Dualism and conflicts in understanding speciation

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Summary
Speciation is a central but elusive issue in evolutionary biology. Over the past sixty years, the subject has been studied within a framework conceived by Ernst Mayr and Theodosius Dobzhansky and subsequently developed further by numerous other workers. In this "isolation" theory, the evolution of reproductive isolation is a key element of speciation; natural selection is given only secondary importance while gene flow is considered prohibitive to the process. In this paper, I argue that certain elements in this approach have produced confusion and irreconcilability among students of speciation. The more prominent debates in speciation (i.e., the species definition, sympatry/allopatry, and the role of reinforcement) all derive from an inherent conflict between the "isolation" theory and Darwin's "selection" view on species and speciation (in which disruptive selection is crucial). New data, mainly from field ecology, molecular population genetics, laboratory studies with *Drosophila* and computer analysis, all suggest that the isolation theory may no longer be the most desirable vantage point from which to explore speciation. Instead, environmental selection in large populations, often unimpeded by ongoing gene flow, appears to be the decisive element. The traditional preoccupation with reproductive isolation has created gaps in our knowledge of several crucial issues, mainly regarding the role of environmental selection and its connection with mate selection. *BioEssays* 22:1134–1141, 2000.

Introduction

Speciation, the evolution of new species, is a central but unresolved issue in evolutionary biology.[1] What is the essence of speciation? What geographical conditions are required for it to happen? What evolutionary forces are crucial? Many answers have been given to these questions and often appear irreconcilable.[2] This has given rise to the conviction that speciation is a very multifarious phenomenon, which defies any generalisation. As I will argue in this paper, this confusion stems largely from a conflict between two theories on speciation that have existed side-by-side for the past sixty years. The first of these theories is the one put forward by Charles Darwin in 1859.[3] The second is the theory developed as part of the Modern Synthesis in the 1930s and 1940s. Since the theories differ chiefly in their emphasis on which factor drives populations apart, I will refer to them as the "selection theory" and the "isolation theory", respectively.

I will first recapitulate some aspects of the historical development of speciation theory, outline the basic tenets of both views, and highlight the conflicts between them. Then I will review three prominent debates related to speciation and argue that all are reflections of those conflicts. At the same time, I will describe recent data from field ecology, molecular population genetics, laboratory experiments with *Drosophila* and computer analysis, which suggest that a modernised version of Darwin's view is more likely to bring progress in the field than an emphasis only on the isolation theory.

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The conflict

Species and speciation form the basis of one of the longest-standing debates in biology. Dedicated attempts to define species were made as early as the 17th century.[4–6] No single early author, however, devoted as much time to it as Darwin, whose expertise in taxonomy made him the foremost authority on species in the mid-19th century. In *On the Origin of Species by Means of Natural Selection*, he elaborated the point that species are no more than "well-marked varieties", and that the term was "arbitrarily given for the sake of convenience to a set of individuals closely resembling each other".[3] He added that the "search for the undiscovered and undiscoverable essence of the term species" was in vain, as it was an attempt at "defining the undefinable".[7]

Most present day biologists consider Darwin's opinion outdated and mainly accept the "biological species concept" (BSC). The BSC, which was developed during the 1930s by Ernst Mayr and Theodosius Dobzhansky, hinges (unlike Darwin's concept) primarily on reproductive barriers. Mayr defined species as "groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups".[8] Dobzhansky consequently applied the BSC to define the process by which species arise (i.e., speciation) as "that stage of the evolutionary process at which the once actually or potentially interbreeding array of forms becomes segregated into two or more separate arrays which are physiologically incapable of breeding".[9]

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It is important to realise that the BSC was not primarily intended as a convenient criterion for sorting taxa. Instead, it was an essential part of a multidisciplinary theory of speciation. This theory developed in a number of logical steps. Mayr's vast ornithological experience with geographic variation and endemism in New Guinea and Polynesia, and similar data from other groups of organisms, had convinced him that geographical isolation (allopatry) was cardinal to the speciation process. His 1942 book *Systematics and the Origin of Species*, was intended to show that the "crucial process in speciation is not selection [. . .], but isolation".[10] The fact that isolation was crucial meant that the processes responsible for allopatric differentiation would break down under gene flow. So, sympatry would only be possible once reproductive isolation had evolved. In the absence of reproductive isolation, two differentiated populations would fuse again upon secondary contact. Therefore, reproductive isolation needed to be the decisive criterion for what constitutes a species, and the evolution of reproductive isolation would define the point where speciation has been completed.

