.

Dobzhansky-Muller incompatibilities: epistatic interactions between alleles at different loci that are fixed independently in diverging populations and cause reduced fitness in hybrids.

Ecological speciation: occurs when barriers to gene flow between populations evolve as a result of ecologically based divergent natural selection. Ecological speciation can occur in any geographical setting.

Genetic accommodation: an evolutionary mechanism wherein a novel phenotype, produced through a mutation or environmental change, is modified into an adaptive phenotype through quantitative genetic changes.

Genetic assimilation: a special case of genetic accommodation, whereby an initially environmentally induced phenotype becomes a constitutively expressed trait.

Genetic drift: random change in the gene constitution of a population, caused by chance differences in survival or reproductive output of individuals.

Genomic neighbourhood: segment of the genome in which a locus resides with characteristics that potentially influence its expression and/or the probability of recombination or mutation, such as epigenetic modifications, gene density, GC content, and so on.

Homoploid hybridisation: the formation of a new population through hybridisation, without a change in ploidy compared with the parental species.

Linkage disequilibrium: non-random association of alleles at two or more loci.

Natural selection: a consistent difference in survival and/or reproductive success among phenotypic classes of individuals. Here, where we contrast natural and sexual selection, natural selection is considered to result from the fit of individuals to their environment, excluding the effects of interactions between the sexes.

Phenotypic plasticity: the ability of a single genotype to produce distinct phenotypes in response to environmental conditions.

Polyphenism: the occurrence of discrete alternative phenotypes in a population that are not to the result of different genotypes, but are caused by environmental influences.

Prezygotic isolating barriers: occur before, and hence prevent the formation of, hybrid zygotes. Mating might not occur because of differences in timing, habitat, behaviour or morphology, or gametic incompatibilities might prevent the fertilisation of the egg.

Postzygotic isolating barriers: operate after zygote formation by reducing either the viability or fertility of the hybrid offspring.

Reinforcement: the strengthening of barriers contributing to reproductive isolation between populations as a result from selection against unfit hybrid offspring.

Reproductive isolation: occurs when two populations produce fewer viable and fertile offspring than expected from their relative abundance in a locality.

Sexual selection: differences in reproductive success among phenotypic classes of individuals arising from variation in the ability of individuals of one sex to fertilise gametes produced by the other sex.

Speciation gene: a locus that contributes to a barrier to gene flow between a pair of populations or species or, in a more restrictive definition, a locus whose divergence contributed to the evolution of reproductive isolation between populations or species.

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into 13 general questions, which we offer as catalysts for discussion and future studies. Three central and interlocking research areas emerge (Figure 1): (i) elucidating the component mechanisms that drive the build-up of reproductive isolation; (ii) understanding the genetics and genomics of speciation; and (iii) connecting speciation processes more closely to research on patterns of biodiversity [4]. We believe that this framework is comprehensive even though our more-specific list of questions clearly is not, partly for reasons of space but also because even a large group such as ours has biases in the range of interests it represents. No doubt other speciation biologists will wish to add to our list.

Box 1. How do we define species and, thus, speciation?

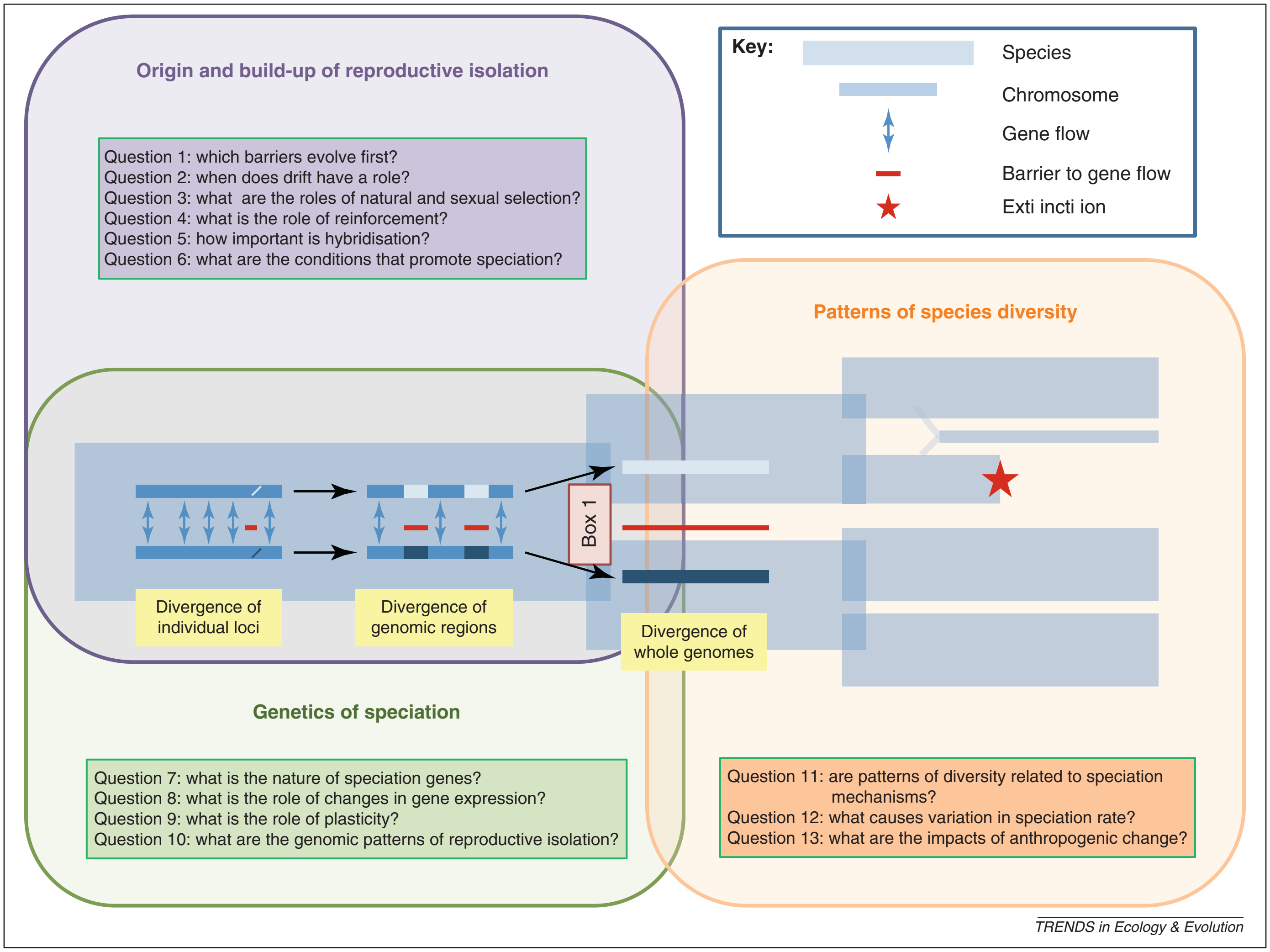
The debate on species concepts is often considered unproductive [3] with the biological species concept [148] being the predominantly accepted definition for sexually reproducing eukaryotes. Yet this relaxed (uncritical?) attitude is risky, because it can result in a breakdown in communication. For example, populations can fulfil distinct ecological roles (ecological species) while still exchanging genes (not evolutionary species) [149], whereas either symbionts or behavioural imprinting can render populations fully reproductively isolated (evolutionary species) without ecological divergence (not ecological species). Evolutionary biologists need to retain an objective criterion for species delimitation, such as complete cessation of sexually mediated gene exchange, to study the process of speciation in its entirety (Question 1), but the use of such a definition might require accepting that different types of biologist have to use different criteria. An alternative, more multidimensional, genome-focused concept of species might be warranted, given the continued progress in understanding genomic divergence (see Questions 7–10). Although several researchers (e.g. [150,151]) have made important inroads in this direction, a generally applicable concept of species does not yet exist. Throughout discussion on speciation research, the lack of a consensus definition should be kept in mind.

