

Ecological explanations for (incomplete) speciation

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Divergent natural selection has been shown to promote speciation in many taxa. However, although divergent selection often initiates the process of speciation, it often fails to complete it. Several time-based, geographic and genetic factors have been recognized to explain this variability in how far speciation proceeds. We review here recent evidence indicating that variability in the completeness of speciation can also be associated with the nature of divergent selection itself, with speciation being greatly promoted by (i) stronger selection on a given, single trait (the ‘stronger selection’ hypothesis) and (ii) selection on a greater number of traits (the ‘multifarious selection’ hypothesis). However, evidence for each selective hypothesis is still scarce, and further work is required to determine their relative importance.

Variability in the completeness of ecological speciation

The causes of speciation have received much attention from biologists [1–13]. One hypothesis posits that divergent selection between ecological niches drives the evolution of reproductive incompatibility (see [Glossary](#)). This process of ‘ecological speciation’ occurs because traits under divergent natural selection, or those genetically correlated with them, affect reproductive compatibility [1–13], and includes the special case where divergent selection operates directly on mate choice. Ecological speciation predicts that ecologically divergent pairs of populations will exhibit greater levels of reproductive incompatibility (e.g. reproductive isolation) than ecologically similar pairs of populations, because ecological divergence is a proxy for the presence of divergent selection [1–3,6–10]. Another prediction is that traits under divergent selection often affect reproductive compatibility [1,3,5,6,11]. There are now numerous examples supporting these predictions, and thus it is generally accepted that divergent selection can promote speciation [1–13].

As support for ecological speciation accumulated, however, it became evident that divergence in this process often varies continuously (even if the endpoint is the development of a discontinuity) [1,2,14–25]. For example, the degree of phenotypic divergence can vary quantitatively [3,7,10], as can the completeness of reproductive isolation

[2,4–6,9,10,13–16], the degree of genotypic clustering [18,19], the sharpness of geographic clines in gene frequencies [21] and the extent of lineage sorting [22,23] ([Figure 1](#); [Table 1](#)). These different means of quantifying divergence can be used to measure arbitrary ‘stages’ of speciation, ranging from continuous variation to population differentiation, ecotype formation, speciation and postspeciation divergence [1,3,8,10,13,15,26,27]. We hereafter use the term ‘stage’ of speciation to refer to a point along this continuum of divergence, with the later stages being associated with strong reproductive isolation and bimodal genotypic clustering (i.e. a strong discontinuity). Notably, different species concepts can disagree on when speciation starts and when it is complete, while still sharing the

Glossary

Correlated evolutionary response: divergence of a trait, which itself might not be under selection, which occurs because it is correlated with another trait that is under divergent selection. Here we use this term primarily to refer to reproductive isolation that evolves as a correlated response to selection on other traits.

Divergent natural selection: selection arising from environmental differences or ecological interactions (e.g. competition) that acts in contrasting directions on two populations (e.g. large body size confers high survival in one environment and low survival in the other) or favors opposite extremes of a trait within a single population (i.e. disruptive selection).

Ecological speciation: a speciation process in which divergent natural selection drives the evolution of reproductive incompatibility (i.e. isolation) between taxa.

F_{ST}: a measure of molecular population differentiation based on the proportion of genetic variation that occurs between populations versus within populations.

Genetic variance–covariance (G) matrix: a matrix whose diagonal elements are the additive genetic variances of different traits, and the off-diagonal elements are the additive genetic covariances of different traits.

Genotypic clustering: the modality of the distribution of gene frequencies, particularly in sympatry or parapatry, with strong bimodality characterizing the existence of two distinct species.

Lineage sorting: related to genotypic clustering, but generally referring to the grouping of taxa in gene genealogies, which can range from polyphyletic through to reciprocally monophyletic relationships.

‘Multifarious selection’ hypothesis: a hypothesis predicting that the completeness of speciation is positively related to the number of genetically independent traits subject to divergent selection.

Niche dimension: used here to refer to an ecological axis, such as habitat use or diet.

Pleiotropy: multiple phenotypic effects of a single gene.

Q_{ST}: a metric of the degree of genetic differentiation among populations displayed by quantitative traits which partitions quantitative genetic variation between versus within populations.

‘Stronger selection’ hypothesis: a hypothesis predicting that the completeness of speciation is positively related to the strength of divergent selection on a given, individual trait.

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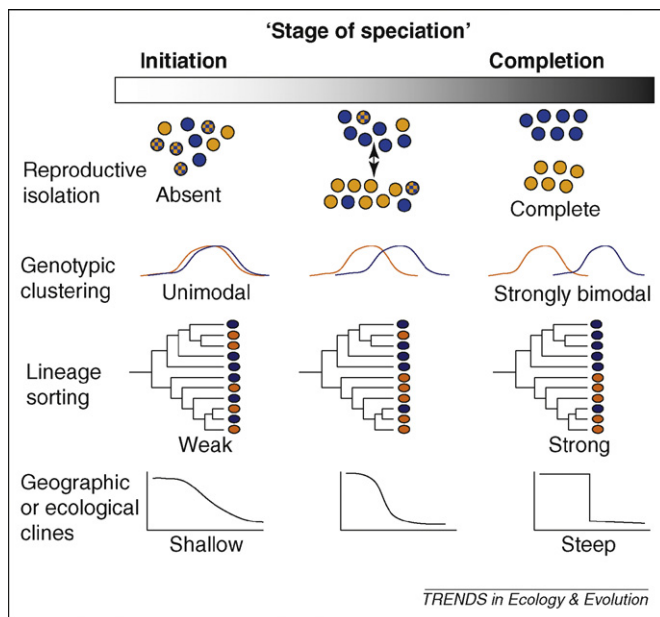


Figure 1. The continuous nature of divergence during speciation. Divergence during speciation can vary quantitatively, for numerous types of differentiation. Thus, different means of quantifying divergence can be used to measure arbitrary 'stages' of speciation, representing stages from the initiation through to the completion of the speciation process (when two populations are depicted, one is shown in blue and the other in orange). For example, reproductive isolation can vary from absent through to complete. Likewise, the distribution of gene frequencies in individuals sampled from two populations, depicted here as genotypic clustering, can vary from unimodal through to strongly bimodal. The extent of lineage sorting can vary from weak to strong. Finally, the steepness of geographic or ecological clines in gene frequency can vary, with the latter stages of speciation being characterized by steep or stepped clines.

characteristic of having stages of divergence [14,25]. Thus, our arguments apply across species concepts.

For the process of ecological speciation in particular, cases in which speciation is clearly incomplete are not uncommon. For example, ecological divergence can be accompanied by only weak reproductive isolation, low genotypic clustering and little neutral genetic differentiation, as observed in *Timema* walking-stick insects, *Pundamilia* cichlids, *Ostrinia nubilalis* corn borers and many other organisms [10,12,15,22,26–31]. Thus, although divergent selection often initiates speciation, it does not always complete it. In other instances, divergent selection does not even initiate speciation [26–28], as observed in Trinidadian guppies (*Poecilia reticulata*) [28], or distinct species pairs collapse [28–31], as observed in stickleback fishes (*Gasterosteus aculeatus*) [31]. In short, there is abundant variability in the stage of ecological speciation achieved, for both cases of speciation with gene flow and in examples of allopatric divergence. This raises the central question reviewed here: what factors explain which stage of ecological speciation is achieved?