The processes responsible for generating reproductive isolation among populations were considered to be a subtle combination of genetic drift, natural selection, and epistasis, acting in small "peripherally isolated" populations. Mayr proved (1954, 1963) that, under the right circumstances, the combined effects of these forces could produce new coadapted gene complexes with reconstituted reproductive systems, i.e., new species under the BSC.[4,11] So, the theory of speciation developed by Mayr and Dobzhansky relies almost exclusively on the evolution of reproductive isolation for explaining the origin and maintenance of species. To many biologists, the development of this theory was an improvement on Darwin, who had not realised the importance of reproductive isolation and hence lacked a clear theory on speciation.

Both these claims about Darwin, however, are not entirely correct. Contrary to popular belief, Darwin was well-aware of reproductive isolation between species. For example, he starts chapter 8 of *On the Origin of Species* with: "The view generally entertained by naturalists is that species, when intercrossed, have been specially endowed with the quality of sterility, in order to prevent the confusion of all organic forms".[3] Darwin, however, knew that hybridisation is common among many groups of animals and plants, without affecting the distinctness of species. This was one of the reasons why he did not consider reproductive isolation of crucial importance, writing that "neither sterility nor fertility affords any clear distinction between species or varieties".[3] To Darwin, then, speciation (or, as he called it, "divergence of character") was not brought about by the evolution of reproductive barriers, but by a mechanism that would force a single species in two directions, reproductively isolated or not. This mechanism was natural selection, which would not just be able to make a single species change by adaptation, but also to make a single species split in two.

Darwin observed that an assemblage of species is more efficient at exploiting a patch of habitat than a single species. By analogy, he reasoned that, under conditions of severe competition, natural selection will favour those individuals within a population that have the most extreme phenotypes, and therefore suffer the least from competition with relatives. "Consequently, I cannot doubt that in the course of many thousands of generations, the most distinct varieties of any one species [. . .] would always have the best chance of succeeding and of increasing in numbers, and thus of supplanting the less distinct varieties; and varieties, when rendered very distinct from each other, take the rank of species".[3]

The main difference between Darwin's view and the one elaborated by Mayr and Dobzhansky, then, is the role of natural selection. To Darwin, natural selection could make a single population change to suit a changing environment (adaptation) or it could force a single population in two, to better exploit the available niches (speciation). Mayr and Dobzhansky, in contrast, decoupled adaptation from speciation; environmental selection is a causative agent only in adaptation. As I hope the following paragraphs will show, much of the confusion about speciation is the result of the fact that the isolation theory has become embedded in the selection-based neodarwinian school. Three conflicts in evolutionary biology are probably the direct result from this irreconcilability. These are the species definition, the allopatry/sympatry, and the reinforcement debates.

The species definition debate

In Mayr's writings, two views on species appear. The first is that all individuals of a species share the same well-integrated complex of epistatically and pleiotropically interacting genes. This is the species *concept*, and Mayr writes that the evolution of two well-integrated gene complexes from a single ancestral one is "the essence of speciation".[4] At the same time, however, the biological species *definition* makes no mention of gene complexes, but rather of devices for reproductive isolation. Consequently, Mayr can also be found writing that "speciation is characterized by the acquisition of these devices".[4] What transpires is that Mayr did not claim that reproductive isolation per se was essential, but that new gene complexes can not evolve nor persist before such barriers to gene flow were in place: "Reproductive isolation refers to the protective devices of a harmoniously coadapted gene pool against destruction by genotypes from other gene pools".[4]

The contrast between Mayr's species concept (coadapted gene complexes) and his species definition (reproductive isolation) is illustrated by his discussion of cytoplasmic incompatibility in insects. This phenomenon, which is now known to be caused by the bacterium *Wolbachia*,[12,13] can produce full reproductive isolation between populations infected by different strains of the symbiont. Hence, Laven[14] suggested that cytoplasmic incompatibility could be a mechanism for instantaneous speciation. Mayr,[4] however, objected that two cytoplasmically incompatible populations "answer the definition of [...] species, yet there is serious doubt whether it would be legitimate to label as species allopatric strains that may differ by only a single genetic factor." Apparently, even though this single factor conveyed complete intersterility, Mayr hesitated to apply the BSC, because the evolution of two different, coadapted gene complexes had not taken place.[15]