In this paper, we consider only sexually reproducing eukaryotes. Asexual eukaryotes and prokaryotes can be divided into phenotypically, genetically or ecologically defined units, which share some characteristics with species (see [23] for discussion). However, we have not considered them here because the processes underlying their origin and diversification are quite distinct.

The origin and build-up of reproductive isolation

Question 1. Which barriers contribute to reproductive isolation?

If we use the biological species concept (Box 1), then understanding speciation requires understanding where gene flow breaks down. The classic scheme for describing the origin of reproductive isolation is whether gene flow is prevented before ('prezygotic') or after ('postzygotic') hybrid zygote formation. Many studies cite prezygotic isolation as either a more important or earlier-evolving barrier to gene flow than postzygotic isolation and thus potentially more



Question 1: which barriers evolve first?
Question 2: when does drift have a role?
Question 3: what are the roles of natural and sexual selection?
Question 4: what is the role of reinforcement?
Question 5: how important is hybridisation?
Question 6: what are the conditions that promote speciation?

Question 7: what is the nature of speciation genes?
Question 8: what is the role of changes in gene expression?
Question 9: what is the role of plasticity?
Question 10: what are the genomic patterns of reproductive isolation?

Question 11: are patterns of diversity related to speciation mechanisms?
Question 12: what causes variation in speciation rate?
Question 13: what are the impacts of anthropogenic change?

Figure 1. Outstanding questions in relation to the processes of speciation and diversification in sexual eukaryotes.

important to the process of speciation. This view stems from: (i) many examples of speciation showing substantial prezygotic isolation (often behavioural mate discrimination in animals or pollinator differences in plants) but no known postzygotic isolation; and (ii) the fact that prezygotic isolation might, by its very nature, contribute more to the total barrier to gene flow than might postzygotic isolation at any stage in the speciation process simply because an organism must mate outside its own population before it produces sterile or inviable hybrid progeny [5]. However, this view does not properly account for extrinsic postzygotic isolation owing to differential adaptation, which might sometimes be the first step in speciation [6]. There are nontrivial issues in understanding the relative importance of different isolating barriers and some researchers argue that decisive evidence on whether pre- or postzygotic isolation is more abundant or important is lacking [3]. The problem is not simply a failure to survey the full range of possible components of isolation or the potential non-independence of different barriers that subsequently skews their relative contribution (e.g. [5,7]). In Box 2, we elaborate on issues that impact the quantification of two of the most commonly measured reproductive isolating barriers, one pre- and one postzygotic: behavioural mate preference and hybrid sterility.

Not only is there a problem in accurately quantifying even the most common isolating barriers, but also extensive heritable variation in both pre- and postzygotic isolation can exist in natural populations (e.g. [8]). One of the greatest challenges in quantifying reproductive isolation is taking this variation into account. Is it meaningful to average across variation among individuals or populations? Distinct phenotypes might be apparent but their underlying genetic architecture can vary, suggesting that selection produces rapid changes in isolation in some circumstances but not others. Future case studies should continue to tackle the essential, but unfortunately not glamorous, task of rigorously documenting all components of isolation and documenting the underlying genetic variation producing these traits (see Question 6).

Understanding the contributions of different components of reproductive isolation still leaves open the crucial question of their order of appearance [9,10]. Which barriers appeared first and initiated the speciation process? Which barriers are most likely to complete the process? Can barriers decay when conditions change (e.g. [11])? These questions can be approached by documenting contributions to isolation in many population pairs across a range of overall levels of isolation: the barriers that appear first will be present when overall isolation is low, the last barriers only when it is nearly complete.

Question 2. When does drift have a significant role?