We first discuss some well-recognized time-based, geographic and genetic factors that affect the stage of speciation achieved (Figure 2). These can all be viewed as nonexclusive alternatives to the selective hypotheses that are the focus of our review. We then discuss a framework for testing the role of ecological divergence in the completeness of speciation, and review studies indicating that speciation is most strongly promoted when an ecological shift along a single niche axis is extreme or when a shift involves divergence in a large number of independent axes.

Table 1. Examples of variation in completeness of speciation which illustrate the continuous nature of variation in divergence

Level of variability ^a	Measure of divergence ^b	Study system(s)	Result	Refs
Among disparate systems	RI (expt.)	Numerous (20 different groups)	Total reproductive isolation varied among systems from 0.08 to 1.00	[17]
Among disparate systems	RI (gene flow)	Numerous (1284 studies reviewed)	F_{ST} varied among taxon pairs from 0.00 to 1.00	[20]
Among disparate systems	Lin. sort.	Numerous (2319 animal species)	Phylogenetic grouping between closely related species ranged from polyphyly to reciprocal monophyly (with 23% of taxa being para- or polyphyletic)	[23]
Among disparate systems	Gen. cluster	Numerous (17 hybrid zones in different taxa)	Modality of the distribution of gene frequencies in hybrid zones ranged from unimodal, to flat, to strongly bimodal	[19]
Within and among disparate systems	RI (expt.)	Numerous (hundreds of taxa from eight disparate groups)	Individual forms of reproductive isolation varied from 0.00 to 1.00, both within and among study systems	[4,9]
Among populations within systems	RI (gene flow)	<i>Gasterosteus</i> sticklebacks	Species pairs exhibit variability in the degree of reproductive isolation; one previously strongly reproductively isolated species pair has collapsed back into a single interbreeding population	[31]
Among populations within systems	RI (expt.)	<i>Timema</i> walking-stick insects	Populations on different host-plant species vary in total reproductive isolation according to exposure to homogenizing gene flow	[10]
Among populations within systems	RI (expt. and gene flow)	<i>Pundamilia</i> cichlids	Phenotypes adapted to opposite ends of ambient light gradients vary in RI and gene flow, depending on steepness of gradient	[12,15]
Among loci within systems	Lin. sort.	<i>Ostrinia nubilalis</i> corn borer strains	Genealogies for five gene regions are discordant, and only one molecular marker (one that potentially affects reproductive isolation) exhibited evidence for pheromone strain exclusivity	[22]
Among reproductive barriers within a system	RI (expt.)	<i>Mimulus</i> monkeyflowers	Estimates of the strength of individual barriers to gene flow, among nine different reproductive barriers, ranged from 0.00 to 0.99	[71]

^aVarious measures of speciation are considered (e.g. reproductive isolation, genotypic clustering, lineage sorting), at various levels of variability (e.g. among study systems, within study systems, etc.). The set of examples was chosen to span a wide range of criteria and taxa.

^bGen. cluster = degree of genotypic clustering; Lin. sort. = lineage sorting; RI (expt.) = level of reproductive isolation inferred from experimental data; RI (gene flow) = level of reproductive isolation inferred from molecular differentiation in sympatry or parapatry.

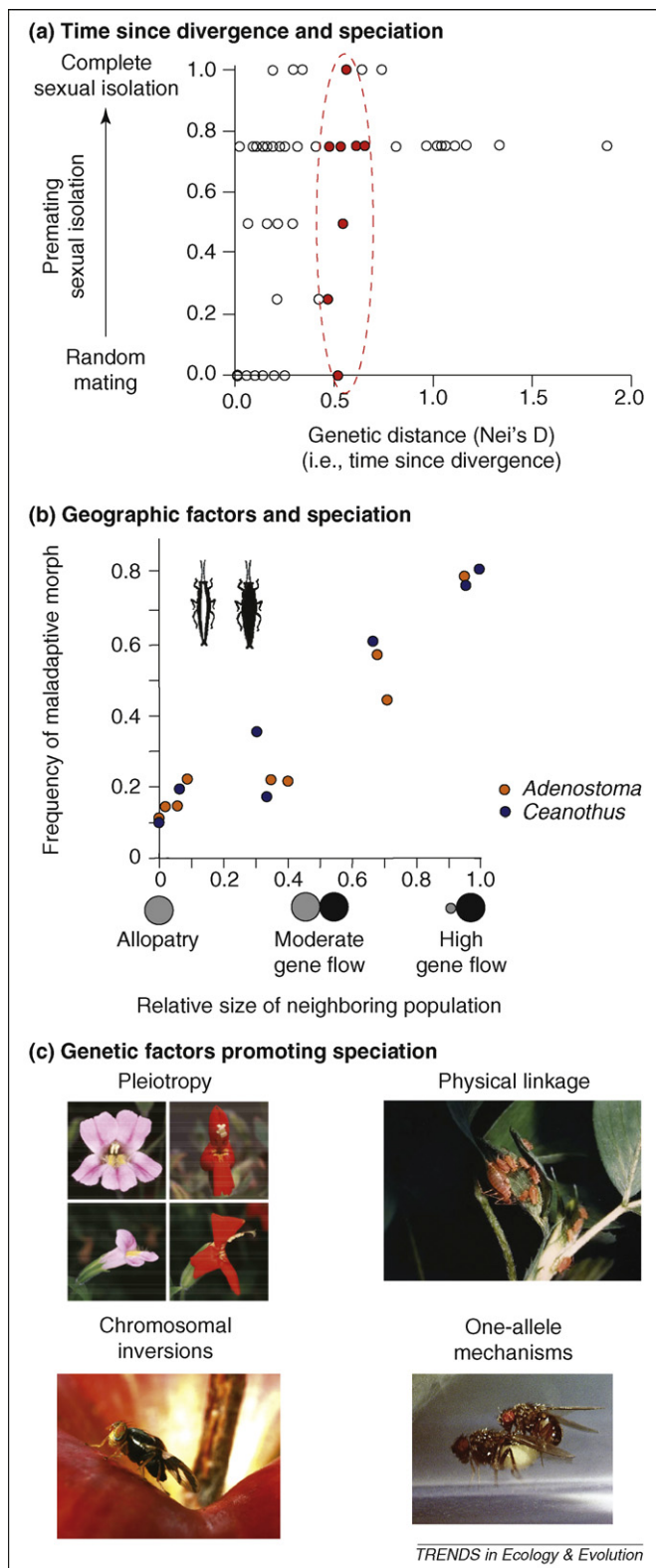


Figure 2. Time-based, geographic and genetic factors affecting the completeness of speciation. **(a)** Time-based factors: levels of premating isolation between taxon pairs of *Drosophila* increase with genetic distance, a proxy for time since divergence. However, much variation is unexplained by genetic distance. Thus, within a narrow window of genetic distance, sexual isolation can vary from absent to complete (highlighted by red oval). Data are from Ref. [16]. **(b)** Geographic factors: *Timema cristinae* walking-stick insects exhibit two color-pattern morphs; an unstriped morph is more cryptic on the host-plant *Ceanothus* and a striped morph is more cryptic on *Adenostoma*. The degree of between-host gene flow is a function of the geographic arrangement of populations (particularly relative population sizes in parapatry). The frequency of the maladaptive morph within populations is correlated with the degree of gene flow into the population from

