A combinatorial view on speciation and adaptive radiation

David A. Marques[1,2,*], Joana I. Meier[1,2,3,*] & Ole Seehausen[1,2,4]

1 Aquatic Ecology and Evolution, Institute of Ecology and Evolution, University of Bern, 3012 Bern, Switzerland

2 Department of Fish Ecology and Evolution, Centre for Ecology, Evolution & Biogeochemistry, Eawag: Swiss Federal Institute of Aquatic Science and Technology, 6047 Kastanienbaum, Switzerland.

3 Current address: Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK

4 Biology Department, University of Hawai'i at Hilo, 200 W. Kāwili St., Hilo, HI 96720-4091, USA

* these authors contributed equally and are listed alphabetically

Correspondence: ole.seehausen@eawag.ch (O. Seehausen)

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Abstract

Speciation is often thought of as a slow process due to waiting times for mutations that cause incompatibilities, permit ecological differentiation or assortative mating. Cases of rapid speciation and particularly cases of rapid adaptive radiation into multiple sympatric species remained somewhat mysterious. We review recent findings from speciation genomics that reveal an emerging commonality among such cases: re-assembly of old genetic variation into new combinations facilitating rapid speciation and adaptive radiation. The polymorphisms in old variants frequently originated from hybridization at some point in the past. We discuss why old variants are particularly good fuel for rapid speciation and hypothesize that variation in access to such old variants might contribute to the large variation in speciation rates observed in nature.

Speciation genomics reveals an important role of old genetic variants

The population genomics of speciation, ‘speciation genomics’, is a flourishing area of enquiry with much potential to address some of the big questions in speciation biology. The first generation of speciation genomics studies has generated a number of new insights but it is becoming clear that we are only beginning to understand the genomic basis of speciation. With the exception of a much improved understanding of the nature of genomic islands of differentiation and their link to speciation [1, 2], genomics studies have so far neither fundamentally changed nor challenged our understanding of the process of speciation. However, we think one aspect shines through that deserves recognition and synthesis at this point, and that may yet turn out to challenge how we used to think of speciation: the age of genetic variants underlying speciation often predates the species splitting time, sometimes by orders of magnitude. We believe that this calls for critical rethinking of the genetic mechanisms underlying rapid speciation and adaptive radiation, and perhaps speciation more broadly. Here, we review the evidence for old variation, often derived from hybridization, facilitating rapid speciation and adaptive radiation into many distinct new species. We argue that the re-assembly of such old variants into new combinations often underlies mysteriously rapid species radiations and we hypothesize that variation in access to old gene variants might contribute to variation in speciation rates within and between lineages.

The problem: rapid speciation, but slow mutation

Many lineages accumulate species diversity at a relatively slow pace of few new species every few million years [3]. However, some lineages appear inherently prone to rapid speciation and species radiations [4-7]. This leads to dramatic variation in speciation rates among lineages and thus highly imbalanced phylogenetic patterns of species richness [8]. Some cichlid fishes (Cichlidae) [9], some postglacial freshwater fishes (e.g. Salmonidae [10, 11]), Darwin‘s finches [12], capuchino seedeaters (genus *Sporophila)* [13], Hawaiian honeycreepers (tribe Drepanidini) [14] and Hawaiian silversword alliance (family Asteraceae) [15] among others (Figure 1), radiated quickly into many species with high levels of sympatry and ecological and mating trait differentiation. In contrast, other lineages, often closely related, remain species poor and do not form adaptive radiations despite ecological opportunity [5, 6, 14, 16].

Several lineage-specific traits and properties have been shown to contribute to high speciation rates [4-6]. Examples include a prominent role of sexual selection [3, 5, 17] and its interaction with ecological opportunity [5], the acquisition of key innovations [4, 18], large ecological versatility [19], high evolvability [8, 20], presence of discrete intraspecific morphs [21], or the ability of sister species to rapidly return to sympatry after speciation [4, 22].

Most of these properties though are constrained by genetic variation available to a single population and waiting times for relevant *de novo* mutations are expected to be long [23]. If the relevant genetic variation depended on *de novo* mutations, it would thus be difficult to explain rapid speciation and adaptive radiations with any of the above lineage properties or their interaction with ecological opportunity alone. Similarly, many of the standard models of speciation (Box 1) assume reproductive barriers accumulate by divergent fixation of new mutations, predicting that speciation usually is either a slow process or a process with long waiting times. The accumulating evidence for rapid speciation and adaptive radiation without waiting times in certain lineages is thus rather difficult to reconcile with classical models of speciation.

The data: ancient genetic variation fuels much more recent speciation events

A key to understanding rapid speciation might lie in asking which loci best reflect the speciation process and reconstructing the source of variation in these genes. Inherent to the idea of 'speciation genes' was a close link between the evolutionary history of alleles causing reproductive isolation [2], *i.e.* their mutational origin, and the speciation process, *i.e.* the evolution of reproductive isolation between populations. That evolutionary history differs markedly among loci in the genome was known for a while [3], but only recently has it become possible to directly contrast the age of allelic variants that are causally involved in a speciation event with the time frame over which reproductive isolation evolved.

Evidence is accumulating that alleles contributing to reproductive isolation are often much older than actual speciation events, *i.e*. when populations started to develop reproductive isolation, particularly in cases of rapid speciation and rapid species radiations (Table 1). For example, inversions containing multiple genes affecting diapause introgressed from Mexican Altiplano highland fruit flies into the ancestor of the apple maggot *Rhagoletis pomonella* species complex in the north-eastern United States and facilitated radiation into a variety of sibling species, host races adapted to recently introduced plants with different fruiting times [24, 25]. Despite the very recent emergence of new species (e.g. the apple maggot, *Rhagoletis pomonella,* in ~200 years; Figure 1), much of the genomic variation underlying the host switches and associated reproductive isolation evolved ~1.6 million years earlier in different populations in a different ecological context [24, 25]. Similarly, genetic variation underlying beak shape (*ALX1*) and beak size (*HMGA2*) variation, associated with adaptation to different food resources and song-mediated reproductive isolation in the adaptive radiation of Darwin's finches [12] by far predates the origin of the major species groups in this radiation [26-28]. Recent speciation events in the cichlid fish radiation in Lake Victoria involved divergent selection on *LWS* opsin haplotypes affecting adaptation to light conditions at different water depth and female mate choice [29]. The *LWS* haplotype polymorphism however was generated about 100-200,000 years ago by hybridization between two cichlid lineages that were ~1.5 million years divergent by the time they hybridized [30]. Threespine stickleback (*Gasterosteus aculeatus* species complex) diverged into many parapatric pairs of freshwater and anadromous incipient species within the past 12,000 years, but the genomic variation that fueled divergent adaptation and indirectly reproductive isolation predates the origins of these populations by orders of magnitude [31]. Combining in several different ways divergent haplotypes through hybridization between the same two parental species has led to multiple new species adapted to extreme habitats in *Helianthus* sunflowers [32]. There is also evidence that hybridization between divergent ancestral lineages was important in most major adaptive radiations of cichlid fishes [30, 33-37], the radiation of clownfish on coral reefs [38], as well as the radiation of the silversword alliance on Hawaii [15, 39] (Figure 1).

