

Hybridization and adaptive radiation

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Whether interspecific hybridization is important as a mechanism that generates biological diversity is a matter of controversy. Whereas some authors focus on the potential of hybridization as a source of genetic variation, functional novelty and new species, others argue against any important role, because reduced fitness would typically render hybrids an evolutionary dead end. By drawing on recent developments in the genetics and ecology of hybridization and on principles of ecological speciation theory, I develop a concept that reconciles these views and adds a new twist to this debate. Because hybridization is common when populations invade new environments and potentially elevates rates of response to selection, it predisposes colonizing populations to rapid adaptive diversification under disruptive or divergent selection. I discuss predictions and suggest tests of this hybrid swarm theory of adaptive radiation and review published molecular phylogenies of adaptive radiations in light of the theory.

Some of the confusion about the role of hybridization in evolutionary diversification stems from the contradiction between a perceived necessity for cessation of gene flow to enable adaptive population differentiation on the one hand [1], and the potential of hybridization for generating adaptive variation, functional novelty and new species [2–4] on the other. Much progress in the genetics [5–9] and ecology of hybridization [9–11], and in our understanding of the role of ecology in SPECIATION (see Glossary) [12–14] make a re-evaluation timely.

Whereas botanists traditionally stressed the diversity-generating potential of hybridization [2,3,14], zoologists traditionally saw it as a process that limits diversification [1] and refer to it mainly in the contexts of hybrid zones (Box 1) and reinforcement of reproductive isolation [15]. Judging by the wide distribution of ALLOPOLYPLOIDY among plants, many plant species might be of direct hybrid origin or descended from a hybrid species in the recent past [16]. The ability to reproduce asexually might explain why allopolyploid hybrid species are more common in plants than in animals. Allopolyploidy arises when meiotic mismatch of parental chromosomes or karyotypes causes hybrid sterility. Mitotic error, duplicating the karyotype, can restore an asexually maintained hybrid line to fertility. Although bisexual allopolyploid hybrid species are not uncommon in fish [17] and frogs [18], the difficulty with which allopolyploid animals reproduce, typically requiring GYNOGENESIS [19], makes establishment and survival of allopolyploid animal species difficult.

However, most cases of natural hybridization are probably not associated with meiotic mismatch, because they occur between closely related species. Resulting hybrid populations are therefore bisexual, with the same number of chromosomes as the parent species have. Their hybrid nature was difficult to detect, but this has recently begun to change owing to improvements in molecular genetic techniques. Indeed, the number of diploid hybrid species discovered is increasing [6,20–26], suggesting that many more species are of hybrid origin than was previously thought. Recent theoretical models and experimental results suggest that induction of POLYPLOIDY as a means of instantaneous reproductive isolation is not required for HYBRID SPECIATION, but that hybrid species can rapidly become reproductively isolated by other mechanisms (Box 2).

Ecological conditions conducive to hybridization are also conducive to adaptive radiation

In 1981, Templeton predicted [27] that events that bring ecologically divergent parental species together also often disturb the environment to yield hybridized habitats and that, in groups susceptible to hybrid speciation, such

Glossary

Adaptive radiation: the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage.

Allopolyploid: having more than two chromosome sets as a result of the duplication of the number of chromosomes in a hybrid lineage following its origin by interspecific hybridization.

Cyotype: haplotype of cytoplasmic (mitochondrial or chloroplast) DNA

Disruptive selection: natural or sexual selection that favours extreme over intermediate phenotypes within a single population.

Gynogenesis: mode of reproduction in which development occurs from eggs penetrated by sperm but not fertilized, hence without genetic contribution of the male.

Hybrid speciation: the origin of a new species through formation of hybrids between existing species.

Introgressive hybridization: exchange of genes between evolutionary lineages as opposed to hybridization yielding exclusively inviable or infertile offspring.

Parapatric speciation: speciation in the absence of geographical barriers to gene flow, resulting in incipient species that occupy adjacent areas.

Polyploid: having more than two chromosome sets, as tetraploid (four), hexaploid (six) and so on.

Transgressive segregation: generation of phenotypes in segregating hybrid populations that are extreme relative to those of either parental line.

Segregation variance: complementary action of different genetic loci that are fixed for alleles with effects in opposite direction in the parent species, that have additive effects when recombined in their hybrids and that generate a phenotype that is different from both parental phenotypes but not intermediate to them (e.g. alleles with opposing sign on quantitative trait loci).

Speciation: evolutionary divergence of subsets of one ancestral species into two different species.

Sympatric speciation: speciation occurring in the absence of geographical isolation (e.g. within the dispersal range of a single generation).