We describe two hypotheses for how such shifts so strongly promote speciation: the ‘stronger selection’ and ‘multifarious selection’ hypotheses. These hypotheses have been discussed in the past, but the relationships between them have not been clearly established [1,2,5,7,15,32–36]. Moreover, given the numerous ‘non-ecological’ explanations for variability in speciation, an evaluation of empirical support for hypotheses that involve divergent selection is appropriate. Thus, we outline critical differences between the selective hypotheses, and review support for each. To keep the selective hypotheses ecologically rooted, we focus on phenotypic traits that mediate interactions between organisms and their environment. However, we note that the hypotheses could be applied to the genetic level, for example by considering strong selection on one gene versus weaker selection on many genes (the term ‘selection’ is hereafter shorthand for divergent selection).

Non-ecological factors promoting speciation

Much theoretical and empirical work on the completeness of speciation has focused on time-based, geographic or genetic factors (Figure 2). Speciation can be promoted by increased time since beginning of divergence [4,16] and by geographic barriers to gene flow [4,5]. Speciation can also be promoted by pleiotropic effects on reproductive isolation of genes under selection [4,5,8,37] and by physical linkage of genes under selection and those conferring reproductive isolation, perhaps facilitated by chromosomal inversions [4–6,38–41]. Finally, speciation is promoted by one-allele assortative mating mechanisms, which resolve the antagonism between selection and recombination during divergence [6,42,43], and by abundant standing genetic variation (sometimes created by interspecific hybridization) [44–46].

There are now examples of the above factors promoting speciation. A role for time is exemplified by the positive relationship between genetic distance and levels of reproductive isolation between species pairs of *Drosophila* and other taxa [4,16], and a role for geographic factors comes from a large number of publications [4,5,47,48]. Empirical examples of genetic factors affecting speciation also exist. For example, adaptation to different pollinators by *Mimulus* monkeyflowers, via divergence in a flower color gene, pleiotropically affects the probability of cross-pollination and thus hybridization [37]. Similarly, female mating preference for male color in cichlids is affected by divergence in a color vision gene [12,15]. Examples of physical linkage and chromosomal inversions promoting speciation include physical linkage of host-plant preference and performance in *Acyrtosiphon* pea aphids [38], physical linkage between sex ratio-distorting genes and color in cichlid fish [49] and chromosomal inversions harboring genetic variation for divergent diapause adaptation in *Rhagoletis* flies [41].

populations of the alternative host. Thus, gene flow constrains the adaptive divergence that drives ecological speciation. Data are from Ref. [48]. **(c)** Genetic factors: speciation is promoted by the pleiotropic effects of genes under selection on reproductive isolation (e.g. *Mimulus*; photo credit: D. Schemske) [37], physical linkage of genes under selection and those conferring reproductive isolation (e.g. *Acyrtosiphon*; photo credit: S. Via) [38], perhaps facilitated by chromosomal inversions (e.g. *Rhagoletis*; photo credit: A. Forbes) [41], and the fixation of the same allele in both of two diverging populations (e.g. *Drosophila*; photo credit: D. Ortiz-Barrientos) [43]

Evidence for a one-allele assortative mating mechanism is provided by a study of *Drosophila pseudoobscura* [43], and good examples for the role of standing genetic variation and hybridization in speciation also exist [44–46,50,51]. These hypotheses have increased our understanding of the factors driving and constraining the speciation process. The hypotheses also provide explicit alternatives, albeit not mutually exclusive ones, to the ecological factors discussed below.

A unified framework for testing ecological speciation

The study of ecological speciation involves isolating the association between ecological divergence and the completeness of speciation, independent from the other factors discussed above (Box 1) [1,2,8–10]. Examples of measures of ecological divergence are the extent of divergence between taxa along one niche dimension (we hereafter use the term ‘niche dimension’ to refer to an ecological axis, such as habitat use [3,32,36]), the number of niche dimensions that differ between taxa, the strength of divergent selection on one trait and the number of traits subject to divergent selection. From a causal perspective we are most interested in selection itself, although niche divergence is more easily measured (e.g. from environmental data). The second factor, the completeness of speciation, can be measured using experimental estimates of reproductive isolation or the extent of gene flow inferred from molecular markers (e.g. in hybrid zones). Higher levels of reproductive isolation, less gene flow and increased genotypic clustering all indicate a later stage of the speciation process [3–6,12,18,19].

When only a few taxon pairs are available for analysis, qualitative comparisons can be made between ecological divergence and the stage of speciation achieved. When numerous taxon pairs are available, more quantitative analyses can be conducted, for example to control for the time since the beginning of population divergence [1,8,9]. This can be important because reproductive isolation can

increase through time via non-ecological processes such as random genetic drift. Specifically, data on niche divergence or selection can be added to the regression method used to study the relationship between reproductive isolation and time alone, where time is generally inferred using genetic distance from molecular data (see Box 1 for caveats) [3,9,16,52–56]. Multiple regression can thus be used to statistically isolate the association between ecological divergence and the completeness of speciation, independent of time [1,9]. Notably, this framework might also be used to control for variation among taxon pairs in genetic architecture, by adding such information to the regression analysis. With this analytical framework in mind, we turn to empirical data.

The nature of ecological shifts and the completeness of speciation

The nature of ecological shifts can affect the completeness of speciation (Box 1). Under one scenario, slight shifts along a single niche dimension initiate speciation, but more extreme shifts along that same dimension are required to complete speciation [5,7,9]. This idea has seen few tests, because most speciation studies consider only two categories of ecological divergence (ecologically similar and ecologically divergent), precluding a test of how reproductive isolation varies with the quantitative degree of divergence along a niche dimension [3,6,8,10,11]. Moreover, studies have not statistically isolated independent (i.e. explicitly uncorrelated) niche dimensions [3,5,9–11], potentially confounding the magnitude of an ecological shift in a single direction with the dimensionality of the shift. Nonetheless, evidence supporting a role for extreme shifts stems from a study which examined over 500 species pairs from eight plant, invertebrate and vertebrate taxa. This study used the multiple regression approach described above and revealed that the magnitude of divergence in the single niche dimension of diet or of habitat was sometimes significantly positively correlated with the