Very few examples also exist for recent, rapid speciation with a known important role of *de novo* mutations. For instance, the monkeyflower *Mimulus guttatus* speciated in the past 150 years as a consequence of a pre-existing hybrid lethality mutation hitchhiking to high frequency in a copper mine population by physical linkage to a novel copper tolerance allele [40]. In two clades of wild tomato, introgression between early-branching lineages, adaptive sorting of standing genetic variation and evolution of genes through selection on *de novo* mutations all contributed to their adaptive radiation [41, 42]. For many examples of recent speciation and rapid adaptive radiation, either the reproductive isolation loci have not yet been identified, or the timing of their evolution has not yet been reconstructed. While it might thus be too early to quantify the relative importance of different sources of genetic variation for rapid speciation and adaptive radiation, the many recent studies showing involvement of old genetic variation make a reassessment of its role timely.

A combinatorial view on the genetics of speciation

The recent speciation genomic findings exemplified by case studies in Table 1 conflict with standard speciation models (Box 1) in many of which the origin of alleles involved in speciation marks the beginning of the speciation process. In the studies we highlight, new species evolved through new combinations of old alleles (Table 1, Supplementary Table 1). Such a pattern is expected under an alternative set of speciation models, including ‘**recombinational speciation**’ (see Glossary) [43] or ‘**hybrid speciation**’ [44], ‘**hybrid trait speciation**’ [45], ‘**adaptive radiation from a hybrid swarm**’ [46, 47], ‘**transporter hypothesis**’ [48] and some other mechanisms of speciation by **selection on standing variation** [49] that results in linkage disequilibrium among old but previously unlinked variants (Figure 2). Each of these models is defined by a restrictive set of conditions with variable overlap among models. However, all of these models can be unified by a common genetic mechanism: speciation through re-assembly of old genetic variants into new combinations which we refer to in the following as ‘combinatorial mechanism’ (Figure 2C-E). That recombining pre-existing variation is a powerful way of generating new species quickly has been recognized early on [43, 50, 51] and adopting a ‘combinatorial view’ of the genetics of speciation might contribute to a better understanding of phenomena left unexplained by individual models or by the mutation-driven view (Box 1, Figure 1).

From a combinatorial view, not the origin but the re-assembly of several old variants into novel combinations constitutes the beginning of a speciation event. Old genetic variants that have never before been together in one population, can be brought together through introgressive hybridization (Figure 2C-D). Gene flow between weakly differentiated populations is often thought to oppose their speciation as it homogenizes allele frequencies between them [3, 52], but this should not be confused with hybridization between divergent lineages which can sometimes facilitate the origin of one or many new species additional to the two that hybridized [46, 47, 52-54]. Alternatively, old genetic variants can also accumulate as standing genetic variation through long persistence in a single large population or in a meta-population (Figure 2E), although the conditions under which recombining such variation will result in new species might be more restrictive (see below).

Old genetic variation in standing or admixture variation

Old genetic variation – divergent haplotypes combined into the same gene pool by hybridization or present as standing variation– might be a particularly good substrate for speciation compared to haplotypes that are gradually building from new mutations (Box 2). Standing genetic variation and admixture variation can represent two ends of a continuum, particularly if admixture happened in the more distant past. Similarly, in a meta-population context, it is arbitrary if populations exchanging genes are considered to share the same standing variation or to be admixing. Important for the combinatorial mechanism is that, within the range where hybrids are viable and fertile, the more divergent two lineages are, the greater we predict the potential will be for hybridization between them to generate polymorphisms that facilitate one or several new speciation events [52, 55-58]. In line with this expectation, a recent experiment using *Drosophila* species hybrids showed that intermediate levels of parental divergence and hybridization between more than two species are most conductive to generating hybrid species reproductively isolated from their parental lineages and from each other [55].

If several underutilized ecological niches are available, divergent and disruptive selection on variation resulting from mixing between distant lineages can facilitate the evolution of several new species through the many different ways in which old alleles from the same admixture event can be combined to completely novel phenotypes [46, 47]. The large frequency of functionally relevant haplotype polymorphisms in admixed populations can facilitate simultaneous adaptation of different subpopulations to several distinct niches each of which requires adjustments in multiple traits [32], which is extremely difficult to achieve from *de novo* mutations but also from standing genetic variation under migration-selection and mutation-selection balance [59, 60]. Empirical examples are rapid adaptive radiations where admixture variation derived from a hybrid swarm ancestry or from secondary introgression is known to have played key roles, such as in the Hawaiian silversword alliance [39], cichlid fishes of Lake Victoria, Lake Malawi and Lake Tanganyika [30, 33, 35-37, 61] or Darwin's finches [26-28].

We suggest that intraspecific standing variation or variation arising from admixture between only weakly divergent young taxa is less likely to facilitate the rapid origin of many different species, but it can facilitate the recurrent evolution of similar species, *i.e*. parallel speciation [62]. For example, upon colonization of a new habitat, re-assortment of old alleles by selection can lead to the evolution of combinations beneficial in the new habitat that simultaneously also evolve upon colonization of a similar habitat elsewhere, or have also evolved previously in such habitats. Parallel speciation would thus increase the speciation rate but it does not increase sympatric species richness. This is illustrated by parallel speciation in threespine stickleback [31, 63, 64], or by *Pogonus chalceus* beetles [65], whereby similar species or ecotypes evolved repeatedly in different sites from re-assortment of standing variation, but novel ecologies rarely evolved and very little or no sympatric species richness emerged. In both these cases, gene flow from populations already adapted to the alternative habitat has enriched the standing variation in the large generalist population and facilitated parallel evolution of new habitat specialists [48, 65].

Admixture variation is a particularly good substrate for speciation

We predict that old genetic variation derived from recent hybridization (‘admixture variation’) will be more powerful than standing genetic variation in facilitating rapid speciation and species radiations. Below we summarize the major reasons. All of them apply to speciation in general, but for rapid speciation and rapid species radiations they are likely to be particularly important.

Large amounts of genetic variation increase the potential for phenotypic evolution and extrinsic reproductive isolation. Drift, purifying and directional selection limit the amount of standing genetic variation that can build and be maintained within a population through time [66]. In contrast, hybridization will immediately generate polymorphisms at a multitude of genes, including often strongly divergent haplotypes [52], with the number and likely effect size of polymorphisms depending on the divergence between hybridizing lineages [55]. A larger amount of genetic polymorphisms affecting phenotypes increases the potential for rapid adaptation to new environments and range expansion via adaptive introgression [23, 67], rapid ecological differentiation and phenotype-based reproductive isolation in emerging species. Admixture-derived allelic variation can also break up covariance between traits and thus relax genetic constraints and increase evolvability in situations where the ancestral line of least resistance in the covariance matrix was not aligned with the direction of selection in a new environment [20, 68]. In addition, hybridization can indirectly augment genetic variation beyond re-assembly by increasing mutation rates, for instance through activating transposable elements, inducing chromosomal rearrangements, or altering genome sizes [reviewed in 52].