Genetic drift and chance events (e.g. colonisation, mutation, chromosomal rearrangement and polyploidy) can be strong contributors to speciation processes (e.g. [12,13]), but the circumstances under which drift is a sole driver of speciation are limited [5], primarily by the impact of gene flow. However, drift might initiate speciation by providing the initial divergence on which selection subsequently acts and this interaction can contribute to the evolution of reproductive isolation even in the presence of considerable gene flow [14,15]. Drift might also act during reinforcement by providing the initial linkage disequilibrium between selected and mating traits (Question 4) [16]. Additionally, when speciation by divergent selection is initially facilitated by founder events, as must often be the case for the colonisation of new habitats, drift might contribute to adaptive radiations. Although there is limited theoretical and experimental support for founder effects [17,18], empirical evidence from natural populations is still scarce. Demonstrating a role for drift in speciation where selection also has a part is especially difficult because drift is used as a ‘null hypothesis’, making unambiguous evidence for its action almost impossible. However, an initial phase of drift might nonetheless strongly influence the probability and

Box 2. Measuring reproductive isolating barriers

Behavioural isolation appears straightforward to measure: what fraction of individuals mate with members of their own population versus another population? However, this apparent simplicity belies many ecological complexities. For example, in the wild, relative abundances and encounter rate can influence the opportunity for behavioural isolation. Moreover, observations are usually temporally discontinuous, so uncommon events might be missed, potentially resulting in a false conclusion about behavioural isolation. By contrast, in the laboratory, such ecological considerations can be controlled, but recent work has demonstrated that experimental design can dramatically affect behavioural isolation measures [152]. Genotype-by-environment interactions, such as influences of larval diet on adult behaviour [153], might influence both pre- and postzygotic isolation measures (see Question 9). However, for most species, there is a lack of detailed enough ecological data to understand what experimental conditions best reflect nature.

Quantifying hybrid sterility, a common postzygotic measure of reproductive isolation, is also problematic. In nature, assessing the relative fertility of hybrids obviously requires knowing that individuals are hybrid, as well as accurately quantifying their fitness. In laboratory studies, hybrid sterility is often quantified very crudely and often, but not always [154], limited to the F1 generation so that, if sterility is manifested in later generations, it is missed. Moreover, measuring the rate of hybrid sterility (and hybrid inviability) evolution is confounded by the genomic structure of taxa. For example, the heterogametic (XY or ZW) sex is more prone to hybrid problems earlier in speciation than is the homogametic (XX/ZZ) sex (Haldane’s Rule). Although this pattern is well documented, it renders any comparisons of the rates of evolution of postzygotic isolation between distantly related species far more difficult. Additionally, without any intrinsic difference in the rate of accumulation of hybrid incompatibilities, F1 hybrid female sterility or inviability will evolve faster if dosage compensation occurs by shutting down one X chromosome in the homogametic sex (as in humans) than if dosage compensation occurs by hypertranscription of the X in the heterogametic sex (as in *Drosophila*). Does this mean that postzygotic isolation provides a stronger barrier in the former category? Similarly, F1 males will evolve postzygotic isolation more slowly in taxa lacking degenerate sex chromosomes (see [155]). Once beyond the F1 generation, the genetic architecture will dictate how succeeding generations fare, and so there is no obvious way to use a single measure that quantifies postzygotic isolation. ‘Barrier strength’ in hybrid zones [58] can provide an overall measure of the difficulty of moving an allele from one genetic background to another, but it is not easily broken down into pre- and postzygotic measures. Ultimately, the degree of isolation between hybridising taxa varies across the genome, and signatures of that fact can be seen in the varying degrees of divergence in different chromosomal regions (see Question 10).

Box 3. The geographic context of speciation

The traditional separation of speciation processes into allopatric, parapatric and sympatric categories does not capture the complexity of spatial relationships that can occur between diverging populations. This is reflected in the further subdivisions that exist in the literature. For example, vicariant and peripatric forms of allopatry are distinguished based on the way in which an ancestral population can be divided up, whereas the term 'allo-parapatric speciation' acknowledges that speciation can have both allopatric and parapatric phases [3]. The potential for changes in spatial relations, and their consequences, is emphasised in [10,156]. Parapatry can include many different spatial patterns. At one extreme, two diverging populations might meet at a narrow hybrid zone. In the contact zone, there might be extensive mixing of gene pools but the vast majority of individuals, living outside the contact zone, have no risk of hybridisation and are not influenced by gene flow from the sister population. At the other extreme, diverging populations might occupy habitats that are intimately intermingled, such as the host plants of phytophagous insects. Even though gene flow between populations might be low, the probability of encountering a potential mate from the other population might be similarly high for all individuals. These differences might be crucial for the role of drift and selection in divergence and for the operation of processes such as reinforcement.

The spatial relationship between populations is not the only determinant of gene flow between them. This has led to a distinction between a geographic and a population genetic definition of sympatry [61,157]. It is possible for two populations to inhabit the same region but to have low gene flow because they mate in different habitats or at different times, for example. We agree with Fitzpatrick and co-workers [157] that, rather than attempting to redefine sympatry, it is best to concentrate on 'evaluating the biological processes affecting divergence'. The spatial context is one factor influencing progress toward speciation. It should not be considered a dominant criterion for the classification of speciation processes, but neither should it be ignored. Indeed, there are many open questions that remain, such as the spatial scale of speciation [56], the arrangement of habitat patches (e.g. [158]) or the abruptness of habitat transitions (e.g. [145]).

speed of speciation. Drift–selection interaction is an important area for future research and making use of invasive species to distinguish roles of bottlenecks and adaptation might be one way forward [19].

Divergence is harder to envisage when populations experience identical selection regimes, but in such situations there is also a role for drift. Dobzhansky-Muller hybrid incompatibilities can accumulate when populations drift to different fitness optima under stabilising selection [20] and such hybrid dysfunction might be initiated by neutral or nearly neutral genetic changes [21]. However, such changes might be the result of the stochasticity of mutation rather than drift *per se*.