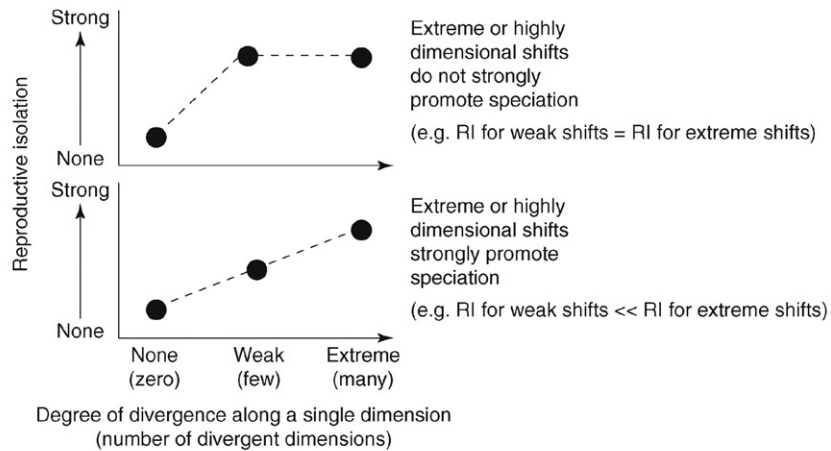
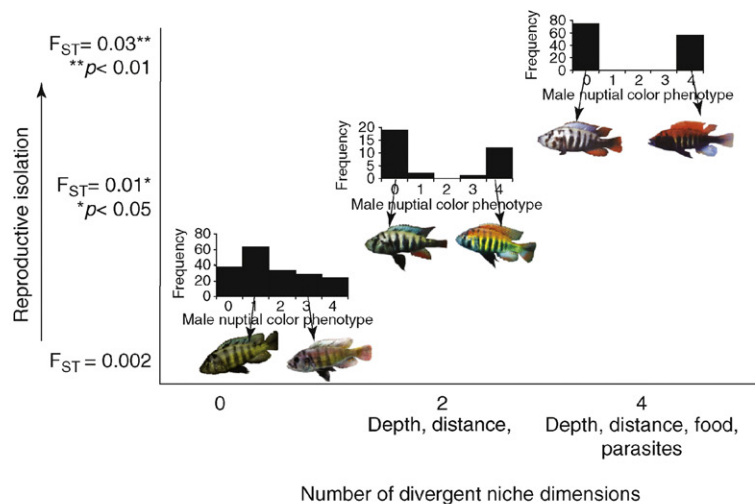
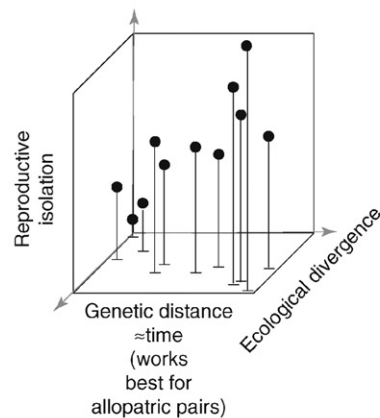
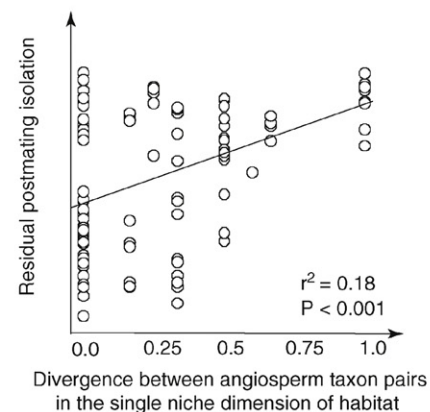
Box 1. A framework for testing ecological speciation, with supporting examples

We describe here published frameworks for isolating the role of various types of ecological divergence in the completeness of speciation. For example, reproductive isolation might increase with the magnitude of divergence in any one niche dimension or with the number of divergent dimensions (Figure 1a). An empirical example where the completeness of speciation increases with the number of divergent niche dimensions involves the incipient species pair *Pundamilia pundamilia* and *P. nyererei* (Figure 1b, blue and red males, respectively). In this example, the completeness of speciation was inferred using neutral genetic differentiation at microsatellite loci, experimental data on mating preferences and the distribution of male nuptial coloration (blue left, red right and three intermediate classes) [12,15,61].

When a large number of taxon pairs are available for analysis, time since population separation might be controlled for in a multiple regression framework, where time is inferred using molecular genetic distance [1,2,9]. For example, Figure 1c depicts a hypothetical scenario where reproductive isolation increases with both genetic distance and various types of ecological divergence. Figure 1d shows an analysis where extreme shifts in habitat between angiosperm taxon pairs promote speciation, independent from time (Figure 1c,d was modified from Ref. [9] and reprinted with permission of the National Academy of Sciences USA). The approach of controlling for time

using genetic data works best for allopatric species pairs. For hybridizing taxa, the degree of reproductive isolation confounds the estimate of divergence time because it directly affects genetic distance. A potential solution is to apply coalescent-based techniques to estimate divergence time independent from gene flow, but the efficacy of these methods needs consideration [51].

To test which specific ecological factors (e.g. Figure 1c) determine the completeness of speciation, one must avoid confounding the extent of divergence along one niche dimension with the number of divergent dimensions, and likewise avoid confounding the strength of divergent selection on a given trait with the number of traits subject to divergent selection. Thus, the first and second measures of divergence should be independent from one another, as should the third and fourth. Various multivariate statistics can generate independent dimensions of niche and trait divergence [3,36,52–56]. For example, a multivariate analog of Q_{ST} can control for correlations among traits when calculating quantitative trait divergence [55]. When it comes to selection itself, multiple regression procedures for quantifying selection on one trait independent from other measured traits are well established (i.e. selection gradients) [56]. Thus, the strength of divergent selection on one trait might be identified, and the number of traits under divergent selection can be inferred.

(a) Hypothetical case : three taxon pairs**(b) Empirical example : three taxon pairs****(c) Hypothetical case: many taxon pairs****(d) Empirical example : many taxon pairs**

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Figure 1. Isolating a role for different types of ecological divergence in speciation. **(a)** A hypothetical scenario where the completeness of speciation varies according to the nature of an ecological shift. **(b)** An empirical example where the completeness of speciation in *Pundamilia* cichlids is positively related to the number of ecological dimensions that taxon pairs differ in. Data are from Refs [12,15,61]. **(c)** A hypothetical example where the completeness of speciation varies as a function of both time and ecological divergence. Various measures of ecological divergence might be used, including the extent of divergence between taxa along one niche dimension, the number of niche dimensions that differ between taxa, the strength of divergent selection on one trait and the number of traits subject to divergent selection. **(d)** An empirical example where residual reproductive isolation (effects of time statistically removed) increases with divergence in habitat use. (c) and (d) were modified from Ref. [9] and reprinted with permission of the National Academy of Sciences USA.

degree of reproductive isolation between species pairs, independent of time [9]. Assuming that increased divergence in the single niche dimensions examined is not correlated with divergence in a greater number of dimensions, the results support a role for extreme shifts along a single dimension in speciation. Another example comes from ecological and phylogenetic studies of galling Australian thrips (Thysanoptera), which suggest that extreme shifts in host-plant use promote speciation more strongly than smaller shifts [57]. One caveat is that extreme niche shifts might be difficult to implement, for example owing to a lack of suitable genetic variation, and thus could be relatively rare [58–60]. The importance of extreme shifts in generating new species might thus reflect a balance between them being rare but more likely to complete speciation when they do occur.