Recent molecular population genetic data, however, suggest that the BSC with its reproductive-isolation criterion does not automatically follow from a concept of a species as a coadapted gene complex, because the latter can persist in spite of the absence of reproductive barriers. In the fruit fly species complex *Rhagoletis pomonella*, for example, an estimated gene flow of 6% does not negate the effects of disruptive selection for an apple- and a hawthorn-feeding species.[16] Another example comes from microsatellite studies of two European oak species. In spite of pervasive interspecific hybridization and gene flow, the two sympatric species remain morphologically, ecologically and genetically distinct.[17] Furthermore, based on mtDNA and microsatellite data, vertebrate species[18,19] have been shown to exhibit considerable gene flow across ecotones. Nevertheless, this has not prevented the divergent environmental selection pressures on either side of the ecotones resulting in the build-up of differently coadapted gene complexes.[20]

These new data suggest that species differences can persist in the face of gene flow. Therefore, the importance of "protective devices" in the form of reproductive isolation mechanisms may have been overstated. Consequently, since the relevant characteristics of species can also be attained without the protection of complete reproductive isolation, the case for using this property as a sine qua non for characterising species has been considerably weakened. What remains is the valuable insight that species are stable coadapted gene complexes. Disconnected from reproductive isolation, it is not possible or desirable to formalise this notion into a strict species definition. In evolutionary biology, it should be sufficient to study the evolution of such gene complexes, without any reference to the category assigned to them. In taxonomy, the BSC has only incidentally been used as a standard to test systematic revisions against and Darwin's motto that "the opinion of naturalists having sound judgement and wide experience seems the only guide to follow",[3] has never ceased to be important.

The sympatry/allopatry debate

The single most conspicuous conflict in speciation undoubtedly is the sympatry/allopatry debate.[1,4,21] Can speciation occur without geographic isolation? If so, how easily, how quickly and under what circumstances does it occur? In Darwin's theory of speciation (see above), sympatry is an implied prerequisite. Here, speciation is fueled by intraspecific competition. The most extreme phenotypes are selected, since they suffer least from mutual exclusion. By necessity, this process takes place only in full sympatry. In the isolation theory, on the contrary, there is no place for sympatry. Since in this framework any gene flow is expected to disrupt the evolution of new coadapted gene complexes, it is inconceivable that two different gene complexes could diverge within the same population, without any prior reproductive isolation. Dobzhansky wrote: "Species are distinct because they carry different constellations of genes. Interbreeding [. . .] results in a breakdown of these systems [. . .]. Hence, the maintenance of species as discrete units is contingent on their isolation. Species formation without isolation is impossible".[9]

Empirical data exist for both allopatric and sympatric speciation, however. On the one hand, Mayr's work remains one of the most comprehensive enumerations of evidence for allopatric speciation, listing numerous instances where populations isolated by geographic barriers have genetically diverged to a small or large extent.[4] On the other hand, evidence for speciation in sympatry has also been accumulating steadily, especially in the past two decades. Some of this evidence is indirect: molecular phylogenetics of sympatric groups of freshwater fish in constricted environments (small lakes, the waters around rocky islands or a single stream system) has revealed monophyly.[22–24] Other evidence is more direct: observed host shifts in several groups of insects have led to the origin and maintenance of genetically differentiated host-specialists.[25–29]

These conflicting observations have produced a consensus among many evolutionary biologists that speciation is multifarious. It can be allopatric, when it is caused by isolation, or sympatric, when selection is the driving force. Differences of opinion revolve primarily about the prevalence of either mode. The two modes may not be so different, however, and, instead, they could be two ends of a continuum of gene-flow opportunities, with selection as the driving factor across the range. To assess the merits of this view, it may be worthwhile to investigate in more detail the respective roles of selection and isolation in allopatry.

To begin with the latter, indications exist that even in classical cases of allopatry (populations isolated in caves, on islands, or in habitat fragments) residual gene flow remains among the supposedly isolated populations. Populations of cave organisms, for example, have been shown to be interconnected by subterranean populations living in minute rock crevices,[30] while land snail populations on isolated limestone hills probably exchange genes via low-density populations on non-calciferous soils.[31] The degree of isolation, then, may often have been overestimated.

