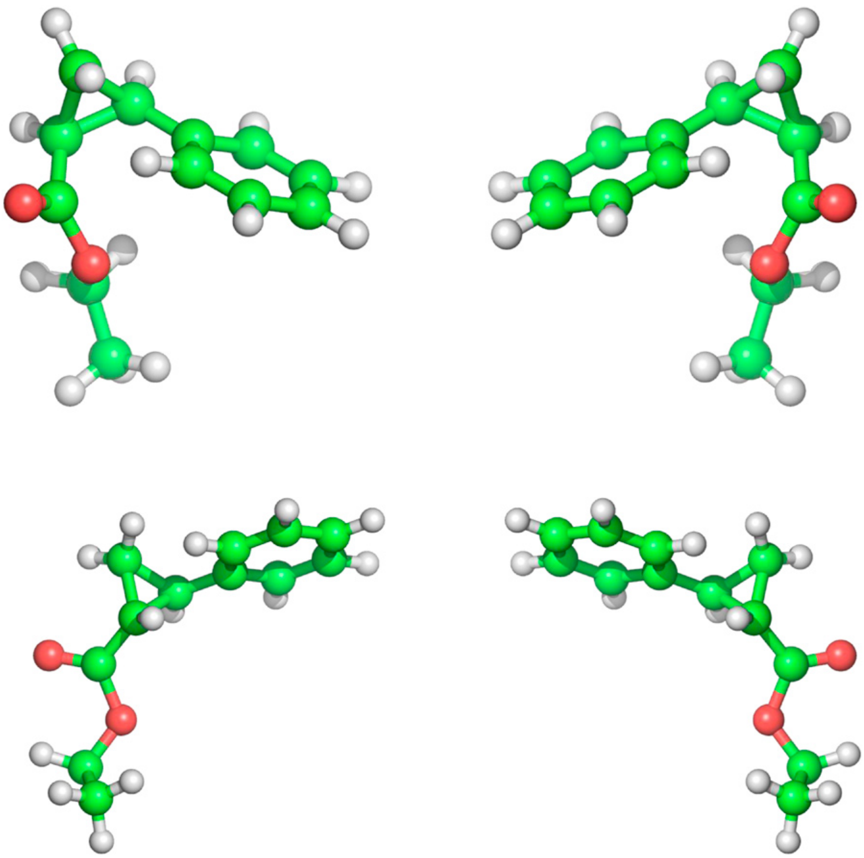

Triangle shapes: The *cis* (upper) and the *trans* (lower) versions of the cyclopropane compound featured in the work by Arnold and colleagues. Each of these two diastereomers is chiral and thus comes in two enantiomers, but the artificial enzyme is highly selective in producing just one of the four possible forms. (Images courtesy of Eric M. Brustad and generated in Pymol (The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC.).)

they can use both design and directed evolution to build on that achievement, improving the performance of the new catalyst or shifting its specificity towards new tasks.

Manfred Reetz came to enzyme catalysis from organic chemistry, with the wishes of synthetic chemists in mind. Working at the Max Planck Institute for Coal Research at Mülheim and at the University of Marburg, he spent much of the most recent phase of his career (until his recent move to emeritus status) developing high-throughput methods for the directed evolution of enzymes (Angew. Chem. Int. Ed. (2011) *50*, 138–174). Initially, this work aimed at improving thermostability for industrial applications, but enantioselectivity soon became an important target.

Following a proof-of-principle study improving the enantioselectivity of a lipase in the mid 1990s, Reetz's group developed a whole toolkit for the directed evolution of enzymes, including iterative saturation mutagenesis (ISM), which reduces the workload of screening by focusing saturation mutagenesis on the most promising sites.

The choice of these sites relies on the combinatorial active-site saturation test (CAST), which takes into account the structural information relating to the active site available from experimental structure determination or from computer modelling.

Applying these tools both to existing natural enzymes and to *de novo* designed ones on a large scale should open up a whole new world of enzyme catalysis, meeting many of the needs of synthetic chemistry, including the synthesis of new pharmaceutical agents.