Another scenario involves the actual number of niche dimensions differing between taxa, with divergence in one or a few dimensions initiating speciation, but with the completion of speciation being characterized by divergence in many niche dimensions [5,7,12,15,36]. Support for this idea stems from Lake Victoria cichlid fish (Box 1). In the incipient species pair *Pundamilia pundamilia* and *P. nyererei*, divergence in numerous niche dimensions has been quantified, including water depth, distance from shore in the lake, diet inferred from stable isotopes and parasite community. Reproductive isolation was measured using both experimental estimates of sexual isolation and levels of gene flow in sympatry inferred from molecular markers [12,15,61]. Both measures of reproductive isolation exhibit a positive relationship with the dimensionality of niche divergence.

We note that the two types of ecological shifts are not independent from one another, and can apply simultaneously. Thus, increased divergence in the single dimension of diet might promote speciation, increased divergence in the single dimension of habitat might promote speciation, and divergence in both diet and habitat might promote speciation more than divergence in diet alone or habitat alone. A central remaining question is: how do these types of ecological shifts promote speciation?

Mechanisms strongly promoting speciation: stronger versus multifarious selection

We consider two mechanisms by which extreme or highly dimensional ecological shifts promote speciation (Figure 3). First, under the stronger selection hypothesis, the completeness of speciation is positively related to the strength of selection on a single trait, with very strong selection on one or a few traits driving the completion of speciation [3–6]. Second, under a multifarious selection hypothesis, the completeness of speciation is positively related to the number of genetically independent traits subject to selection, with selection on many traits required to complete speciation [12,15,32–36]. These two hypotheses can be visualized in terms of the metaphor of an adaptive landscape: is the completion of speciation caused by increased divergence between adaptive peaks in a single dimension (i.e. trait), or via the generation of peaks that are separated in multiple dimensions [36]? Although both factors can contribute simultaneously to speciation, the

strength of divergent selection on a trait can vary independently from the number of traits under selection, and thus it is useful to treat each mechanism as a distinct hypothesis.

An important point is that one-to-one mapping is not expected between the nature of an ecological shift (i.e. how extreme or multidimensional it is) and the nature of divergent selection (i.e. its strength and how many traits it acts upon). Thus, both types of ecological shift noted above might cause stronger selection on a given single trait, selection on a greater number of traits or both (as illustrated in Figure 3a). For example, an extreme ecological shift along a single niche dimension might cause stronger selection on a trait that was previously under weaker selection, or it might result in more (i.e. new) traits being subject to selection. This means that selection estimates, rather than environmental data, are required to distinguish the stronger versus multifarious selection hypotheses (see Box 2 for an approach using divergence in phenotypic traits as a surrogate for selection on traits).

Probability of speciation under stronger versus multifarious selection

The probability of speciation under stronger versus multifarious selection can vary according to the total strength of divergent selection, per-trait selection coefficients and the nature of correlations between selected traits and other traits.

Total selection strength

Two arguments suggest that multifarious selection can be important for completing speciation. First, multifarious selection can be required to generate increased total strength of divergent selection in natural populations, because the strength of selection on any single trait is dictated by the ecological setting, and thus can be low and never increase [3,17]. Therefore, multifarious selection might be required to generate a total selection strength that is sufficient to complete speciation. Second, even if divergent selection on one trait is strong, extreme divergence in that trait can be constrained by a lack of suitable genetic variation [58–60] or functional constraints [62,63]. In such a scenario, multifarious selection on many traits can be required to generate an overall degree of trait divergence that is large enough to complete speciation. Empirical studies of selection strength and levels of genetic variation in traits under selection are required to test these ideas.

Per-trait selection coefficients and correlated evolutionary response

We outline here critical differences in how genetic divergence is expected to occur under the stronger versus multifarious selection hypotheses, even when the total strength of divergent selection is held constant (Figure 3b). These differences arise for two reasons. First, the hypotheses differ in the expected magnitude of per-trait (gene) selection coefficients. Specifically, for a given total strength of selection, per-trait selection coefficients will increase as the number of traits under selection decreases. Thus,