The role of selection, in contrast, may have been undervalued in models of allopatric speciation. In the basic allopatry model, a species' range becomes bisected by a physical barrier, producing two very large daughter populations. With this model, since selective differences are likely to be small, and the populations so large that genetic drift is close to zero, speciation will proceed slowly, if at all. Stebbins,[32] for example, pointed out that American and Asian sycamore trees, after millions of years of isolation, have failed to evolve reproductive isolation. According to Mayr,[33] the rise of the Isthmus of Panama, which partitioned entire marine biotas into a Pacific and a Caribbean portion 3.5 million years ago, had produced "two collossal gene pools", and "differences are still either nonexistent or they are so slight that one doesn't really like to rank these as species."

In the isolation framework, where founder effects and genetic drift play an important role, large isolated populations are not expected to be the ideal situation for the evolution of new coadapted gene complexes. Stebbins's sycamore enigma, for example, was explained by Mayr[4] by arguing that the two populations had been too large to be genetically restructured and hence continued to share the same balancing systems. An alternative for basic allopatry is the bottleneck model, in which geographically isolated populations are founded by a very small number of colonists. In such a small population, random changes in gene frequencies and the ensuing changes in epistasis could, theoretically at least, cause a genetic revolution, leading to a new coadapted gene complex, which subsequently could possibly shift into a new niche.

Little evidence for bottleneck speciation exists, however. Five small-scale[34] and three large-scale[35–37] laboratory studies have largely yielded negative results. Molecular data from field populations also do not support the idea. Ancient allele polymorphisms in island species flocks, long regarded as prime examples of speciation by founder effects, were discovered to be high. Enzyme polymorphisms in the Hawaiian *Drosophilas* are just as high as those in their mainland counterparts,[38] and in the Galápagos finches, 21 ancient allele variants were found at an *Mhc* locus.[39] The persistence of high numbers of ancient haplotypes is inconsistent with very small numbers of colonists. In the case of the Galápagos finches, the founding populations must at least have been as large as forty birds, and probably several hundred.

At the same time, new data tend to favour the basic allopatry model. The Panama Isthmus, regarded by Mayr as ineffective in producing allopatric speciation, is now known to have caused the evolution of numerous reproductively isolated species in various groups of marine organisms.[40] Knowlton and co-workers[41,42] for example, have shown that the isthmus separates almost twenty pairs of sister species of snapping shrimp. All species pairs are reproductively isolated while morphologically very similar, and their mtDNA divergence corresponds well with the geological age of the barrier. Nevertheless, there can be no doubt that the populations have always been very large, which rules out any bottleneck effects or genetic drift. In contrast, transisthmian environmental differences are considerable, including tidal influence, nutrient content and temperature fluctuations, which might better explain the genetic differentiation.

Laboratory studies, too, have shown that reproductive isolation can build up in "allopatric" populations exposed to different selection regimes. Rice and Hostert[34] cite numerous experiments using *Drosophila* that resulted in prezygotic reproductive isolation. Some experimenters[43,44] also tested the development of reproductive isolation between allopatric populations that experienced the same selection pressure, and obtained negative results. These developments indicate that in both allopatric speciation and sympatric speciation, adaptation to different niches is the driving force, although stronger selection pressures are required to produce speciation in the latter. This selection pressure will often be met because of strong competition in sympatry.

The reinforcement debate

The reinforcement model of speciation says that populations that have attained a certain degree of postzygotic reproductive isolation in allopatry (as shown by reduced hybrid fitness), are expected to improve prezygotic reproductive isolation on secondary contact, given natural selection for assortative mating.[9,45–47] In view of its reliance on reproductive isolation alone, reinforcement can thus be seen as fully consistent with the "isolation" view of speciation.

To better define the role of reinforcement in speciation, Butlin distinguished between the processes of reproductive character displacement (namely, the adaptive increase of assortative mating between populations that have already experienced full postzygotic reproductive isolation) and reinforcement (that is, adaptive increase of assortative mating between populations that have experienced only partial postzygotic reproductive isolation).[45,46] With this distinction in mind, we see that reproductive character displacement is not a speciation process under the isolation theory, whereas reinforcement is. Nevertheless, the basic evolutionary mechanism (selection for assortative mating) is identical in both processes.