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Book review

The origin of species by means of ecological selection

Douglas Futuyma

Ecological Speciation
Patrik Nosil
(Oxford University Press, Oxford; 2012)
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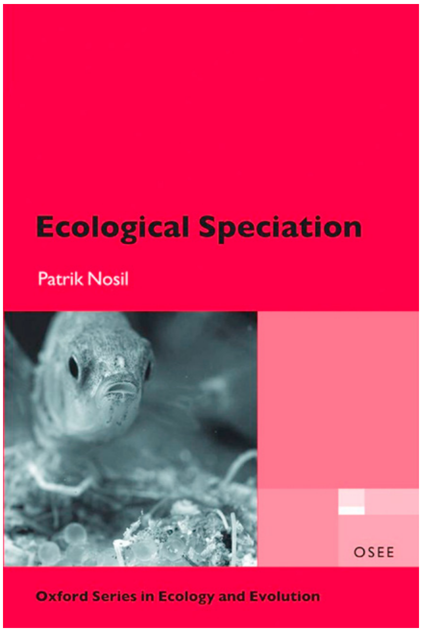
Fifty years ago, Ernst Mayr published *Animal Species and Evolution* [1], which influenced a generation of evolutionary biologists' thinking about speciation. Mayr justified the biological species concept, described forms of reproductive isolation, argued strenuously for the importance of allopatric divergence compared to parapatric or sympatric speciation, affirmed the importance of ecological sources of natural selection in the divergence of populations and species, and developed the thesis that epistasis for fitness (the 'unity of the genotype') was so important that genetic drift in newly founded populations could initiate selection toward a new genetic constitution, incompatible with that of the 'parent' species. (This hypothesis of 'founder speciation' gave rise to the punctuated equilibrium hypothesis, but was later rejected by most population geneticists.) Mayr identified the ecological basis of some forms of reproductive isolation, such as differences in breeding season or habitat; he appeared ambivalent on whether or not to interpret sexual isolation as an adaptation to prevent hybridization, and he implied that postmating isolation, and perhaps also sexual isolation, may stem from pleiotropic effects of the genes underlying selected characters. A role for sexual selection in mate preference had been widely dismissed for several decades, and Mayr almost completely ignored it. He interpreted most hybrid zones as regions of secondary contact between divergent, previously allopatric



populations, and recognized that some alleles could introgress far beyond the contact zone if they were not strongly selected against. Mayr's arguments were widely accepted, not only by zoologists, but also by many botanists, as Verne Grant's book *Plant Speciation* [2] shows.

Research on speciation since that time has resulted in enormous growth in formal theory and in evidence, especially on genetic aspects of species differences and population structure [3,4]. Many of the ideas that Mayr, Grant, and others of that era promoted have been supported, even if some are today expressed in different terms. But there have also been some changes in emphasis and opinion. For example, 'speciation with gene flow', a term that embraces (in increasing order of theoretical likelihood) sympatric speciation, parapatric speciation, and reinforcement of premating isolation in hybrid zones, has become more widely accepted, even if still controversial. And some understudied topics are now a focus of considerable research.

Among these topics is the question of what causes reproductive isolation to evolve. There is general agreement that genetic drift alone is very unlikely to cause speciation, so the problem is to identify the sources of natural selection and how they result in various forms of reproductive isolation. Several possibilities have been identified. Divergent sexual selection could yield sexual isolation and perhaps gametic isolation (but why would sexual selection differ between populations?). Different populations could achieve genetically different, incompatible, adaptations to similar environmental selection (a process that has been labeled 'mutation-order selection'), or they may diverge by adapting to different ecological conditions (whether physical or biological). The divergent adaptive characteristics, or the underlying genetic difference, may result in reproductive isolation. This last process has been termed 'ecological speciation' and is the subject of this new book by Patrik Nosil. The author, in the eleven years since he first published on this subject, has studied divergent adaptation to different host plants and reproductive isolation in a stick insect (*Timema*), and has collaborated with other researchers on the relationship between ecological selection and reproductive isolation, and on speciation with gene flow. Although Nosil touches on seemingly every topic that could possibly be germane to ecological speciation, his book strongly emphasizes the subjects of his own research experience.

It may be argued with considerable justification [5] that the evolution of reproductive isolation by ecological divergence has long been recognized — especially isolation by adaptation to different habitats, breeding seasons, or pollinators — and that most speciation involves ecology in one way or another. The term 'ecological speciation' may therefore be an unfortunate choice of label for a restricted role of ecology in speciation. (A more egregiously unsuitable label, that may well sow confusion, is 'adaptive speciation', used by Dieckmann *et al*. [6] for a very particular model of sympatric speciation — as if allopatric speciation did not entail adaptation). Nonetheless, the focus on how ecological selection results in reproductive isolation – especially sexual, gametic, and postmating isolation – is rather recent. How often does reproductive isolation stem from divergent ecological selection? What are the selective agents and the selected traits? And, especially, what mechanisms create an association between selected traits and the various forms of reproductive isolation?

The last question is easily answered if the selected trait is a 'magic trait', a term introduced by Gavrilets [4] to denote a trait that is both an ecological adaptation and a barrier to gene flow. Some magic traits are 'automatic', such as mating in different habitats, or a strong fitness tradeoff between adaptations to different habitats, possibly resulting in what Nosil and his collaborators have termed 'immigrant inviability'. Other magic traits are not logically predictable — for example, the different mimetic wing color patterns of various *Heliconius* butterflies happen also to be cues that males use in choosing mates, so the color-pattern genes are, in a sense, pleiotropic. The color differences, however, cannot fully account for reproductive isolation, unless the underlying genes also affect mate preference (in at least one case, preference is based on a gene that is very tightly linked to the color-determining loci [7]). In yet other cases, the mechanistic connection between ecology and reproductive isolation is utterly unclear. In some herbivorous insects, for example, populations adapted to different host plants are more sexually isolated than same-host populations, and Nosil summarizes cases in which ecological divergence is also correlated with intrinsic hybrid inviability. In no case has the genetic and causal basis of such correlations been identified.