The extent of gene flow between diverging populations clearly influences the role of drift and can vary over the speciation process, for example being very low during an allopatric phase and higher following secondary contact (Box 3). Thus, understanding the potential impact of drift requires characterising the amount of gene flow during divergence, a difficult problem that is an important challenge in speciation research. Improved methods for inferring population histories [22], such as Approximate Bayesian Computation [23], tests based on the directions of allelic effects [24], and extension of the Qst/Fst comparison approach [25] might help in this. Future work should aim to infer the roles of gene flow, drift and selection in overall divergence and the fixation of individual speciation genes (see Question 7) and compare their contributions across speciation events in different taxa and environments.

Question 3. What are the relative roles of natural and sexual selection?

Both natural and sexual selection have been suggested to be drivers of speciation. Divergent or disruptive selection arising from habitat heterogeneity has been highlighted recently as a dominant force driving population divergence, supported by many examples of 'ecological speciation' [6]. The focus has been on viability selection, but sexual selection can clearly be influenced by environmental factors [5]. Sexual selection [12,26,27], including sexual conflict [28,29], has a high probability of generating reproductive isolation by virtue of its influence on the evolution of sexual traits. However, some studies suggest that changes in male signalling and female preference might not be powerful enough on their own to shape reproductive isolation between populations, and traits influencing mating success are also subject to viability selection [30]. Gene flow might alter the roles of sexual and natural selection: it is hard to envision stable divergence in sympatry without ecological divergence (but see [31]), whereas sexual selection alone might result in strong isolation between populations that exchange few genes. Sexual selection might be less decisive in speciation than might natural selection, given that species need to be ecologically differentiated to coexist [5]. Nevertheless, experimental evidence has demonstrated that, in the absence of natural selection, but in the presence of sexual selection via conflict, assortative mating can sometimes evolve ([32] but see [33,34]). Whether this can be sufficient to complete speciation is unknown. These arguments suggest that categorisation of some speciation processes as 'ecological', and others as driven by sexual selection, is unhelpful [5,35]. Indeed, recent models support the idea that a combination of both natural and sexual selection is particularly powerful to initiate and complete speciation [36,37]. The focus should be on determining the contributions of different modes of selection to the origin of reproductive isolation, recognising that a clear separation between natural and sexual selection is not always possible.

It is difficult to quantify the relative contributions of natural and sexual selection to speciation from the frequency that these topics appear in the literature, because of potential publication biases. A meta-analysis of whether sexual selection contributes to speciation found no evidence of bias [38]. By contrast, Hendry [35] has suggested that the evidence supporting the nearly ubiquitous idea that speciation requires natural selection might be affected by an interpretation bias, resulting in a high number of publications asserting positive evidence for ecological speciation without robust inference. Thus, natural selection might appear to contribute more frequently to speciation than does sexual selection, regardless of whether it really does. The reason for this apparent publication discrepancy is unclear. A possible explanation could be that sexual

Table 1. Criteria to identify the roles of natural and sexual selection in speciation[a]

Criterion	Evidence for a role of sexual selection	Evidence for a role of natural selection	Comments
Divergence among populations or closely related species	Substantial differences in male sexually selected traits	Differences in ecological traits	Most common criterion but not sufficient alone to identify the source of selection. Sexual dimorphism is not necessarily a reliable indicator of sexual selection
Correlations with fitness components	Variation in male trait is correlated with female preference, and with variation in mating success	Differences in traits are correlated with fitness, with crossing pattern over environments	Measurement of female preferences in choice experiments might be more difficult to achieve than transplant or controlled environment experiments testing local adaptation
Parallel reproductive barriers between similar pairs of environments	Not expected (unless female choice evolves through sensory drive, where natural and sexual selection operate together)	Expected	Systematic comparisons between suitable population pairs have been limited to candidate examples of ecological speciation
Level of genetic differentiation	Low overall, suggesting rapid divergence	Increases with environmental differences	
Hybrid viability and fertility	Little or no reduction (but hybrid fitness can be reduced by improper signals or preferences)	Reduction of hybrid viability or fertility is environment dependent	
Character displacement on secondary contact	Might occur for reproductive traits	Expected for ecological traits	

[a] Based on [35,169].

selection as a driver of speciation might be underestimated simply because it is potentially more difficult to verify (Table 1).

Categorising the drivers of speciation as either ecological or sexual is complicated further by variation in the temporal action of these selective pressures. Given that speciation signatures change over evolutionary time, later processes might mask the initiating steps. For example, sexual selection could be involved early in the emergence of reproductive isolation, but not in the maintenance of coexistence, which requires the action of natural selection [5]. The signature of sexual selection as a driver of speciation decays over time [39] and might be weakened by greater extinction rates caused by sexual selection [40].

Future work needs to consider the criteria for demonstrating a role for sexual compared to natural selection, paying attention to the stages in the speciation process at which each could operate singly or in combination [30]. It would be beneficial to make use of both experimental studies on partially isolated population pairs and comparative analyses across related species.

Question 4. What is the role of reinforcement?

Reinforcement remains a contentious mechanism contributing to speciation. Recent work has established that the opportunity for reinforcement might be considerably broader than was thought previously [16], as illustrated by recent examples [41,42], but fundamental questions over its action and frequency remain.