Syngameon: a complex of selection-maintained, genetically weakly but ecologically highly distinctive species capable of exchanging genetic material.

Box 1. Introgressive hybridization, hybrid zones and hybrid fitness

Introgressive hybridization can influence evolution in several ways: it can cause fusion of species [58], genetic swamping of one species by another, elicit reinforcement of reproductive isolation between incompletely isolated species [15], transfer of genetic material between species, potentially facilitating their adaptive evolution [10,11], and the origin of new species (Box 2). However, it can also remain without an obvious impact if recombinant genotypes are retained in a narrow contact zone between two species by a balance between dispersal into the contact zone and selection against hybrids [35].

Introgressive hybridization is most commonly observed in zones of geographical contact between otherwise allopatric taxa. Studies of such zones have provided important insights into the evolutionary process and have helped resolve part of the debate about fitness of hybrids. In many cases, most hybrid genotypes tend to be less fit than are the parental genotypes in parental habitats [9,35], owing either to endogenous or exogenous selection or both [8]. However, theory predicts that some can be of equal or superior fitness in new habitats and, occasionally, even in parental habitats [8,26].

Most attempts to explain hybrid zones can be roughly classified as derivatives of two models. The tension zone model explains hybrid zones entirely by a balance between dispersal into the zone and selection against hybrids [35]. The bounded hybrid superiority model implicates elevated hybrid fitness in those sections of the environment where the hybrid zone lies [66]. Many clinal hybrid zones are well explained by the tension zone model, but in others, environment-dependent hybrid superiority has been demonstrated. In such cases, hybrid zones often represent a mosaic of genotype distribution in space (mosaic hybrid zones), tracking patchy environments, rather than showing the clinal pattern predicted by the tension zone model [11]. An excellent example of environment-dependent hybrid superiority, although not in a classic hybrid zone situation, are the ground finches of Daphne Major in the Galapagos Archipelago [10].

events might induce bursts of speciation followed by long periods of stasis. INTROGRESSIVE HYBRIDIZATION between animal species is now known to be common [10,28] and many cases involve taxa of ADAPTIVE RADIATIONS. Evidence has been found in most genetic studies of adaptive radiations, including Darwin's finches [29], Hawaiian picture-winged fruit flies [30], Hawaiian crickets [31] and African cichlid fish [24,25], among others [32–34].

Hybridization is also particularly common in populations at the ecological or geographical periphery of species ranges, where population densities are low, mates are rare and/or selection against hybrids by competition with parents is weak or nonexistent [6,11]. Investigations of hybrid zones suggest that, in parental niches, most hybrid genotypes have lower fitness than do their parents [35], but that some hybrid genotypes can be fitter than both parents in third niches, often novel, extreme or perturbed habitats ([8,9,11,36,37] Box 1). Adaptive radiations are thought to often require new adaptive zones comprising sets of novel, thoroughly altered ('perturbed') or previously underutilized niches ([12] Box 3). Hence, ecological conditions that are conducive to hybridization and establishment of hybrid populations tend also to be conducive to adaptive radiation. In this article, I develop the argument that adaptive radiation is facilitated by interspecific hybridization upon invasion of new adaptive zones.

The rationale

Sympatric speciation and the genetics of hybrid populations

Most adaptive radiations occur in geographically narrowly confined regions, and sympatry or parapatry during part of the speciation process is invoked in many ([12] Box 3). SYMPATRIC and PARAPATRIC SPECIATION had long been considered highly unlikely, because creating associations between ecological and mating genes against gene flow can require unrealistically strong DISRUPTIVE SELECTION [38]. Publications that demonstrated monophyly of geographically confined adaptive radiations from mitochondrial gene sequences [39–45] stimulated renewed interest in sympatric speciation. More recent theoretical investigations suggest that such speciation is more likely than was previously thought when populations experience disruptive selection on quantitative traits that have broad variation determined by the additive effects of many genes [46,47].

Hybridization creates such variation instantaneously and simultaneously in several functional traits, including phenotypic marker traits that can be recruited for mate choice. If genetic variation in quantitative fitness and mating traits is an important constraint to ecological speciation without geographical isolation, such speciation will be more likely in hybrid than from nonhybrid populations. In many cases, phenotypic variation in a hybrid population will not only exceed that in each parent population, but will also exceed the combined variation of both parent populations, referred to as TRANSGRESSIVE SEGREGATION ([7] Box 2). This is often due to SEGREGATION VARIANCE [48] or reassortment of functional gene complexes in hybrid populations. Independent of this, hybridization might, in some cases, lead to increased mutation rates [16,26,49].