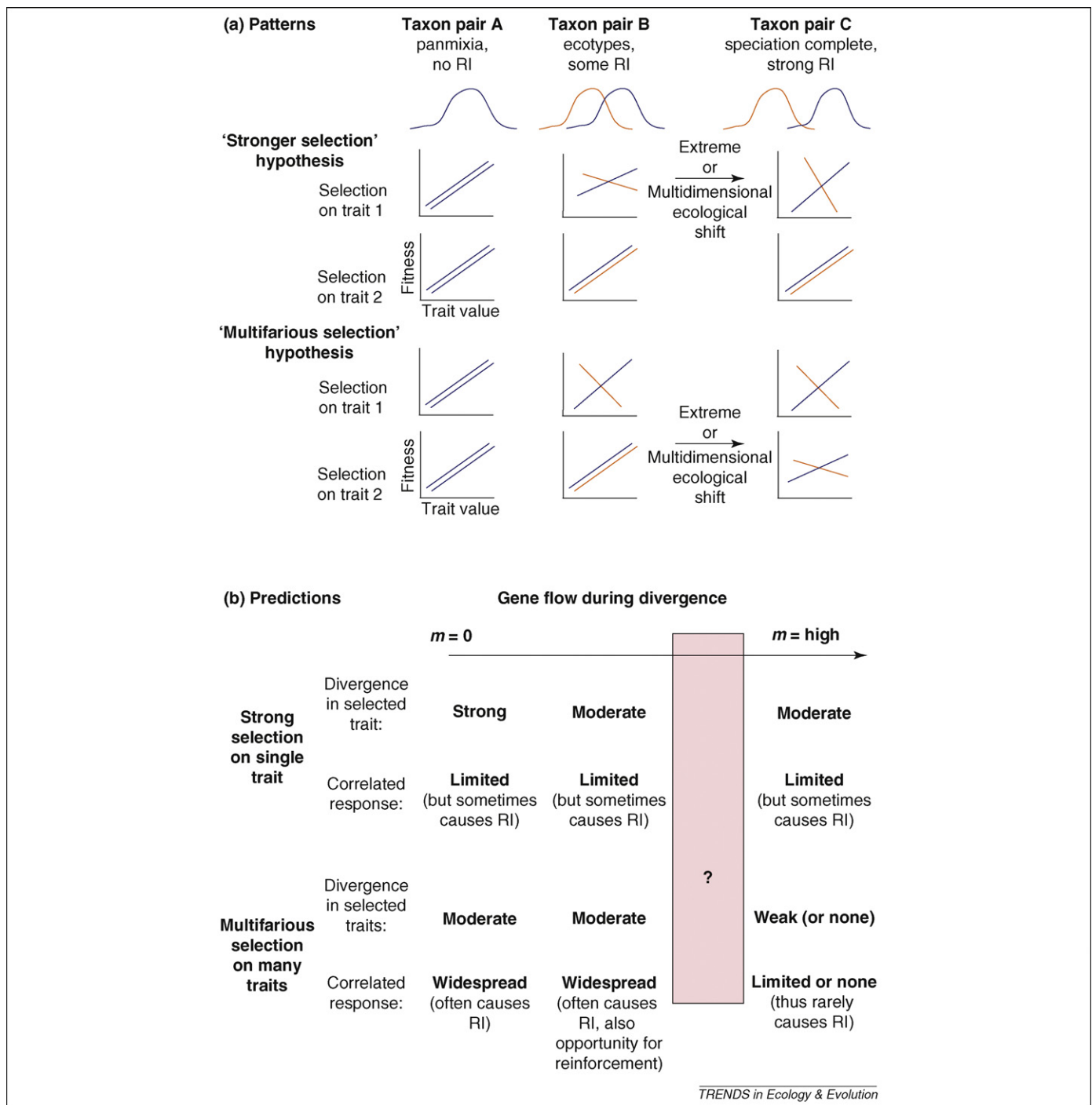


Figure 3. Patterns and predictions of the 'stronger selection' and 'multifarious selection' hypotheses. **(a)** The three taxon pairs depicted vary in which stage of the speciation process has been achieved (RI = reproductive isolation; the distributions represent stages of the speciation process, as outlined in Figure 1). Selection might act on two phenotypic traits (e.g. morphology and physiology). Graphs represent fitness functions, where the x axes represent trait values and the y axes represent fitness. Crossing fitness functions are indicative of divergent selection, with steeper lines indicating stronger divergent selection. The critical change predicting the completion of speciation under each hypothesis is labeled by an arrow. Note that both extreme shifts along one niche dimension and multidimensional niche shifts can cause either stronger selection on a given single trait ('stronger selection'), selection on a greater number of traits ('multifarious selection'), or both. The case depicted here might be extended to a multiple regression framework, as described in Box 1. **(b)** The probability of speciation under each hypothesis depends on a balance between the total strength of selection, the number of traits subject to selection (which affects per-trait selection coefficients), rates of gene flow (m , which also affect the opportunity for reinforcement) and the probability that divergent selection incidentally affects reproductive isolation (i.e. causes a 'correlated response'). Multifarious selection on many traits is more likely to result in reproductive isolation as a correlated response. However, speciation under strong selection on a single trait becomes more likely when gene flow is too high to allow divergence except under strong selection. The actual point at which multifarious selection loses efficacy in causing divergence in the face of gene flow is wide ranging (denoted by the question mark), being dependent on a balance between the factors noted above.

per-trait selection coefficients are expected to be higher when selection acts on one or a few traits (stronger selection hypothesis) relative to when selection acts on many traits (multifarious selection hypothesis). Divergence in a

given trait is a function of its (per-trait) selection coefficient and rates of gene flow [5,36,64]. The implication is that strong selection on a few traits will sometimes be more effective at causing and maintaining adaptive divergence

Box 2. Testing the multifarious selection hypothesis using independent axes of trait divergence

The approach uses trait divergence as a surrogate for divergent selection, with more highly multifarious trait divergence representing more highly multifarious selection. Trait divergence is quantified between multiple pairs of taxa for multiple traits, and then principle component analysis is used to determine the amount of the variance in trait divergence explained by each of x independent directions. Each direction is a composite trait, made up from a linear combination of the original traits. The dimensionality or 'evenness' of each matrix can be calculated from its eigenvalues using Levene's index ([3], pp. 220–221):

$$L = 1 / \sum p_i^2,$$

where p_i is the proportion of total variance accounted for by eigenvector i . $L = 1$ if all variance is in the first direction, and $L =$ the number of eigenvectors (i.e. traits) if variance is equitably distributed among directions. L therefore measures 'dimensionality,' with higher values indicative of more multifarious divergence (Figure 1a versus Figure 1b below), and thus presumably more multifarious selection.

Ideally, this analysis is conducted for different classes of taxon pairs which vary in the stage of speciation achieved. Support for the multifarious selection hypothesis arises if there is a positive relationship between the stage of speciation achieved and the unevenness of the distribution. For example, in *Timema* walking-stick insects, trait divergence was more multifarious for comparisons between distinct species relative to divergence between ecotypes within species ($L = 2.04$ versus 1.66, respectively) (Figure 1c) [36]. Ideally, many stages of divergence would be examined to test for a quantitative association, with increasing importance attached to multifarious selection as the slope and strength of the positive relationship increases (Figure 1d).

A strong assumption of this approach is that the classes of taxon pairs being compared do not differ strongly in the genetic variance–covariance (G) matrix, because trait divergence is a function of both selection and the G matrix [3,36,62]. In practice, this means that phenotypic data can only be a useful surrogate of selection when comparing very closely related taxa with similar demographic histories, or when variability in the G matrix is measured and controlled for.