A major difficulty in quantifying the role of reinforcement in divergence is that the crucial pattern predicted by reinforcement (i.e. increased isolation in areas of sympatry compared with areas of allopatry) might decay over time as a result of gene flow and can also result as a byproduct of other processes, such as ecological character displacement. Studies that can document the frequency of hybridisation between populations across time (e.g. [43]) can discriminate these processes, but such opportunities are rare. A current challenge is how to move beyond studies of individual species to elucidate general patterns concerning the frequency and importance of reinforcement in nature. Surveys following the classic example of Coyne and Orr's [44] *Drosophila* analysis represent one way forward, but they need to be combined with finer-scale dissection of process in as many cases as possible. Relating reinforcement to patterns of sexual selection within species is also important, because reinforcement can involve preferences driven by the advantage of mating with genetically compatible partners.

One unresolved issue is the stage at which reinforcement acts. Is it important only in the later stages of speciation? Is it capable of completely eliminating hybridisation? The strength of selection for reinforcement is weak at early stages of divergence, because hybrid fitness is only slightly less than parental fitness, but it also diminishes later in the speciation process as hybridisation becomes rare (e.g. [45]), whatever the fitness of hybrids. Future studies might be able to resolve the timing of selection on individual loci underlying reinforced signals or preferences, and so approach this type of question.

Question 5. How important is hybridisation in speciation?

Hybridisation is an important event in the history of plant species [46]. Evidence for rapid speciation in plants, particularly by allopolyploidy, is strong [47], whereas speciation involving polyploidy is relatively rare in animals. A more controversial process, in both animals and plants, is the homoploid hybrid origin of new lineages [48]. Barriers that putatively limit speciation via this mechanism have recently been discussed [49], but a key problem to overcome is lack of reproductive isolation from parental populations. A solution to this problem must include sufficient ecological separation from the parental species (e.g. novel niche occupation [50] or transgressive variation), which appears to be common [51,52]. In contrast to homoploid hybridisation where the new lineage is initially close to a 1:1 mixture of the parents, hybridisation can be a source of genetic diversity allowing one or a few selectively favoured traits to introgress from a related species and contribute to the success of a novel lineage with no general mixing of parental genomes. *Heliconius* butterflies might provide an example [53]. Clearly, processes other than hybridisation are necessary to complete speciation in either scenario; hence, hybridisation should be viewed as a contributor to the origin of reproductive isolation rather than a sole cause of speciation.

Determining the predicted differences in genomic signatures between general mixing and limited introgression is difficult because historical gene flow and incomplete lineage sorting can cause similar patterns of shared genetic variation [22]. Another obstacle is demonstrating that introgression results in adaptation and contributes to reproductive isolation. Advances need to identify analytical techniques that can distinguish current and past hybridisation from other genetic effects that result in shared variation across taxa. The controversy regarding genomic signatures of hybridisation during human–chimpanzee speciation [54,55] demonstrates that progress will not be easy.

Question 6. What are the environmental and genetic conditions that promote speciation?

Questions 1–5 have considered the steps that might lead to complete reproductive isolation and the mechanisms that contribute to its build-up. However, why do some populations eventually evolve complete reproductive isolation whereas others do not [9]? Are there common features, either in the environments of species or their genetic make-up, that make speciation more probable in some cases than in others?

Reproductive barriers between populations might evolve as a result of divergent selection overcoming gene flow and so the intrinsic dispersal tendency of organisms might have a high impact on the probability of speciation [56]. Moreover, whereas strong selection on one or a few traits might be more effective at causing adaptive divergence in the face of gene flow, weaker selection on multiple traits ('multifarious selection') might be more effective in driving speciation by generating a more general barrier to gene flow [57]. Previous theoretical work supports this distinction [17,58] and there are new opportunities in the genomic era to distinguish these possibilities empirically (see Question 10).

Ecological divergence is usually regarded as a prerequisite for the evolution of reproductive isolation through assortative mating (Question 3), reinforcement (Question 4) and/or sexual preference for condition-dependent ornaments [36]. Ecological adaptations can also act as 'magic traits' [59], which reduce gene flow between populations owing to the patchiness of ecological resources on a scale comparable to dispersal distance and/or owing to the direct effects of adaptation on mating preferences [60,61]. Kirkpatrick and Ravigné [62] suggest that sexual selection is more effective than natural selection in generating disequilibria and, hence, new species. They show that the most effective combination in driving speciation is a one-allele mechanism (same allele fixed in both populations) driven by direct selection on both the sexually selected characters and traits important in survival or fecundity.

Populations can also diverge when different mutations are fixed in separate populations adapting to similar environmental conditions ('mutation-order' speciation [63]). Although populations connected by gene flow are less likely to diverge, because favourable mutations in one population can spread to other populations (e.g. [6]), incompatible mutations might arise in different parts of the range of a species. Range contraction and expansion might then generate hybrid zones, bringing together clines of individual loci that together provide substantial barriers to gene flow [64]. Mutation-order speciation requires strong divergence with very low gene flow. This kind of speciation can be promoted when incompatible mutations have similar fitness advantages, when less fit mutations arise slightly earlier in evolutionary time than more fit alternatives, and when allopatric divergence occurs before secondary contact [65]. Mutation-order speciation becomes the most probable speciation mode in situations where large populations are in very similar ecological conditions and evolve reproductive isolation where the effects of drift are small. However, distinguishing these different speciation processes requires much greater knowledge of the genetic basis of reproductive isolation than is currently available (Questions 7–10). It also requires discriminating predictions from the different processes, ideally allowing each process to be identified by a positive signature, and not just by elimination. Generating such predictions is a crucial theoretical challenge in speciation research [65].

The amount and quality of genetic variation might set limits to speciation. The likelihood and rapidity of speciation are influenced by whether variation generating reproductive barriers derives from new mutations or from standing genetic variation, which can be enhanced by hybridisation and introgression. Evolution of reproductive barriers might be faster in the former case [66], but new mutations can have a prominent role in speciation, especially in large populations and/or those with a large mutational target (e.g. multiple polygenic traits). Once the earliest genetic differences have accumulated between populations, subsequent mutations might be favoured in one, but not the other population because of their epistatic interactions with the genetic background [67]. Such incompatibilities can be expected to accumulate at an accelerating rate (the 'snowball effect') [68], but empirical data supporting this pattern are equivocal [69,70]. The roles of standing variation, new mutations and the accumulation of incompatibilities are certainly worthy of further study.