Recombining and sorting of intrinsic incompatibilities might cause leaps in reproductive isolation. **Bateson-Dobzhansky-Muller incompatibilities** (BDMIs [51, 69-72]) are unlikely to arise or segregate as standing variation within a single population because selection purges mutations that are deleterious in their native background [51, 73, 74]. In admixed lineages however, incompatible alleles initially segregate and their sorting into new compatible combinations can lead to reproductive isolation from the parental species [71, 75-78], and potentially among multiple new species arising from the same hybrid ancestry [46]. Initially, the fitness of hybrids can be reduced if many partial incompatibilities are still segregating, but unless individual incompatibilities are very strong, variation among hybrids will lead to the emergence of some hybrid combinations that are at least as fit as the parents, including combinations that are different from both parental combinations [51, 79].

Transgressive segregation can facilitate crossing fitness valleys. Interactions among genes from different ancestry can lead to **transgressive trait values** [80]. Extreme trait values can facilitate adaptation to novel ecological niches in hybrid species [44, 81] and in adaptive radiations [82]. Similarly, transgressive phenotypes or novel phenotype combinations can cause behavioral reproductive isolation if new allele combinations produce novel mating cues and novel preferences [55, 58, 83].

Hybridization might lead to enrichment of large effect haplotypes. Haplotypes of large phenotypic, ecological and context-dependent fitness effect increase the propensity of a population to respond to novel selection pressures and the propensity for ecological speciation given new ecological opportunity [84]. Empirical evidence shows that rapid ecological speciation often involves admixture-derived **large-effect haplotypes**, e.g. in *Rhagoletis* [25], cichlids [37] and Darwin's finches [28]. Parental haplotypes are likely to contain multiple co-adapted alleles which might together have a large effect on phenotype and function. The expected breakdown of such haplotypes by recombination in admixed populations might be impeded by sorting into emerging species that fix alternative haplotypes, and/or through restricted recombination, e.g. due to inversions such as in *Rhagoletis* [24]. We propose that selection in hybrid populations might further enrich admixture variation for large-effect haplotypes. First, theory suggests that large-effect haplotypes with ecological context-dependent fitness effects are more likely to overcome purging selection on linked incompatibilities [85]. Second, in a situation of ongoing gene flow between species emerging from the hybrid population, divergent selection is more efficient in maintaining and strengthening differentiation if it is based on large-effect haplotypes than when based on dispersed small effect variants [86].

Admixture variation might facilitate rapid genome-wide reproductive isolation. When an admixed population experiences ecological opportunity, new species might emerge through sorting of different genetic variants contributing to ecological differentiation, assortative mating and prevalent incompatibilities, all at the same time [52]. In principle, selection might favor linkage disequilibrium between loci involved in adaptation to different niches and those involved in assortative mating and perhaps also intrinsic incompatibilities [47]. We hypothesize that multiplicative effects of selection against recombination at many loci might lead to nearly immediate reduction in gene flow, similar to the last phase in models of 'genome-wide congealing' [87]. This might also facilitate the emergence of multiple species with different combinations of genes from the same hybrid population. We expect that this becomes more likely, the larger the number of differentiated loci is, and the larger the difference between alleles are, between the parental lineages.

Implications

Speciation via a combinatorial mechanism has many implications. One consequence is the decoupling of the speciation process from the slow rate of accumulation of mutations relevant to phenotypic differentiation and reproductive isolation (Figure 2, Box 1). A second consequence is the facilitation of the evolution of linkage disequilibrium between genes even in the face of gene flow, and with it the partial alleviation of constraints to speciation imposed by sympatry [88]. Thereby, a combinatorial mechanism offers one possible explanation for how reproductive isolation can evolve extremely rapidly, for how multiple species can arise in short succession from the same ancestral population, and how such speciation can happen without geographical isolation.

A combinatorial mechanism allows early and rapid speciation at the time ecological opportunity arises even when geographical isolation is lacking, as there is no waiting time for relevant mutations, and because some deviation from linkage equilibria is there from the onset. Sorting and recombining of pre-existing alleles with effects on gene flow can lead to leaps in reproductive isolation (Figure 2) such as seen in the rapid genomic stabilization of *Tragopogon* [89] or sparrow hybrid species [76, 90]. The mass of ecologically relevant alleles with linkage disequilibrium between some facilitates crossing otherwise constraining fitness valleys by large peak shifts and thereby facilitate ecological novelty and differentiation. Examples include *Mimulus* monkeyflowers [91] adapting to different pollinator syndromes, *Helianthus* sunflowers adapting to xeric habitats [32], a hybrid species of Darwin's finches with extreme body and beak size that arose within two generations [92], or pupfish that acquired a completely new feeding adaptation in the presence of the ancestral feeding type [93]. To the extent that adaptive radiation on islands and in lakes requires that evolution of new species outpaces arrival of existing species from the mainland, this effect of jumpstarting adaptive radiations may not only affect the rate at which an adaptive radiation unfolds but it may be decisive about whether a radiation occurs at all.

Variation in access to old genetic variation for combinatorial mechanisms might be one factor contributing to variation in speciation rates, and in the propensity of adaptive radiation among lineages. Predictors might be the amount of standing genetic variation in a meta-population or whether divergent lineages with somewhat leaky reproductive isolation exist in geographic proximity. Also, the longer lineages retain the potential for hybridization after extended periods of isolation, the more likely they are to receive old genetic variation and to generate such variation in other lineages. Phylogenetically strongly isolated species ("living fossils") cannot receive gene flow from other species and we hypothesize that this limits their potential for rapid speciation. Differences among lineages in the rates of evolution of complete intrinsic genetic incompatibility [94-96] might thus contribute to variation in lineage-specific speciation rates in a way contrary to predictions from classical speciation theory [97]: if the combinatorial mechanism is widespread and important in rapid speciation, we expect that high speciation rates should be associated with taxa showing slow completion of intrinsic incompatibility.

If speciation and hybridization occur repeatedly within the evolutionary history of a lineage, such as in 'fission-fusion-fission radiations', the genetic variation in the lineage is expected to increase [98]. Whereas small effect haplotypes and variants might get lost through drift, large-effect haplotype polymorphisms generated by hybridization and favored in different niches are likely to persist at high frequencies in a fission-fusion-fission radiation, a process akin to **balancing selection** in a metapopulation. Such enrichment might contribute to the persistent high propensity of speciation in lineages with a history of repeated hybridization and adaptive radiation such as some lineages of African cichlid fish [22, 30, 33-37] and Darwin's finches [12, 28, 92]. Introgression might thereby also protect functionally relevant variation from extinction in single species or populations and thus promote the long-term persistence of biodiversity at the gene-level. Future research will have to subject these hypotheses to critical scrutiny (Box 3).