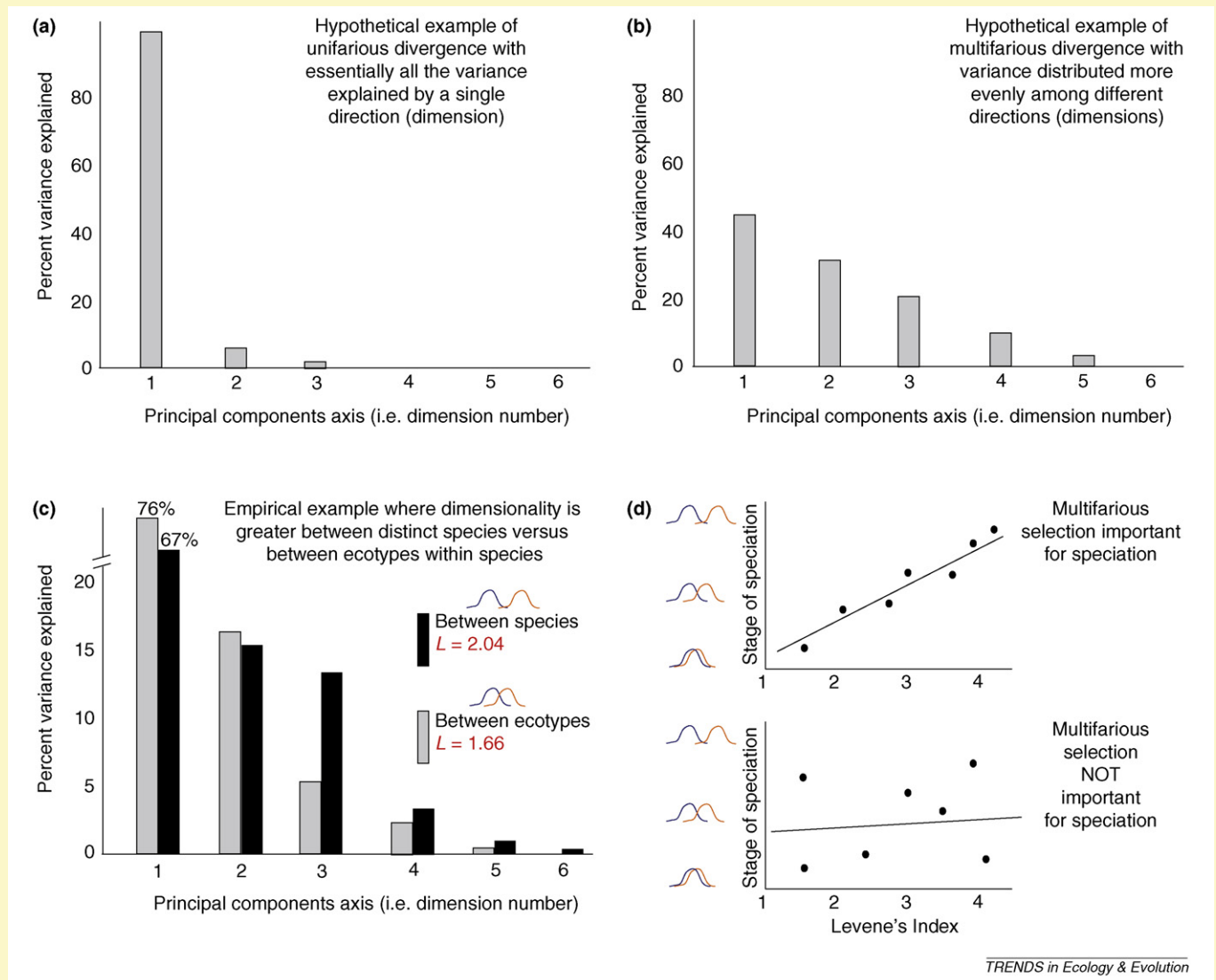


Figure 1. Quantifying the dimensionality of trait divergence. (a) and (b) depict hypothetical examples of relatively unifarious and highly multifarious divergence, respectively. (c) An empirical example in *Timema* walking-stick insects where morphological divergence between distinct species is more multifarious than divergence between ecotypes within species. (d) Applying the framework to numerous sets of taxon pairs that vary in the completeness of speciation.

in the face of gene flow, because the higher selection coefficients associated with it can more strongly overcome gene flow.

Second, divergent selection on a trait (gene) can cause divergence in other correlated traits, referred to as a 'correlated evolutionary response.' At the genetic level, correlated response occurs as a result of pleiotropy or hitchhiking [33]. Under the reasonable assumption that the number of genomic regions under selection increases with the number of traits, the correlated response resulting from multifarious selection will often cause more genomically widespread divergence than the response caused by strong selection on one trait [3,33,65]. Thus, by sampling through the genome more widely, multifarious selection is more likely to incidentally cause divergence in a few key genomic regions that are particularly important for speciation. Examples of such regions are those affecting mating preference or causing intrinsic hybrid inviability (see Ref. [36] for review of this 'sampling model'). A related point is that each individual genomic region might cause only limited reproductive isolation such that strong reproductive isolation can arise only via the combined and cumulative effects of divergence in many genomic regions. Thus, controlling for total selection strength, some predictions emerge.

- (i) Strong selection on one or a few traits is better at causing adaptive divergence in the face of gene flow than is multifarious selection. However, because selection on a single trait often causes little correlated response, it will often result in single-trait polymorphism rather than speciation.
- (ii) Multifarious selection will sometimes be too weak to strongly overcome gene flow, precluding divergence in the selected traits and any correlated response. However, when multifarious selection does cause divergence, its widespread correlated response might be more effective at driving speciation than the more limited correlated response caused by selection on one or a few traits.

Thus, the probability of speciation under each hypothesis will depend on a balance between total selection strength, the number of traits subject to selection, rates of gene flow and the probability that the correlated response to selection causes reproductive isolation (Figure 3b). For example, in the presence of high gene flow between populations, only strong selection on a few traits might be capable of causing the adaptive divergence required to drive ecological speciation. If gene flow is somehow restricted, for example in parapatry, multifarious selection might be strong enough to overcome gene flow, and drive speciation. When gene flow is very low, the correlated response caused by multifarious selection might be very effective at incidentally causing the evolution of reproductive isolation [5,65]. Due to the numerous interacting factors at play, explicit theory is required to make clearer predictions about speciation probabilities under each hypothesis.

A final point is that the importance of the two hypotheses might vary among stages of the speciation process. For example, strong selection on one or a few traits might

initiate speciation, thereby either causing some reduction in gene flow or the evolution of a genetic polymorphism, either of which in turn allows divergence in other traits that are under (weaker) multifarious selection. In such a scenario, single-trait polymorphisms might become converted to speciation. Because most past work has focused on the early stages of ecological speciation, future studies that examine multiple stages are required to avoid a bias toward understanding only the early stages of the process. We now turn again to empirical data.

Support for the stronger selection hypothesis

Support for the stronger selection hypothesis stems from Ref. [9], a study which, in addition to the niche dimensions of diet and habitat, also reports on divergence in one phenotypic trait (body size). In some cases, body size divergence was positively correlated with reproductive isolation, independent from time. Assuming that greater divergence in size arises via stronger divergent selection on size, the results support the stronger selection hypothesis. Similar results stem from positive associations between body size divergence and levels of premating isolation in stickleback fishes [66], body size divergence and levels of intrinsic postzygotic isolation in *Centrarchid* fishes [67], body shape divergence and premating isolation between *Gambusia* fish ecotypes [68] and the magnitude of color pattern shifts in relation to levels of premating isolation in *Heliconius* butterflies [69]. A final example concerns the *Pundamilia* cichlids discussed above, where reproductive isolation is positively related to the degree of divergence in genes encoding opsin [12].

Support for the multifarious selection hypothesis

This hypothesis most clearly traces its roots to a review of experimental evolution studies in *Drosophila* that

Box 3. Preliminary support for the multifarious selection hypothesis in herbivorous insects

In herbivorous insects, divergent selection between populations on different host plants might act on many different types of traits, for example on cryptic coloration used to evade visual predation or on physiology used to detoxify plant chemicals. Selection was estimated on both these traits in three taxon pairs of *Timema* walking-stick insects (Figure 1a). These pairs vary in their degree of reproductive isolation and the completeness of speciation, inferred using experimental estimates of host-plant preference, levels of mtDNA differentiation, and taxonomic status (Figure 1b). The taxon pairs also differed in the number of traits subject to divergent selection (Figure 1c, y axis measures fitness, with crossing fitness functions indicative of divergent selection). The results revealed that strong divergent selection on the single trait of cryptic coloration is associated with host ecotype formation and intermediate levels of reproductive isolation. By contrast, stronger reproductive isolation between a species pair was associated with divergent selection on both cryptic coloration and physiology, rather than on cryptic coloration alone [10,36,70]. The results are consistent with the multifarious selection hypothesis, but further replication is required for a robust test. Another potential example comes from *Rhagoletis* flies, where diapause life-history traits create a strong ecological barrier to gene flow. Different diapause traits, such as initial diapause depth, timing of diapause termination and postdiapause development rate, are genetically uncoupled and are each subject to divergent selection such that the barrier to gene flow is created by multifarious selection [35].