The most surprising result of investigations of adaptive radiations is the rapidity with which multiple speciation events occur during adaptive radiations. For instance, 33 species of sculpins, functionally so diverse that two endemic families were proposed for them, evolved from standard riverine sculpins in Lake Baikal within the Pleistocene [50], and >500 species of cichlids have arisen in the Lake Victoria region within 15 000–200 000 years [51,52]. At such timescales, the amount of heritable variation in fitness traits in the founder population might not only determine the probability of speciation by divergent or disruptive selection, but also the functional volume attained by a radiation. Here, I develop two related hypotheses for the role of hybridization in this process.

A hybrid swarm origin hypothesis for adaptive radiations

If a population that contains sufficient variation at functional loci arrives in an adaptive landscape that comprises multiple underutilized fitness peaks ('niches'), and if only subsets of these peaks can be effectively utilized by any one functional genotype, several populations of functional genotypes might emerge rapidly by multiple events of ecological speciation [12,13,46]. By instantaneously elevating heritable variation in ecologically relevant traits in situations where the potential for ecological diversification is unusually high, hybridization upon invasion of new environments [6,21,53] should therefore facilitate rapid

Box 2. Novelty through hybridization: transgressive segregation and ecological hybrid speciation

Hybrids often exhibit novel or extreme characters when compared with parental taxa, referred to as 'transgressive segregation'. This is usually due to segregation variance and sometimes to overdominance or epistasis [7,48]. Segregation variance is caused by the complementary action of different genetic loci that are fixed for alleles with effects in opposite directions in the parent species and that have additive effects when recombined in their hybrids (e.g. alleles with opposing sign on quantitative trait loci). Transgressive segregation is very common in interspecific hybridization. Rieseberg and colleagues [7] found at least one transgressive trait in 155 out of 171 hybrid cases reviewed, and found that 541 of 1229 traits examined were transgressive. Transgressive segregation could explain elevated rates of adaptive evolution after hybridization [57] (Figure 1).

For lasting increase in adaptive diversity, the novel genomes have to become genetically stabilized and reproductively isolated from the

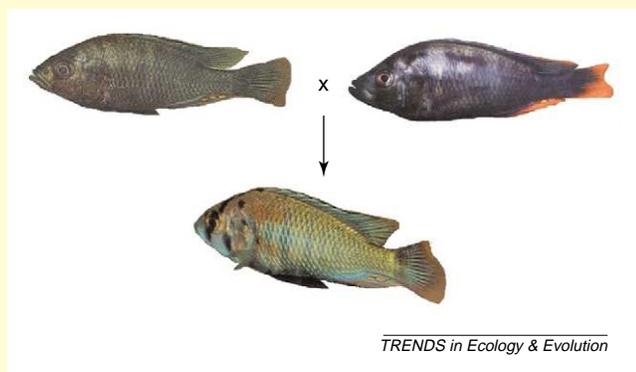


Figure 1. Occurrence of a novel male nuptial colour pattern in hybrids between two Lake Victoria cichlid species [70]. The male in the centre is an F1 hybrid produced by mating a *Haplochromis* sp. 'black lividus' (top left) with an *Astatotilapia nubilila* (top right). Males of both parental species are blue-black. The 'orange dorsum' phenotype appeared among the hybrids alongside blue-black phenotypes. The transgressed phenotype closely resembled a male nuptial colour pattern that is known from several other species in Lake Victoria. Top left and centre photo reproduced, with permission, from [70].

parental ones. This was considered the second major theoretical constraint to HOMOPLOID HYBRID SPECIATION, after the presumed fitness disadvantage of hybrids. However, molecular gene mapping studies of hybrid species, together with the experimental creation of hybrid lines and computer simulations, have demonstrated that homoploid hybrid speciation can be rapid, with no more than 25 generations required for stabilization of hybrid genomes [67]. Simulation models suggest that ecological separation from both parent species is essential [68,69]. Empirical studies show that hybrid species usually differ ecologically from both parents by occupying different spatial, temporal or diet niches [6,20,34,37]. Transgression is probably instrumental in enabling some hybrid genotypes to utilize habitats or niches that neither parent species can utilize. Ecological niche partitioning is possible if hybrid genotypes have elevated fitness in some ecological conditions, but lower than parental fitness in parental ecological conditions. Hybrids between *Helianthus* sunflower species combine additive effects of different parental loci for salt tolerance, enabling them to colonize habitats in which neither parent taxon performs well [37]. Interspecific hybrids in *Xenopus* frogs combine specific parasite resistances of both parents but express susceptibility to a third parasite that both parents are resistant to [18].