As indicated above, a combinatorial mechanism might also help to explain sympatric speciation. An important role of introgression from divergent lineages has been demonstrated for some of the better examples of sympatric speciation and sympatric adaptive radiation [34, 37], raising a conflict with the most narrow sense definitions of sympatric speciation that exclude cases where alleles did not evolve in the sympatric context [99]. Sympatric speciation from *de novo* mutation and panmixia (with complete linkage equilibrium) is expected to be very difficult [59, 60, 88, 99]. However, old haplotypes with several co-adapted SNPs, might substantially increase the likelihood of sympatric speciation.

Finally, evolutionary diversification through combinatorial mechanisms of speciation generates a network-like evolutionary history of species rather than the tree-like evolution with dichotomous splitting of lineages that dominates evolutionary thinking. This might affect the suitability of tree-based comparative methods for research on rapid speciation and adaptive radiation, and perhaps more general [100].

Concluding remarks

Speciation through combinatorial mechanisms, by which new combinations of old gene variants quickly generates reproductively isolated species, offers a perspective on speciation that contrasts with the gradual growth of reproductive isolation through accumulation of differences from *de novo* mutations. Such a mechanism has the potential to explain how speciation can sometimes be very fast, and how multiple new species can arise nearly simultaneously and can persist in sympatry very soon after their origins. We propose that explicitly considering this class of mechanisms might help understand the often tremendous variation in speciation rates – something to be tested in future comparative work on speciation rates. Ongoing research in speciation genomics will soon allow more conclusive answers regarding the importance of combinatorial mechanisms relative to others in facilitating speciation and species radiations, and hence their contributions to patterns in biodiversity.

Figure 1. Examples of species that arose from new combinations of ancient alleles. From left to right, top to bottom: Darwin finch (*Geospiza conirostris*, photo by David Marques), freshwater stickleback (*Gasterosteus aculeatus*, David Marques), Lake Victoria cichlid (*Pundamilia nyererei*, Ole Seehausen), Hawaiian silversword (*Argyroxiphium sandwicense*, Ole Seehausen), scale-eater pupfish (*Cyprinodon desquamator*, Anthony Terceira), Heliconius butterfly (*Heliconius timareta*, Thomas Horton), capuchino seedeater (*Sporophila hypoxantha*, Hector Bottai), short-winged *Pogonus* beetle (*Pogonus chalceus*, Roy Anderson), red monkeyflower (*Mimulus aurantiacus* ssp. puniceus, Sean Stankowski), munia (*Lounchura castaneothorax*, Graham Winterflood), clownfish (*Amphiprion akallopisos*, Ole Seehausen), *Helianthus* sunflower (*Helianthus deserticola*, Jason Rick), apple fly (*Rhagoletis pomonella*, Andrew Forbes).

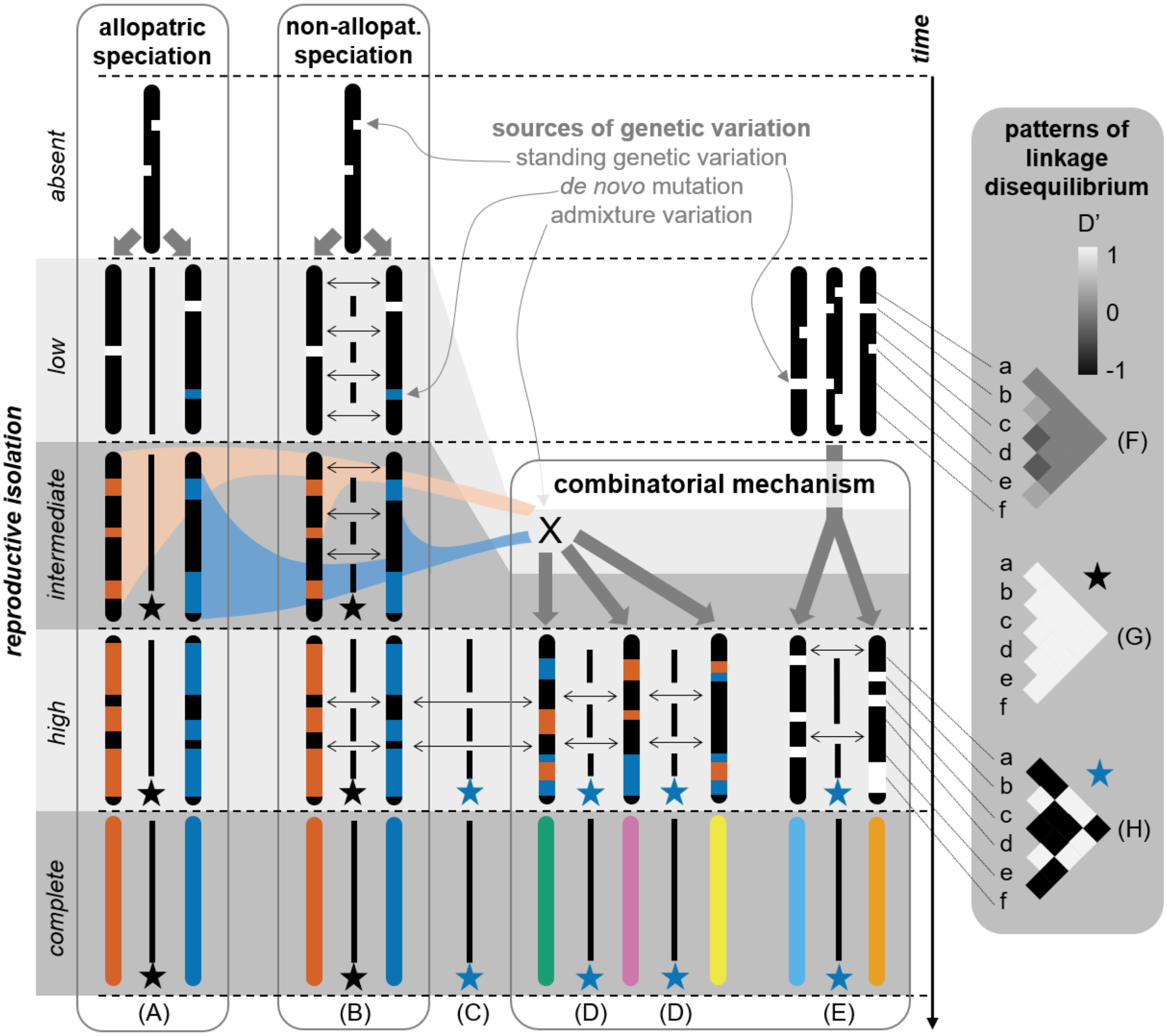

Figure 2. A combinatorial view on the genetics of speciation. In models of allopatric speciation (A), or non-allopatric speciation (B), reproductive isolation usually evolves by the accumulation of barriers through selection and drift acting on new mutations and is thus coupled to time by mutation rate (Box 1). In speciation by a combinatorial mechanism, the re-assembly of old genetic variation into new combinations (witnessed by novel patterns of linkage disequilibrium (H)) marks a speciation event, thereby decoupling the evolution of reproductive isolation from mutation rate and thus time. Speciation by a combinatorial mechanism can start from admixture variation, such as during hybrid speciation (C) or adaptive radiation from a hybrid swarm (D), or from standing genetic variation in large populations or meta-populations (E) maintained e.g. by balancing selection. Speciation through selection on admixture variation generated by hybridization between two lineages will lead to sorting and thus linkage disequilibrium (H) between some alleles from either parental lineage (G). From standing genetic variation, combinatorial reassembly should lead to strong linkage disequilibrium among sets of loci (H) that were previously in linkage equilibrium (F). Horizontal arrows indicate gene flow between diverging genomes, black vertical bars represent barriers to gene flow. Stars indicate predicted linkage disequilibrium patterns (right hand box) between the populations on either side of a star.