Genetic and genomic signatures of speciation

Question 7. What is the nature of speciation genes? Can speciation result from specific genes?

Research on speciation genes asks which genes and gene networks contribute to speciation. However, different researchers use different criteria to define or establish this association. It has recently been argued that only genetic changes contributing to an increase in reproductive isolation should be accepted as 'speciation genes' [71]. Distinguishing such genes might be difficult, especially for genes that contribute early in an ongoing speciation process. Nevertheless, using this more constrained definition will help guide future work.

Speciation genes can be associated with any form of reproductive isolating barrier, but most is known about those related to hybrid dysfunction [21]. Incompatibility between nuclear and mitochondrial genes associated with reproductive isolation is well known in plants [72] but has recently been recognised in animals [73,74]. Recent work has indicated that genetic changes causing hybrid incompatibility might be fixed for non-adaptive reasons (i.e. duplicate gene silencing or coevolution of meiotic drivers and their suppressors [21,75]). This emphasises the need for future work to connect speciation genes to the process that caused their divergence. However, more genes need to be found, in a wider range of taxa, for additional components of isolation, with understanding of gene–environment interactions and with clear evidence for their role in the evolution of reproductive isolation. Only then will it be possible to make generalisations about the types of gene causing incompatibility and the modes of selection causing their divergence.

How often prezygotic reproductive isolation is a result of changes in many rather than a few genes, the nature of such genes, and how many substitutions are required per locus remain open questions. Some progress has been made in identifying key genes, especially for chemical signalling [76–78], but in many cases analyses remain at the quantitative trait locus (QTL) level (e.g. [79]). Only when future studies can document the molecular basis of isolating traits will there be solid grounds for linking genetic changes to the processes that drive divergence. One possible approach is to examine candidate gene families for signs of positive selection or patterns of gain and loss (e.g. [80–82]). Although comparative genomics (e.g. [83]) is a seductive approach, we caution against assuming that evidence for rapid evolution is equivalent to evidence for a contribution to reproductive isolation. Once identified, such candidate loci need to be tested for their role in speciation and we recommend that sequencing should not be allowed to run too far ahead of functional analysis.

Question 8. What is the role of changes in gene expression and in genomic processes?

Evolution of gene expression has been shown to have a significant role in speciation [84], although the underlying substitutions responsible have only rarely been identified [85]. For example, transcriptional profiling has been successfully exploited to identify candidate genes that show miss-expression in hybrids, including several species of *Drosophila* [86–88] and whitefish *Coregonus* [89]. As well as gene-by-gene analyses, regulation of sets of genes assembled into functional networks is crucial [90]. This network approach will also help to overcome the problem that genes miss-expressed in hybrids might be downstream targets of the loci that actually cause reproductive isolation.

Current evidence suggests that expression divergence is predominantly the result of changes in *cis*-regulatory rather than coding factors (e.g. [91,92]). However, some of this evidence is ambiguous [93] and other influences, such as genomic neighbourhood [94], might be important. It remains a challenge to discover whether differences in regulatory genomic regions are more (or less) important than differences in coding sequences, specifically for reproductive isolation. Moreover, gene duplication and loss, epigenetic effects, small RNAs, transposable element activity [95], creation of new exons or introns [96] and perhaps many other unsuspected genomic processes might contribute to speciation. New sequencing technologies make expression (and genomic) analysis possible in a much wider range of species (e.g. [97]), but such studies must also link the changes detected to their possible role in speciation, which is potentially much more demanding. A narrow-minded approach about the genetic mechanisms that potentially contribute to speciation will not advance the understanding of speciation.

Question 9. What is the role of plasticity?

Phenotypic plasticity has been considered by some researchers as an 'obstacle' for the diversification and formation of new species, for example because it weakens selection for local adaptation. However, phenotypic plasticity might promote novel phenotypes, divergence, colonisation of new habitats and eventually speciation [98,99]. For example, polyphenism can create different adaptive phenotypes within a population and these phenotypes can undergo genetic accommodation and assimilation ([98,100], see also [101]).

The role of phenotypic plasticity in the evolution of reproductive isolation has not been widely studied. Plasticity might disrupt the link between mating-signal value and individual quality, which might in turn erode female preference [102], or it might enhance the link through condition dependence, which could facilitate population divergence. Likewise, the frequency of environmental change might affect whether plasticity impedes or promotes population divergence [103]. Condition-dependent habitat choice might also contribute to reproductive isolation [104]. Learning is another form of plasticity whose potential importance for speciation has been recognised [105] but which deserves further study, especially in groups other than birds. Future work will need to establish the extent to which plasticity is involved in facilitating or obstructing population divergence, the relative contribution of plasticity in different types of trait to reproductive isolation, and whether such patterns vary across taxa.

Question 10. What are the genomic patterns of reproductive isolation?

Recent genomic studies have shown that, in the early phase of divergence, reproductive isolation might be concentrated around a small number of locally adapted genes [106,107]. Later reproductive isolation is expected to progress from a genetic mosaic pattern to genome-wide divergence, especially after reproductive isolation is complete. Dissecting this progression is a priority where new sequencing technologies offer enormous potential. Combining genomic surveys with experimental tests (e.g. [108]) will be important because genome scans might be predisposed to identify small areas of high divergence. Barton and Bengtson [109] have shown that, for gene flow to be significantly reduced over much of the genome, hybrids must be substantially less fit and the number of genes involved in building the barrier must be so large that the majority of other genes become closely linked to some locus that is under selection. The effects of selection on specific loci can spread to other parts of the genome through restricted recombination, through general reduction in gene flow owing to reduced fitness of immigrants and their offspring, and/or through the evolution of assortative mating [110,111].