Butlin's papers, which also carried criticism against the probability of reinforcement actually operating in nature, were followed by a number of theoretical,[47–49] comparative[50,51] and empirical[52,53] studies. Liou and Price[49] showed that, under conditions of low hybrid fitness and considerable initial genetic divergence between the two hybridising populations, reinforcement could indeed reduce gene flow to zero. The empirical studies, which were done on flycatchers[53] and *Drosophila*,[52] supported this, as they showed an increase in assortative mating in sympatry, whereas hybrid fitness was low but not zero. The comparative studies on *Drosophila*, finally, showed that sympatric species have relatively stronger prezygotic isolation than allopatric species, which also lends support to reinforcement as a relevant speciation mode in this group.

From the viewpoint of the isolation theory, then, these recent data suggest that reinforcement can and does indeed produce new species. From the viewpoint of the selection theory, however, the relevance of reinforcement is reduced. As the process acts only on pairs of populations that are already genetically and ecologically diverged and that have a strong (though not complete) degree of reproductive isolation, it can be argued that reinforcement is not a speciation mode because it is not instrumental in the populations' divergence. It only serves to reduce gene flow to zero. If the selection viewpoint is adopted, reinforcement represents the same phenomenon as reproductive character displacement: it is adaptation within two populations that have already speciated.

Redefining the role of reproductive isolation

In the previous paragraphs, I have argued that the selection view may eventually be a preferable platform for discussing species and speciation than the isolation view. Selection, rather than reproductive isolation, appears to be what drives and keeps species apart, both in allopatric and in sympatric situations. It will be interesting, however, to examine in more detail the precise role of reproductive isolation, for two reasons. (1) Models and observations exist where full prezygotic and/or postzygotic isolation evolves between populations without any obvious environmental selection. (2) Reproductive isolation is still important, as it will act as a catalyst of speciation processes that are initiated by selection.

Two types of "non-environmental" reproductive isolation, i.e., without any direct connection to environmental selection, can be envisaged. First, there are situations of the "instantaneous kind, where a single trait becomes fixed in a population, rendering it reproductively isolated from other populations. Examples include bidirectional cytoplasmic incompatibility in arthropods due to infection by the bacterial symbiont *Wolbachia*, as mentioned above,[14,15,54,55] coil reversal in globular snails, which causes mechanical incompatibility of the genitalia,[56–58] and polyploidy in plants, which leads to inviability of hybrids due to aneuploidy.[4] Second, recent advances in the field of sexual selection suggest that isolated populations can easily diverge in their systems for sexual signalling. Computer analysis of "runaway" sexual selection has shown that this process exhibits unpredictable, cyclical behaviour, which is likely to run out of phase in allopatric populations.[59] This means that, soon after geographic separation, male signals in one population may no longer coincide with a preference in females of the other population, leading to prezygotic isolation. Moreover, allopatric populations are likely to diverge in the complicated sets of traits that are involved in male–male sperm competition, sexual manipulation of females by males, and the female prevention of the latter—a set of selective pressures referred to as sexually antagonistic selection.[60] Again, if males and females do not coevolve (as in allopatric populations), their compatibility will decrease, resulting eventually in both prezygotic and postzygotic isolation.

All the situations mentioned above should result in a situation where isolation is attained first, unrelated to environmental selection, after which the resultant genetic partitioning would allow for independent adaptation in both daughter populations. Will the latter actually happen? Two facts make subsequent niche shifts unlikely in the "instantaneous" situations. First, the reproductive isolation trait will usually be the only genetic difference between populations that are incompatible due to *Wolbachia* infection, coil reversal, or polyploidy. Second, the environment will remain unaltered. In coil reversal and *Wolbachia* infection, respectively, the conditions for the establishment[61] and maintenance[15] of the isolation are restrictive, and empirical evidence is rare.[54,58,62] Allopolyploid (rather than autopolyploid) plants, however, are an exception. The combination of two different genomes may allow the new polyploid to be preadapted to a niche that is intermediate between those of its parents. In fact, studies of recently originated allopolyploids show that these establish successfully in such intermediate habitats (e.g., *Tragopogon* in North America see Refs. 63,64). Possibly, allopolyploid speciation may be a case where the isolation view is more appropriate than the selection view. However, the same may not be true for situations where isolation is attained through sexual selection and/or sexually antagonistic selection.