Table 1: Study systems with evidence for ancient genetic variation involved in recent rapid speciation or in recent radiations with several speciation events in short succession. In all cases, the origin of relevant genetic variation clearly pre-dates the onset of speciation, *i.e.* the beginning of the build-up of reproductive isolation. Included are only taxa that are sufficiently reproductively isolated from each other to coexist in sympatry or where reproductive isolation has been shown experimentally. For more details see Table S1.

System	Start of speciation	Age of alleles	Source(s) of alleles	References
Darwin's finch radiation (genera *Geospiza*, *Camarhynchus*, *Platyspiza*, *Certhidea*, *Pinaroloxias*)	~10 y, ~100-300 ky, <1 Mya	~1 My	Hybridization	[26-28, 92]
Marine / freshwater threespine stickleback (*Gasterosteus aculeatus*)	34-50 y, <12 ky	1-14 My	Standing variation and hybridization	[31, 63, 64]
Tragopogon goatsbeard flowers	~90 y	~2 My	Hybridization	[89, 101]
Rhagoletis pomonella species complex	~200 y	~1.6 My	Hybridization	[24, 25]
Lake Ejagham *Coptodon* cichlid radiation	1-2 ky	~10 k	Hybridization	[34]
Bahamas *Cyprinodon* pupfish radiation	~10 ky	>>10 ky	Hybridization	[93]
Italian sparrow (*Passer italiae*)	~10 ky	~800 ky	Hybridization	[76, 90]
Lake Victoria Region Superflock (tribe Haplochromini) encompassing multiple cichlid radiations in different lakes including the Lake Victoria radiation	~150 ky, ~15 ky (Victoria)	>2 My	Hybridization	[30]
Helianthus sunflowers	60-200 ky	>1 My	Hybridization	[32]
Mimulus aurantiacus monkeyflowers species complex	recent	old	Hybridization	[91]
Sporophila capuchino seedeater radiation	44k gen.	>>44k gen.	Standing variation or hybridization	[13, 102]
Australo-Papuan munia radiation (genus *Lonchura*)	<500 ky	>>500 ky	Standing variation or hybridization	[103]
Heliconius butterflies	<2 Mya, <1.5 Mya	~4 Mya, >2 Mya	Hybridization	[104, 105]
Hawaiian silversword alliance (genera *Argyroxiphium*, *Dubautia*, *Wilkesia*)	~5 Mya	~15 Mya	Hybridization	[15, 39]

Box 1: History of speciation models

Ernst Mayr defined speciation as the process generating "groups of interbreeding natural populations that are reproductively isolated from other such groups" [74]. In the view of Mayr and Dobzhansky [51], reproductive isolation evolves between populations in allopatry as they accumulate incompatible mutations at interacting genes, so-called BDMIs [3] (Figure 2A). Gene flow between populations was thought to hinder speciation, because it opposes the formation of independent sets of compatible genes that are incompatible when combined [51, 74]. Even though Dobzhansky recognized that "by hybridization a species can 'discover' new evolutionary possibilities" [51], hybridization was not considered important in the eyes of Modern Synthesis and Post-Modern Synthesis zoologists. Non-allopatric speciation was deemed unlikely [3, 88, 106, 107]. In the 1980s and 1990s, empirical evidence for non-allopatric speciation began to accumulate but the genetics remained unresolved [3].

Wu's [108] proposal of the 'genic view' of speciation (Figure 2B) suggested a solution by emphasizing that speciation with gene flow might start with reproductive isolation at single genes where strong divergent selection overcomes homogenizing gene flow. The proportion of the genome diverging might then increase gradually until reproductive isolation is complete [108]. Loci that initiate speciation in this view of speciation include genes involved in ecological divergence, assortative mating, or intrinsic incompatibilities.

Both allopatric and non-allopatric speciation depend on the accumulation and divergent fixation of variants at genes relevant to speciation. If the source is *de novo* mutation, speciation is expected to be a slow process with long waiting times. However, some theoretical studies of sympatric speciation have suggested that speciation by disruptive selection on standing variation for quantitative traits can be immediate and rapid [109, 110]. Mayr also proposed immediate and rapid speciation following a founder event (**founder effect speciation**, see Glossary) [111]. In this model, reproductive isolation arises due to drift-induced allele frequency changes which alter selection pressures on epistatically interacting genes [111], albeit evidence from nature is rare [112]. However, while speciation can be immediate and rapid in such models, they leave unexplained the accumulation and maintenance of the large amounts of standing variation required for rapid radiations into many species [59, 60].

Box 2: Old genetic variation is good substrate for speciation

We predict old genetic variation to be a better substrate for speciation than haplotypes that are gradually building up through new mutations, because of multiple reasons. Most new mutations are expected to be neutral or deleterious [113], especially those that affect genes. Old variation, in contrast, has already been filtered by selection and old haplotypes have been shaped by selection in their native genomic and ecological context [52]. Strongly deleterious mutations will have been purged and old haplotypes are thus enriched for potentially (i.e. context-dependent) beneficial mutations. Variants that have passed the 'intrinsic fitness filter' are thus more likely to allow for ecological and mating trait adaptation, and more rapidly so than *de novo* mutations [49].

In addition, alleles introduced by hybridization, or having been present as standing variation, occur at much higher frequency than new mutations. They are thus less likely going to be lost through drift and they are more easily seen by selection even if recessive [49]. Old genetic variation might also be enriched for **large-effect haplotypes** and thus more likely to promote a fitness peak shift and the crossing of fitness valleys too wide to be crossed by most *de novo* mutations. In populations or species diverging with gene flow, evolution under migration-selection balance is expected to promote the clustering of many small effect mutations into single large-effect haplotypes [86, 114-116]. This is because adaptations underlain by such a clustered genomic architecture, possibly protected by locally reduced recombination (as in an inversion), better persist in the face of gene flow than adaptations that rely on long distance linkage disequilibrium between variants dispersed across the genome. Old haplotypes that have evolved under selection-migration balance might thus often confer large context-dependent fitness effects. Introgression of such large-effect haplotypes into a population with ecological opportunity might facilitate jumps across fitness valleys which are otherwise difficult to cross under mutation-limited evolution [82, 117].