Chromosomal rearrangements can contribute to speciation (Box 4) and inversions in particular have received much attention. Genes that contribute to pre- and postzygotic isolation tend to map to inversions that distinguish species of sunflowers [112,113], monkeyflowers [114] and *Drosophila* [115,116] but in the apple maggot fly *Rhagoletis pomonella*, inversions only accentuate divergence that occurs genome-wide [108]. Moreover, genes involved in reinforcement might be found in inversions (and on sex chromosomes [117,118]). This pattern is consistent with theory because these factors can promote reinforcement by protecting linkage disequilibrium between genes involved in increased sexual isolation and those responsible for reduced hybrid fitness [119].

Box 4. Chromosomal arrangements involved in speciation

Several kinds of chromosomal rearrangement are involved in speciation. Duplications can generate new genes that cause reproductive incompatibilities when they diverge [159]. An example is *Xmrk*, a gene in the platyfish that was the first example of a Dobzhansky-Muller incompatibility to be genetically characterised [160]. Fusions and reciprocal translocations that differ between species can cause problems in meiosis when heterozygous and so cause postzygotic barriers [3]. Inversions can likewise have underdominant effects on fertility that contribute to postzygotic isolation. Furthermore, because inversions suppress recombination when heterozygous, they tend to accumulate genetic differences that contribute to both pre- and postzygotic isolation [161].

Although it is clear that rearrangements can contribute to speciation, it is less evident how often they do so and what mechanisms are involved. In mammals, there is a correlation between the rates of speciation and chromosomal rearrangement [162], but the cause and effect have not been proven. Many sister species differ in rearrangements and produce hybrids that have reduced fertility because of meiotic problems [163]. Still to be determined is whether the rearrangements were involved in the process of speciation or were established later. Finally, inversions might contribute to the maintenance of young species not through direct fertility effects but by creating large linked blocks that do not break up upon hybridisation (e.g. [164]; see [165] for an overview of recent debates about the role of rearrangements in speciation).

However, there is still the need to distinguish two ways in which such patterns could arise: either inversions were somehow established in populations that were already partially isolated, and alleles that further increased isolation accumulated within the inversions, or loci that generated partial reproductive isolation between populations favoured the spread of an inversion that captures them [120]. Sister species in several taxa show more divergence in DNA sequence in regions of the genome that have been rearranged [121]. This intriguing observation is consistent with both of the scenarios, but is also consistent with alternative explanations, some of which do not involve selection [121,122]. Clearly, there remains a need to understand how initial localised genomic divergence evolves towards completion of reproductive isolation.

Connecting speciation and biodiversity

Question 11. How are biodiversity patterns related to speciation mechanisms?

The biodiversity patterns seen today are the result of the interplay of speciation, range changes and extinction. Among these, speciation is the only process generating ‘new’ diversity. A good understanding of the mechanisms of speciation is of obvious importance for understanding the dynamics and patterns of biodiversity. In reverse, biodiversity patterns can provide important clues about the underlying processes, including speciation, that generated the patterns.

Numerous theories have been proposed to explain biodiversity patterns such as the latitudinal diversity gradient (e.g. [123,124]). Although speciation has a crucial role in all of these theories, it is typically only included in an indirect way. For example, the Metabolic Theory of Ecology [125] and the Neutral Theory of Biodiversity [126] presuppose that speciation rates are proportional to mutation rates and population sizes (e.g. [127]). These assumptions might be justified for certain speciation mechanisms but not for others, and they are not supported by the scarce empirical evidence for the relationship between speciation rates and ecological or genetic parameters (e.g. [128]). Incorporating realistic speciation scenarios in ecological biodiversity theories remains a major challenge.

To infer process from patterns, phylogenetic methods can be useful. For example, Venditti and collaborators [129] analysed the frequency distribution of branch lengths of phylogenetic trees. From these data, they concluded that speciation rates tend to be remarkably constant, thus excluding various speciation scenarios. Phylogenies have also served to infer that sexual conflict could be a driving force of speciation [38,130] and that ecological characteristics account for diversification in birds [131]. In addition to phylogenies, species abundance distributions (e.g. [132]) and the geographic range distributions of sister clades (e.g. [133]) have been used to infer speciation mechanisms from diversity patterns. However, all these methods are based on assumptions that might not hold in the context of speciation. For example, phylogenetic methods tend to assume that evolutionary processes are homogeneous in time, whereas speciation models demonstrate that, around speciation events, selection can switch rapidly between directional, stabilising and disruptive modes [134].

Similarly, geographic approaches to speciation tend to assume that past geographic distributions can be inferred from present-day distributions, an assumption that is rarely met [135]. The validation of the techniques to infer speciation processes from diversity pattern needs to be seen as a higher priority than it is today.

Question 12. What causes variation in speciation rate and duration?

Patterns of diversity are influenced by speciation rate and understanding the causes of variation in speciation rates among taxa, habitats or regions is a major research challenge. In addition to speciation rate (i.e. the number of branching events per lineage per unit time), the time it takes one species to branch into two reproductively isolated groups (i.e. speciation duration) also varies. Direct estimates of the latter have followed the trend initiated by Coyne and Orr's [44] study on *Drosophila* and subsequently applied to other taxa [136]. Collectively, these studies suggest rather substantial variation within and between taxa in the time for completion of reproductive isolation, but offer little explanation for such variation (but see Question 6 and [136]). They also suffer from incomplete surveys of possible components of isolation (Question 1). Thus quantifying speciation duration and explaining variation in that duration remain important open questions.