On the one hand, there is no doubt that speciation is often associated with strong divergence in traits for assortative mating and/or postzygotic isolation. For example, *Odysseus*, a gene responsible for hybrid male sterility between *Drosophila simulans* and *D. mauritiana* has turned out to be a homeobox gene, expressed in the testes, which evolves extremely rapidly due to an unknown selection pressure.[65,66] (See the article by Orr and Presgraves, this issue.) The fact that molecular phylogenies of the *Drosophila simulans* clade using this gene show better resolution than those using other genetic markers, suggests that it has been important in the speciation process from a very early stage onwards.[67] Many other genes involved in reproduction show similar evidence for strong selection, although usually it is not known if these genes are responsible for reproductive isolation between species.[68–70] Other evidence comes from comparative studies of speciation rates in birds, which generally show that polygamous clades (where "runaway" sexual selection will be more prevalent), show higher speciation rates.[71,72] In effect, sexual selection can play a major role in incipient isolation.

On the other hand, however, sexual selection and sexually antagonistic selection may often be chanelled by natural

Box 1. Two concepts of speciation

	"Isolation" concept	"Selection" concept
Speciation initiated by:	Disruption of gene flow due to geographical, temporal, ecological, or any other type of gene-pool segregation; most rapid in peripheral isolates, but also possible in other geographic settings	Adaptation to different environments
Speciation progresses by:	Genetic drift and founder effects, natural selection or both	Natural selection and superimposed sexual selection
Speciation completed when:	Pre- and/or postmating reproductive isolation has evolved	Differently adapted gene pools have evolved
Accompanying species concept:	Biological species concept	Darwin's species definition
Geographic setting:	Most rapid in peripheral isolates, but also possible in other geographic settings	Most-rapid in sympatry, but also possible in other geographic settings

selection. Colour variation in male guppies[73] and possibly also cichlids,[74] for example, is influenced by the presence or absence of predatory birds, and mate selection in fishes is often by body size, which is also an environmentally selected trait.[75] In addition, many types of reproductive isolation have been shown to be caused secondarily by environmental selection. For example, flowering time in monkeyflowers has diverged due to water regimes of the soil[76] and diurnal mating rhythms in melon flies have been shown to diverge as a correlated response to larval development time.[77] Therefore, possibly, even in cases where species appear to have formed primarily due to the evolution of reproductive isolation, this reproductive isolation may have been actually superimposed on an underlying environmental selection.

In general, then, the role for reproductive isolation may be seen as catalytic, rather than instrumental in speciation. The buildup of differently adapted gene pools will be disrupted by recombination. Because assortative mating and postzygotic isolation can prevent this, selection and reproductive isolation are probably best viewed as mutually reinforcing, as has been pointed out by Rice and Hostert: once an initial episode of strong environmental selection causes partial reproductive isolation as a by-product, weaker selection (which otherwise would have been hampered by gene flow) will then be able to differentiate the two populations further, which in turn causes further reproductive isolation, and so on.[34]

Conclusions

In this paper, I have attempted to argue that many of the debates concerning speciation are the result of conflicts between the "selection" and "isolation" views on species and speciation. The biological species concept, the scepticism towards sympatric speciation, the emphasis on genetic drift, and the popularity of reinforcement are all features of the isolation theory, which views speciation as a process that begins and ends with the acquisition of reproductive isolation. Recent data, however, allow a re-appreciation of the role of natural selection. Reproductive isolation is then seen to take a catalytic, rather than an instrumental role. This view on species and speciation is surprisingly compatible with Darwin's ideas on the subject.

Future work on the role of environmental selection should fill conspicuous gaps in our knowledge of speciation. The experiments by Kilias and co-workers[43] and Dodd,[44] which showed that prezygotic and weak postzygotic isolation evolved in "allopatric" laboratory populations of *Drosophila* under conditions of different selection regimes, but not under identical selection, urgently need a detailed follow-up. These studies indicate that reproductive isolation may often be a by-product of selection, whereas theory[59,60] suggests that it might also build up independently. We may only have scratched the surface of the full extent of interactions between natural and sexual selection.

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