Box 3: Roadmap for studying combinatorial mechanisms in speciation

A diagnosis of speciation with an important role of combinatorial mechanisms should include comparison of species splitting times with coalescent ages of haplotypes involved in speciation and of linkage disequilibrium patterns at such loci between new species and ancestral species to assess whether new species are characterized by new combinations of old variants.

If speciation occurred through reassortment of old haplotypes, their coalescent time should considerably exceed the distribution of genome-wide coalescent times marking the start of speciation [118]. Underestimating species splitting times, e.g. due to gene flow in secondary contact or due to incomplete isolation during early stage speciation, can also lead to higher than expected coalescent ages of reproductive isolation loci even when the latter evolved from *de novo* mutation [119]. However, if the haplotypes form paraphyletic or polyphyletic gene trees when outgroup taxa are included or show clear signs of introgression, they are unlikely to represent new mutations. Detecting this will require studying speciation in a strongly phylogenetic context. Many early speciation genetics studies overlooked the combinatorial process because they were confined to the diverging sister species.

Novel combinations of old alleles can be identified from patterns of linkage disequilibrium between reproductive isolation loci among the new species and between them and the ancestral species (Figure 2F-H). Combinatorial mechanisms from standing genetic variation should lead to the evolution of strong linkage disequilibrium between such loci from initial linkage equilibrium in the ancestral population (Figure 2F & 2H). Combinatorial mechanisms from admixture variation predicts in the new species the evolution of linkage disequilibria with reversed sign when compared to other and to parental species between some of the loci originating from different parental species (Figure 2G & 2H).

Empirical distributions of effect sizes of admixture-derived and other variants are needed to confirm the predicted shift to large effect sizes, e.g. via QTL mapping or GWAS [120, 121]. Comparing variation in phenotypes, fitness [e.g. 32] and mating behaviour [e.g. 55, 58] between experimental hybrids and their parental lineages can elucidate the potential of hybrid populations to become new species or to initiate a new radiation. Evolution experiments with synthetic hybrid lineages and multiple ecological niches [e.g. 122] might help to assess how the sorting of admixture-derived large-effect haplotypes contributes to adaptive radiation.

Comparing rapidly speciating lineages with close relatives that do not speciate could reveal to what extent combinatorial mechanisms contribute to heterogeneity in speciation rates and species richness. Such lineages should be investigated for differences in genetic variation, distributions of effect sizes, admixture history or admixture potential, in particular where they co-occur with hotspots of adaptive radiation on islands or lakes.

Glossary

Adaptive radiation from a hybrid swarm: several ecologically differentiated species evolve from a single hybrid population, whereby admixture variation facilitates adaptation to a variety of new niches but importantly also reproductive isolation among the emerging species.

Balancing selection: selective process by which two or more alleles are maintained in the gene pool of a population at frequencies larger than expected under neutrality. Mechanisms include negative frequency dependent selection, spatial or temporal heterogeneity in the direction of selection or global heterozygote advantage.

Bateson-Dobzhansky-Muller incompatibilities (BDMIs): alleles at different loci that are incompatible with each other when present in the same genome.

Founder effect speciation: speciation following a founder effect, in which reproductive isolation arises due to strong drift-induced allele frequency changes altering selection pressures on epistatically interacting genes.

Hybrid speciation: two species through hybridization form a third, stable lineage, isolated from both parental species, either with a mosaic of parental chromosome blocks (i.e. homoploid hybrid speciation) or combining both parental chromosome sets (i.e. allopolyploid hybrid speciation). Deeply divergent haplotypes are immediately available throughout the genome facilitating response of the hybrid population to divergent selection between parental species and the hybrid population and associated ecological differentiation of the hybrid lineage. The hybrid species might become reproductively isolated from both parental species through sorting of incompatibilities additional to mating trait divergence and divergent adaptation.

Hybrid trait speciation: introgression from a distant relative of a 'magic trait', *i.e.* a trait conferring both ecological divergence and reproductive isolation, triggers speciation in the introgressed lineage.

Large-effect haplotypes: Haplotypes that strongly influence the phenotype, its ecological function, its mating function and/or fitness.

Recombinational speciation: a special form of hybrid speciation involving karyotype evolution (e.g. chromosome arm translocations) between the hybrid species and its parental lineages.

Transgressive trait values: Extreme trait values in hybrids that lie outside of the range of the values of both parental species combined.

Transporter hypothesis: a mechanism by which the standing genetic variation of a population or species is replenished by recurrent gene flow from a population or species adapted to an alternative habitat and thereby facilitates repeated adaptation to the alternative habitat in additional locations, including possible parallel speciation.

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Supplementary Table 1: Study systems with evidence for ancient genetic variation involved in recent rapid speciation or in recent radiations with several speciation events in short succession. In all cases, the origin of relevant genetic variation clearly pre-dates the onset of speciation, *i.e.* the beginning of the build-up of reproductive isolation. Included are only taxa that are sufficiently reproductively isolated from each other to coexist in sympatry or where reproductive isolation has been shown experimentally.

System	**Start of speciation**	**Age of alleles**	**Source(s) of alleles**	**Evidence for importance of old alleles in speciation**	**Evidence for (partial) reproductive isolation and involvement of old haplotypes**	**Refe-rences**
Darwin's finch radiation	~10 y, ~100-300 ky	~1 My	Hybridization	The most recent estimate of ~10 years was documented in Lamichhaney et al. (2018), who described a very recent hybrid speciation event ("Big Bird"), including the allopatric / much earlier origin of alleles involved in transgressive segregation of beak size and shape leading to both ecological differentiation and behavioural reproductive isolation to one of the parental lineages. The divergence times between the most basal clades of Darwin's finches was estimated by Lamichhaney et al. (2015) to 0.9 Mya, whereby most rapid radiations of ground and tree finches started only 100-300 kya. The divergence of two haplotypes at crucial genes for beak size (HMGA2) and beak shape (ALX1) within these ground and tree finch radiations was estimated to ~1 Mya by Lamichhaney et al. (2016) and 913 kya by Lamichhaney et al. (2015), respectively, thus clearly pre-dating the time frame for speciation. Sorting of these old haplotypes or old variation at other genes seems to be generally important in speciation in Darwin's finches as shown in genome scans by Han et al. (2017).	Darwin finch species differing in beak morphology can coexist on the same island, haplotypes underlying ecological divergence and behavioural mate choice are very old	[1-4]
Marine / freshwater threespine stickleback	34-50 y, <12 ky	1-14 My	Standing variation and hybridization	Terekhanova et al. (2014) showed that extremely young (34-250 y) to very young (600-700 y) freshwater populations in the White Sea basin (Russia) evolved through selection on freshwater haplotypes that diverged from the marine haplotypes 1 Mya. Both marine and freshwater ecotypes breed in sympatry and must be reproductively isolated to some extent in the 34-years old Lake Ershovskoye. Bassham et al. (2018) studied 50 years old Alaskan freshwater populations, which are morphologically as distinct from their marine ancestors as the much older Alaskan populations. They show that the same haplotypes are selected in three independent freshwater populations and that those freshwater haplotypes are selected against in the marine environment and are only present there due to a migration-selection balance with many freshwater populations. Studying two max 12 ky old freshwater stickleback populations in Alaska, Nelson & Cresko (2018) estimated the mean divergence times between freshwater and marine haplotypes to 6.4 My ranging from 1-14 My. Genomic regions that were differentiated between marine and freshwater sticklebacks were substantially older than the genome-wide average. These exact same haplotypes are shared with marine and freshwater populations around the globe that have independently evolved in the last <12ky (Jones et al. 2012 documenting the identity of alleles, and many other references	Coexistence of freshwater and anadromous sticklebacks, haplotypes involved in parallel adaptation are ancient	[5-7]