Whether speciation duration and rate are coupled is also an open question. Some conditions might influence both. For example, the rate of adaptive divergence should covary with ecological opportunity [137,138]; natural selection can accelerate divergence [29] and this, in turn, has been linked to the extent of reproductive isolation [136]. Therefore, empty environments might lead to both rapid and frequent speciation. However, they might lead to frequent initiation of speciation, which is not completed [9], either because gene flow persists or because the divergent population does not persist (perhaps owing to specialisation [139] or to environmental change [140]), so decoupling duration and rate. Sexual selection (Question 3) is usually predicted to decrease speciation duration and increase speciation rate, but it might not always do so [28]; it might also increase extinction rate, resulting in a weak phylogenetic signature. However, the total duration of speciation might be determined by intrinsic factors, such as genetic architecture, trait diversity [9], extent of phenotypic plasticity (Question 9), levels of ploidy [47] and epigenetic processes, such as genomic imprinting (e.g. [141]). It is likely that the interplay of extrinsic and intrinsic factors influences speciation duration and rate; thus, they should be studied jointly.

As with understanding patterns of speciation, phylogenetic methods can also be used to test hypotheses about speciation rate, including the assumption that it is constant [129], but might suffer from model simplification. A major problem is to distinguish the impact of candidate ecological or genetic factors on speciation rate from their impact on extinction rate (e.g. [131]). Nowhere is this more evident than in the debate about the origins of the latitudinal diversity gradient [142,143]. Solving this problem will require both improved comparative methods and better understanding of the mechanics of speciation and extinction.

Question 13. What is the impact of anthropogenic change?

Humans have undoubtedly increased extinction rates and are the ultimate cause of the current biodiversity crisis. How human activity promotes or prevents speciation,

Box 5. Where are we and where should we go?

What is the best way forward to address the newer questions highlighted here? Evolutionary biologists use a variety of techniques to understand speciation. Experimental approaches can be powerful to distinguish the roles of different sources of selection, but are limited to some taxa and to short timescales. Comparative approaches examine much longer timescales and cannot discern details. Studies of divergent populations that have not completed speciation can be powerful but are subject to doubt about the completion of speciation [166]. These problems pervade most aspects of speciation research. Additionally, much research on the genetics of speciation has focused on model systems. New technologies and analytical tools now make it possible to extend these to a much wider range of organisms, representing different speciation processes and/or levels of divergence. We suggest that new insights into the genetic processes involved in speciation would be especially profitable if they examine the genomics of diverging populations, the genomics of hybrid zones, the genetic architecture of genes and networks underlying isolation, and the sources of variation in gene expression.

Although molecular genetic studies are enticing (and necessary), such studies require abundant knowledge of the ecology and population biology of the study taxon for them to be truly valuable. Moreover, we suggest that speciation research is now at a stage where systematic documentation of the contribution of different processes is more important than the collection and categorisation of isolated examples. Such data require identification of whole clades that can be analysed genetically, ecologically and behaviourally, considering all aspects of reproductive isolation within a strong phylogenetic context. Ultimately, many such clades need to be analysed, including both species-rich and species-poor groups, as well as taxa that are important components of biological diversity but rarely studied by speciation biologists (e.g. nematodes and fungi [167]).

Is there ever a time to say that some questions have been conclusively answered and should not be a significant part of the future research agenda [168]? The history of speciation biology has been largely dominated by arguments about: (i) allopatric versus sympatric speciation; (ii) patterns in the magnitude or genomic location of genes influencing reproductive isolation; and (iii) the relative importance of ecological adaptation or sexual reproduction. Evolutionary biology has gained much from these debates, and many important lessons have been learned. The extended lifespan of many debates in speciation biology is perhaps a source of frustration, preventing necessary progression. So what research issues have had their moment in the sun in speciation biology? We argue that debates over geographic categories of speciation process are unproductive. The criteria for 'proving' sympatric speciation can be made so exacting that an unambiguous case is almost impossible. However, there is clear evidence that ecologically important divergence, perhaps beyond the point of no return, can occur despite gene flow. Debates over the importance of a few genes of large effect versus many genes of small effect, or of coding versus noncoding divergence, should now be of historical interest only. The same is true for characterising speciation via sexual versus natural selection. These polarised arguments need to be replaced by more productive exploration of the relative importance of the different processes, and how they interact.

however, is less clear. For example, introduction of exotic plants might promote diversification and cause speciation by host shifts in herbivores [144]. Habitat disturbance can also reverse speciation [11,145]. Fragmentation of natural populations might promote speciation by impeding gene flow for some organisms, whereas human-aided dispersal might prevent or break down geographic isolation in others. In addition, continued anthropogenic changes might increase the occurrence of secondary contact and therefore the opportunity for reinforcement (Question 4 [146]. Different taxonomic groups might be more (or less) susceptible to such changes. Although there are some studies documenting rapid evolutionary responses to anthropogenic changes [147], such work is in its infancy. Uncovering the processes that have generated current biodiversity is no longer enough; future work should also seek to understand how speciation mechanisms might be impacted by rapid environmental change. In its turn, the study of responses to rapid anthropogenic change will help to understand natural speciation and extinction events

Concluding remarks

Clearly, there are many fascinating questions about speciation that we have not been able to include in this overview. Some of them appeared in our initial list (see the supplementary material online) and have been excluded only for reasons of space; others have no doubt been missed because we cannot represent all possible approaches to the problem. However, we also feel that some traditional foci of speciation research have reached a stage where they can now be left behind (Box 5). Arguably, every speciation event is unique. The common ground cannot be found by trying to force these events into categories, but it might be reached by focusing on the evolutionary forces, ecological circumstances and genetic mechanisms that they share. We are better placed now than ever before to move from a narrow perspective of speciation biology based on a handful of organisms and really start taking advantage of the endless forms of developing species. The biology of speciation can now enter a new era.

Online discussion forum

There is an online discussion forum linked to this article at discussions.cell.com.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tree.2011.09.002.

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