On this and other questions, Nosil provides thorough literature review, often in the context of confronting theory with data. He devotes considerable attention to the usually large difference between what we want to know and what existing evidence tells us, although he is usually clear on what he thinks the evidence will eventually support. For instance, he suggests that antagonistic pleiotropy, causing fitness tradeoffs between environments, "may be the most general way that pleiotropy contributes to ecological speciation" (p. 114). He dissects ecological speciation and provides analysis and literature review of every organ — ecological selection against immigrants and hybrids, the positive feedback between divergent adaptation and gene flow, ecological character displacement, 'adaptive speciation' (he thinks no cases of this have been demonstrated), the importance of multiple isolating factors in the incremental process of speciation (an important, inadequately studied issue), and many other topics, among which I will comment on two.

On the ever-disputed issue of the geography of speciation, Nosil emphasizes that ecological speciation should not be equated with sympatric speciation and remarks that allopatric speciation "may be the easiest form of ecological speciation, and common in nature" (p. 145). But he devotes less than a page to allopatric speciation, because it "is theoretically uncontroversial," and instead devotes a chapter to speciation with gene flow. This imbalance is, I think, a mistake. It will surely, despite his disclaimer, lead careless readers to equate ecological speciation with speciation with gene flow, and it may well encourage neglect of allopatric divergence even though it is probably more common. There is much more to be learned about allopatric divergence, and this knowledge is crucial for some of the very questions that Nosil is concerned with and finds difficult to answer. Important questions about speciation with gene flow include the relative effects of selection and gene flow in accounting for genetic differences, and the timing of gene flow relative to the accumulation of reproductive isolation. (Did gene flow occur throughout the speciation process or only when allopatric populations expanded their ranges and met?) Allopatric populations provide a baseline for addressing these and other questions, as one of Nosil's own studies shows.

How common is ecological speciation, compared with alternatives? Undoubtedly common, but so may be alternatives. 'Mutation-order speciation', in which initial conditions affect which of two or more trajectories a population takes, does not require that adaptation in two populations be based on the *de novo* occurrence of different mutations — often, differences in allele frequencies suffice. In this context, a bit of genetic drift in small or newly founded populations should not be ruled out, and there are other reasons not to dismiss peripatric (founder-effect) speciation, 'founder-flush' speciation [8], or speciation on 'holey landscapes' [4]. There are countless cases, moreover, in which sexual selection may account for reproductive isolation. Although sexual selection can include ecological selection based on environment-dependent signal transmission, there are many examples of divergence in sexual signal and preference (*e.g.*, many acoustic signals of Orthoptera and sex pheromone differences among insect species that do not differ in the host plant) that I greatly doubt have been driven by ecology. 'Runaway' sexual selection may well be common [9], and the species recognition inherent in sexual isolation may be inseparable from sexual selection within species [10].

Ecological Speciation will be essential reading for researchers in this subject. It is a comprehensive and often insightful treatment of its deliberately restricted subject. It is not a consistently gripping read, though. There is repetitive discussion of some topics, such as the antagonism between selection and gene flow and numbers of divergently selected genes. The writing style is not particularly graceful, and the text could perhaps have borne more proofreading. (The sentence "a number of approaches have been proposed, but few are yet to be implemented" (p. 49) was presumably intended to mean exactly the opposite.) And even though Nosil usually provides a satisfying history of the relevant literature, contemporary work on some topics is sometimes breathlessly described as if it were more novel than it really is. 'Speciation with gene flow' is such a topic — recent theoretical analyses add some quantification, but little conceptual novelty, to longstanding models of gene flow/selection balance, hybrid zones, and selective sweeps, and the relevant genomic data, interesting as they are, so far seem to simply provide more detail to patterns that had been described in the 'allozyme era' of empirical population genetics. Indeed, Nosil's summary of "what we know about ecological speciation" concludes that "the sources of divergent selection can be numerous," that there is some evidence "for most forms of reproductive isolation evolving as a result of divergent selection," that pleiotropy, linkage disequilibrium, and restricted recombination may contribute to the genetic basis of ecological speciation, that ecological speciation can occur "under any geographic arrangement of populations," but that "divergence occurs most easily when rates of gene flow are low," and that "speciation proceeds to highly varying degrees" (pp. 214–215). Mayr would doubtless have protested the "any geographic arrangement" assertion, but probably would have expressed little surprise otherwise. (He probably would have said that all this supports his ideas.) But although contemporary work on ecological speciation has supported existing theory, Nosil's cautious summary actually does not do justice to the advances that he has comprehensively summarized, — advances to which he has contributed and that are steps to the deeper understanding that both he and I expect soon to be achieved.

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