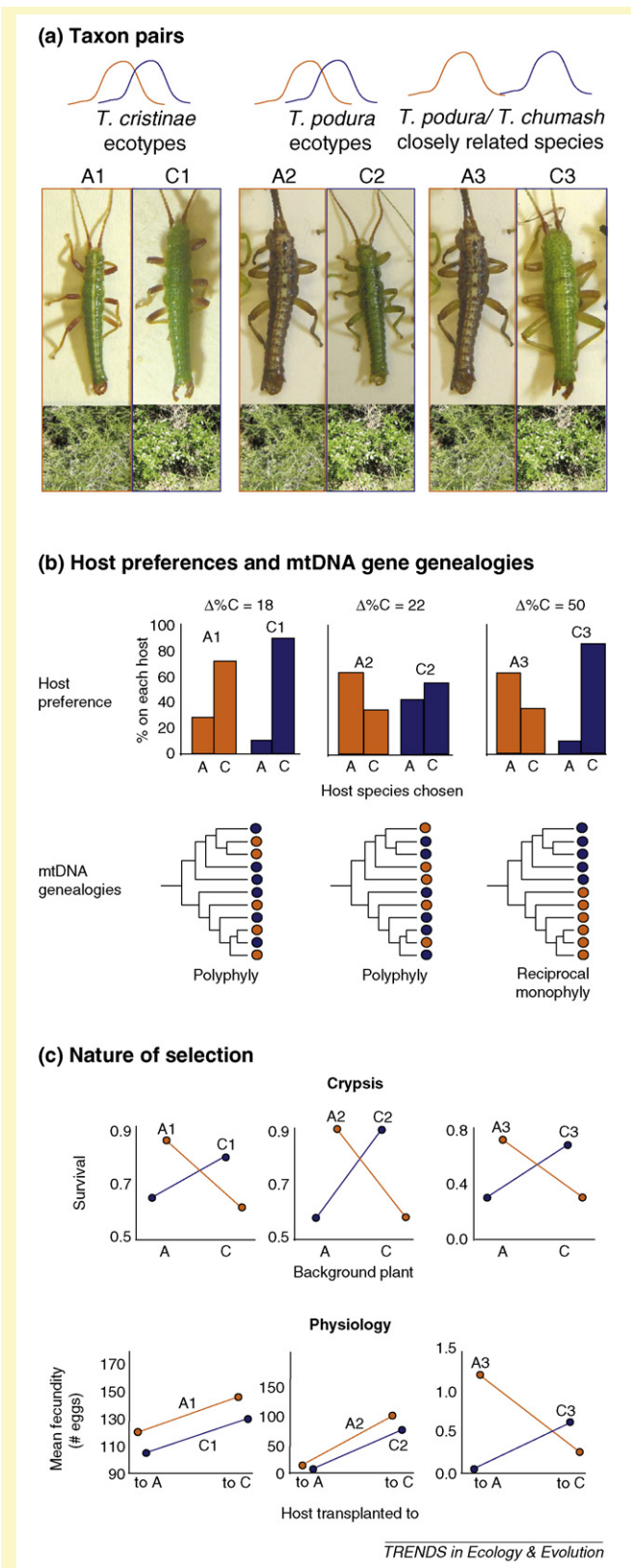


Figure 1. In *Timema* walking-stick insects, the completeness of speciation increases with the number of traits subject to divergent selection. **(a)** The two ecotype pairs and the species pair studied. A1 and C1 refer to ecotypes of *T. cristinae* (in all cases A refers to use of *Adenostoma* as a host, and C refers to use of *Ceanothus*). A2 and C2 refer to ecotypes of *T. podura*. A3 and C3 refer to the species pair *T. podura* and *T. chumash*, respectively. **(b)** Data on the completeness of speciation, here the degree of divergence in host preference and the extent of lineage sorting in mtDNA genealogies (the latter are schematic for simplicity). $\Delta\%C$ refers to the difference between each taxon pair

concluded that 'laboratory experiments collectively indicate that multifarious...divergent selection can readily lead to complete reproductive isolation, but that single-factor...divergent selection will typically lead to only incomplete reproductive isolation' ([33], p. 1647). Despite being intuitive, there are almost no tests of this hypothesis in nature, perhaps due to the difficulty of generating the required selection estimates. Nonetheless, a few key systems, such as taxon pairs of herbivorous insects, provide some preliminary information (Box 3). For example, in *Timema* walking-stick insects, the degree of reproductive isolation between taxon pairs increases with the number of traits subject to divergent selection [36,70]. Similarly, multifarious selection on diapause life-history traits creates a strong barrier to gene flow between host races of *Rhagoletis* flies [35]. However, these studies did not explicitly isolate an effect of multifarious selection independent from selection strength on individual traits.

Integration of different factors affecting speciation

The selective hypotheses reviewed here are refinements of the ecological speciation hypothesis. Nonetheless, such refinements are important, given the abundant unexplained variability in the stage of speciation achieved (Table 1). Similar refinement has been important for understanding the specific role of geographic, genetic and time-based factors in speciation [4,5,71]. For example, it would be overly crude to classify levels of gene flow during divergence as present versus absent; a migration rate, m , of 0.001 is very different from $m = 0.20$, yet both represent nonzero gene flow. Likewise, time since divergence of 1 million years is relatively 'ancient,' but cannot be equated with a divergence time of 20 million years. Detailed empirical [4,10,16,71] and theoretical [5,21,60] treatments of the role of time, gene flow and genetics in the completeness of speciation have increased our understanding of the speciation process. Thus, work on the role of ecological factors in completing speciation is warranted. A particularly unexplored area is how ecological and non-ecological factors interact during speciation. Treating such interactions is beyond the scope of this review, but some limited data do suggest they represent an important direction for further research. For example, in *Rhagoletis* flies, genetic variation for diapause traits under divergent selection in sympatry originated in chromosomal inversions that arose in allopatry, so that selection, geography and genetic architecture interact [41]. Other examples of such interactions come from Lake Victoria cichlid and stickleback fishes [12,15,50].

Conclusions and future directions

We have outlined a framework for testing the role of ecology in the completeness of speciation, reviewed how speciation can be strongly promoted by extreme or highly dimensional ecological shifts and outlined two hypotheses

in the percent of individuals choosing *Ceanothus* over *Adenostoma* in host preference trials. **(c)** Estimates of selection on two traits, cryptic color pattern and physiology. Modified from Ref. [70] and reprinted with permission of the Public Library of Science.

for why such shifts drive speciation: the stronger and multifarious selection hypotheses. Each hypothesis has seen some limited and relatively indirect support, and certainly one hypothesis does not appear more strongly supported than the other. Thus, further tests are required to yield a comprehensive understanding of how speciation unfolds from beginning to end.

In addition to the directions highlighted throughout the article, we suggest four main avenues of further research. First, the existing data do not robustly identify independent axes of trait divergence and selection, which are required to avoid confounding different hypotheses. Second, we focused on the degree to which speciation reaches completion. Future work could focus on the maintenance of species boundaries after speciation is completed. For example, some types of reproductive barriers, such as intrinsic hybrid inviability, might be less reversible than others, and thus particularly effective at maintaining species boundaries. Third, even in the examples cited here, it is uncertain whether variation in niche divergence is the cause or the consequence of variation in levels of gene flow (i.e. reproductive isolation) [12,15,36]. Potential solutions involve comparing allopatric taxa in which levels of gene flow cannot affect niche divergence (because gene flow is absent), measuring selection itself and using experimental manipulations to infer causality [72]. Fourth, increased sophistication of molecular tools will eventually allow the stronger and multifarious selection hypotheses to be tested at the genetic (i.e. gene) rather than phenotypic (i.e. trait) level. Thus, systems that span a range of divergences, and that can combine genetic and ecological data, hold much promise for testing whether the stronger and multifarious selection hypotheses might represent 'ecological rules' of speciation.

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