System	**Start of speciation**	**Age of alleles**	**Source(s) of alleles**	**Evidence for importance of old alleles in speciation**	**Evidence for (partial) reproductive isolation and involvement of old haplotypes**	**Refe-rences**
				documenting the age of such freshwater populations). Together, these studies show that the extremely rapid parallel speciation in sticklebacks is based on much older genetic variation that is found at very low frequency in the marine populations due to constant inflow from a multitude of freshwater stickleback populations as suggested by the transporter hypothesis proposed by Schluter & Conte (2009).		
Tragopogon goatsbeard flowers	~90 y	~2 My	Hybridization	Soltis et al. (2004) studied two allotetraploid *Tragopogon* species *T. mirus* and *T. miscellus* that evolved from hybridization between the parental species *T. dubius*, *T. pratensis* and *T. porrifolius*, respectively which were introduced to the Palouse prior in the early nineties and did not co-occur prior to 1928 (90 years ago). Bell et al. (2012) dated the phylogeny of *Tragopogon*, with the TMRCA between *T. porrifolius* and the other two parental species falling only slightly short of the *Tragopogon* crown age of 2.6 My and thus reported as ~2 My.	Evolution and coexistence of new hybrid species or 'new combination of old alleles' in sympatry with parental lineages.	[8, 9]
Rhagoletis pomonella species complex	~200 y	~1.6 My	Hybridization	About 200 years ago, a new morph evolved in the *Rhagoletis pomonella* species complex which switched from the original hawthorn host to the earlier fruiting apple that was introduced to North America. Other species had evolved previously adapted to plants with differing fruiting times, generating *R. mendax*, *R. zephyria* and the flowering dogwood fly. In addition, a new species feeding on introduced honeysuckle seems to have evolved from hybrid ancestry of *R. mendax* and *R. zephyria*. Even though some of these host-adapted morphs are sometimes called host races and others are called species and have received a scientific name, Xie et al. (2008) showed that there is no qualitative genetic difference among them. The host switch is associated with temporal and behavioural reproductive isolation as they find their mates on the host plants that fruit at different times. The radiation was preceeded by introgression of inversions with genes affecting diapause from the Mexican altiplano fly. Feder et al. (2003) estimated the age of the Mexican inversions to 1.57 Mya.	Sympatric coexistence, reproductive isolation related to diapause time and mate choice on the host plants	[10, 11]
Lake Ejagham cichlid radiation	1-2 ky	~10 k	Hybridization	Poelstra et al. (2018) showed that even though *Coptodon* cichlids colonized the Cameroonian Lake Ejagham 9 ka, right after its formation, they only radiated into four new species in that lake after a secondary colonization with admixture 1-2 kya. The second lineage is closest to a riverine taxon that diverged about 10 kya from the original Lake Ejagham Coptodon lineage. All four species in the lake evolved shortly after the admixture event and Poelstra et al. (2018) found the three species they studied all to be admixed. Speciation seems to be associated with introgression of a cluster of 8 olfactory genes possibly involved in chemosensory mate choice. The introgressed haplotype is fixed in two species, and absent in the third species supporting a role in behavioural reproductive isolation between some of the species.	Sympatric coexistence in a small crater lake, speciation after introgression of genes that are possibly involved in behavioural mate choice	[12]

System	Start of speciation	Age of alleles	Source(s) of alleles	Evidence for importance of old alleles in speciation	Evidence for (partial) reproductive isolation and involvement of old haplotypes	Refe-rences
Bahamas *Cyprinodon* pupfish radiation	~10 ky	>>10 ky	Hybridization	Whereas generalistic *Cyprinodon* pubfish are widespread on Bahamas islands, they speciated only on a single island. On San Salvador Island, 3-4 ecologically different species evolved within the past 10,000 years. Richards and Martin (2017) discovered adaptive introgression of four genes related to jaw morphology and one gene with behavioural effects from a much more divergent species *C. laciniatus* of another Bahamian island (divergence time >>10ky, but not dated formally) that seem to have played an important role in shaping this radiation with a novel trophic specialist. The authors date the introgression event to ~5,700-23,500 years suggesting that introgression may have preceded the radiation. Introgression may thus not only have been a facilitator but potentially even a trigger of this adaptive radiation.	Sympatric coexistence of the different trophic specialists, alleles involved in trophic adaptations and behavior introgressed from a distant relative	[13]
Italian sparrow (*Passer italiae*)	~10 ky	~800 ky	Hybridization	The Italian sparrow is a hybrid species between the House and the Spanish sparrow that likely arose less than 10,000 years ago [14]. Using a genomic cline approach, Hermansen et al. (2014) show that sorting of pre-existing incompatibilities contributed to reproductive isolation between the hybrid species and its parental species. Sex-linked and mito-nuclear incompatibilities isolating the hybrid Italian sparrow from its parental species represent different subsets of loci contributing to reproductive isolation between the parental species. Ravinet et al (2018) estimated the divergence between the house and the Spanish sparrow to 0.83 Mya and the divergence time of the alleles combined through hybridization is thus ~0.8 My.	Sorting of parental incompatibilities contributes to reproductive isolation between the hybrid Italian sparrow and its two parental species.	[15, 16]
Lake Victoria Region Superflock (tribe Haplochromini) encompassing multiple cichlid radiations in different lakes including the Lake Victoria radiation	~150 ky, ~15 ky (Victoria)	>2 My	Hybridization	Meier et al., (2017) showed that the multiple adaptive radiations in different lakes in the Lake Victoria Region Superflock of haplochromine cichlids are all derived from hybridization between two or three lineages that are 1.5-3 My divergent. The oldest radiation in the region (Lake Edward) is only about 100-150,000 years old. The largest radiation of 500 species in Lake Victoria itself is likely less than 15,000 years old as the lake was completely dry for 4,000 years before that. Meier et al. (2017) showed that the hybridization event contributed the haplotype diversity at the red opsin gene involved in adaptation to different water depths and potentially also in color-based mate choice. They further showed that other polymorphisms too that originated from the ancient hybridization event became divergently sorted between sympatric Lake Victoria species and are enriched among the exceptionally strongly differentiated regions.	Sympatric coexistence of dozens of species at each site within each lake, genetic variation derived from admixture was e.g. involved in visual adaptation to different water depths which also affected mating behaviour.	[17]
Helianthus sunflowers	60-200 ky	>1 My	Hybridization	Rieseberg et al. (2003) summarizes evidence for hybrid origins of three *Helianthus* sunflower species derived from the same two parental species during the past 60-220 ky. They show that experimental hybrids can recover the extreme (transgressive) phenotypes displayed by the hybrid species which allowed them to adapt to extreme habitats. Rieseberg et al. (1996) showed that five generations of	Genes involved in major ecological transitions and in hybrid inviability are derived from hybridization between two parental species. They	[20]

System	Start of speciation	Age of alleles	Source(s) of alleles	Evidence for importance of old alleles in speciation	Evidence for (partial) reproductive isolation and involvement of old haplotypes	Refe-rences
				hybridization between the parental species were sufficient to experimentally recreate hybrid populations with patterns of introgression that were significantly similar to the composition of one of the hybrid species. Strasburg and Rieseberg [18] estimated the divergence time between the two parental species to about 1 Mya.	show strong pre- and postzygotic isolation from their parental lineages [19].	
Mimulus aurantiacus monkeyflowers species complex	recent	old	Hybridization	Studying the recent radiation of the *Mimulus aurantiacus* monkeyflower species complex, Stankowski and Streisfeld (2015) show that the allele at the *MaMyb2* gene conferring red coloration introgressed into *M. puniceus* from another member of the species complex. They postulate that this introgression event initiated pollinator-mediated divergence between *M. puniceus* and its yellow-flowered sister species *M. australis*. Even though the authors have not dated the alleles, they report that “the level of sequence divergence at *MaMyb2* is an order of magnitude greater than the genome-wide average”.	The allele contributing to RI through a switch of pollinator syndrome introgressed from a divergent species.	[21]
Sporophila capuchino seedeater radiation	44k gen.	>44k gen	Standing variation or hybridization	Campagna et al. (2015) estimated that the southern capuchino seedeater radiation (genus *Sporophila*) is about 44,000 generations old, and thus has a Pleistocene origin (Campagna et al., 2017). They inferred a demographic history with large ancestral population size of the capuchino radiation and introgression from the allopatric outgroup *Sporophila bouvreuil.* They further showed that pigmentation genes have repeatedly been targeted by divergent selection between different species pairs. Species grouping in PCA of those high divergence regions suggests that different species share old haplotypes that have been sorted differentially in the rapidly emerging species. Even though the haplotypes predate the radiation, the authors do not yet know the ages of the pigmentation gene haplotypes or if they are derived from the hybridization event or not.	High levels of sympatry among species, RI mostly based on differences in song and plumage coloration. Evidence for repeated selection on haplotypes at pigmentation genes that are shared among species and thus predate the individual speciation events.	[22, 23]
Australo-Papuan munia radiation (genus *Lonchura*)	<500 ky	>>500 ky	Standing variation or hybridization	Stryjewski & Sorenson (2017) show that the diversification of *Lonchura* munias in the region of Australia and New Guinea into 13 species occurred over maximum 0.5 million years. The species differ in plumage coloration and bill size. Genomic regions under divergent selection between species show gene trees that are discordant from the species phylogeny and haplotypes at these genes are shared among multiple species. Each species has a unique combination of old alleles at different high differentiation regions which include genes involved in pigmentation, bone development or cold adaptation. The authors are not able to distinguish if repeated selection on ancestral polymorphisms or introgression explain the mosaic patterns at regions under divergent selection but it seems certain that the haplotype diversity predates the species diversification.	Lack of mitochondrial introgression, difference in plumage coloration and bill size, and rarity of hybrids despite sympatry all suggest the presence of some reproductive isolation. At high differentiation regions, each species is a unique mosaic of old haplotypes shared across multiple species.	[24]
Heliconius butterflies	<2 Mya <1.5 Mya	~4 Mya, >2 Mya	Hybridization	Wallbank et al. (2016) studied the *dennis-ray* mimicry ring of Amazonian *Heliconius* butterfly species displaying red hindwing rays (*ray*) and a red forewing patch	Haplotypes underlying wing color patterns under strong	[30, 31]

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				(*dennis*). These color patterns are under strong natural selection for warning coloration and also involved in mate choice. The authors show that the two wing patterns are controlled by two closely linked regulatory regions of the *optix* gene. The underlying haplotypes each evolved once in different species that are 4 My divergent and were then reshuffled among a group of 16 species. They estimate that about 2 Mya the *dennis* allele introgressed from *H. elevatus* into *H. melpomene* and around the same time, the *ray* allele evolved in *H. melpomene*. *H. elevatus* then also acquired the *ray* allele through introgression from *H. melpomene* a million years later which allowed it to join the *dennis-ray* mimicry ring in the Amazon rainforest. Both *dennis* and *ray* also introgressed into *H. timareta* which speciated from the ancestor of *H. tristero* and *H. heurippa* around the time of the *ray* introgression. Enciso-Romero et al. (2017) showed that introgression between *H. melpomene* and *H. cydno* which are about 2 My divergent facilitated the evolution of new races in more recent times. Introgression of haplotypes controlling the yellow hindwing bar from *H. melpomene* into *H. cydno* about 1 Mya facilitated the origin of *H. pachinus* in Costa Rica and a new race of *H. cydno* in Colombia. Likewise, they found introgression from *H. cydno* into *H. melpomene* about 410 kya facilitating the formation of new races in *H. melpomene*. There is also evidence for hybrid speciation forming *H. heurippa* [25, 26] and for widespread hybridization across the *Heliconius* radiation [27-29].	divergent selection between mimicry rings and involved in mate choice have been reshuffled among species and populations through introgression facilitating adaptation to novel mimicry rings.	
Hawaiian silversword alliance	~5 Mya	~15 Mya	Hybridization	Barrier et al. (1999) show that the entire Hawaiian silversword alliance is an allotetraploid group which evolved from hybridization between two North American tarweed species which Baldwin and Sanderson (1998) estimated to have diverged about 15 Mya. Baldwin and Sanderson (1998) estimated the age of the Hawaiian radiation to 5.2 million years ago. Silversword alliance species occur in a wide range of habitats from exposed lava, dry woodland to bogs, in altitudes ranging from < 100 m to > 3700 m, and they show a broad diversity of growth habits, including cussion plants, shrubs, trees and lianas.	(Partial) reproductive isolation due to differences in reproductive traits such as floral organ size and morphology and difference in habitat preference.	[32, 33]

Criteria for study systems to be used in this table:

Evidence for genetic variation with an important role in speciation that clearly predates the onset time of speciation, including old haplotypes at genes known to contribute to either reproductive isolation or ecological differentiation.

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