Extension of ecological speciation theory [12,13,46] predicts that the rate at which hybrid taxa can become reproductively isolated from parent species is enhanced in groups with mate choice and with variation in phenotypic marker traits that can be recruited for mate choice. Given that divergence in quantitative traits under selection in isolated populations is much faster than accumulation of genic incompatibilities that cause endogenous selection against hybrids, there will typically be ample opportunity for conditions to arise under which hybridization generates genetic and functional diversity without incurring endogenous selection against hybrids.

Glossary

Homoploid hybrid speciation: speciation in which the hybrid species obtains the same number of chromosomes as its parent species (in contrast to polyploid hybrid speciation).

adaptive radiation, as exemplified by waterfleas of the *Daphnia longipes* complex in northwest European lakes [34,54]. In a multimodal fitness landscape, where fitness peaks are seasonal niches mediated by changing abundances of predators of, and prey for *Daphnia*, different

Box 3. Adaptive radiation

Adaptive radiation is the rapid diversification of an ancestral population into several ecologically different species, associated with adaptive morphological or physiological divergence [12]. Although often occurring within confined geographical regions (e.g. large lakes or island archipelagos), much of the functional diversity of life might have arisen during episodes of adaptive radiation. Relatively broad agreement exists on several factors that might facilitate adaptive radiation, although these have proven difficult to quantify. They include release from competition in an underutilized environment (e.g. a new lake), key evolutionary innovations that enable utilization of resources that were there but could not be utilized previously (e.g. echolocation in bats) and mating systems that are conducive to rapid divergence (reviewed in [12]). However, variation in these criteria is, in many cases, insufficient to explain the adaptive radiation of some and the absence of radiation of other taxa that share the same characteristics and find themselves in the same environmental circumstances. For example, Lake Victoria in East Africa was colonized by five unrelated lineages of cichlid fish at the same time, but whereas one radiated into (500 new species, the other four did not diversify.

fitness peaks are occupied by parent species and hybrid taxa [54]. In *Daphnia*, the hybrid taxa are typically asexual clones, which has facilitated the discovery of the phenomenon. However, if disruptive selection can recruit genes for mate choice, the same principle could apply to sexual hybrid populations.

The hybrid origin hypothesis predicts that the probability of occurrence of rapid adaptive radiations is increased if secondary contact between related species or divergent populations coincides with environmental situations in which (some) hybrid genotypes have no ecological disadvantage and ecological opportunities enable niche partitioning between more functional gene combinations than those represented by the genotypes of the parental species.

A syngameon hypothesis for the generation of functional diversity

After a radiation has progressed to a stage with two or more incipient species, occasional or localized hybridization between these species would facilitate further ecological diversification. Such SYNGAMEON conditions would enable the rapid generation, by hybridization, of new adaptive trait combinations, which are suitable for utilizing resources that were not utilized previously, whilst

simultaneously preventing exhaustion of variation at quantitative trait loci under the strong selection associated with rapid adaptive radiation and sympatric speciation. Hence, hybridization would maintain and prolong the adaptive radiation momentum. Such a mechanism has recently been suggested to explain the rapid evolution of mimicry rings in *Heliconius* butterflies [55]. As the number of species increases and unexploited resources become scarce, selection as experienced by individual populations would change from directional or disruptive to stabilizing. The probability that recombinant hybrid genotypes find underutilized niches in which they enjoy equal or higher fitness than do their parents would diminish. The depression of ecological fitness of hybrids relative to that of parental types would tend to reinforce pre-mating isolation. Hence, the frequency of interspecific hybridization would decrease and fewer hybrid genotype populations would become established as new species, whilst genomic incompatibilities would begin to accumulate between existing species. The same idea, however, also predicts that radiations might never reach this stage if the environmental conditions are unstable [29] or if successively evolved innovations open up new niche dimensions, allowing for successive rounds of radiation [55].

The syngameon hypothesis predicts that the volume (species numbers and functional diversity) acquired in an adaptive radiation depends not only on ecological opportunity and genetic variation, but also on the opportunity for occasional or locally restricted hybridization between diverging populations within the radiation, such as in strongly structured metapopulations that experience temporal and spatial variation in selection regimes.

Predictions and tests

Provision of support for the hybrid swarm theory requires a combination of tests that: (i) establish evidence of hybridization preceding many radiations; (ii) demonstrate that, in many radiations, most of the diversity arose in a syngameon phase; (iii) demonstrate generation of functional diversity by hybridization; and (iv) demonstrate that hybridization elevates the likelihood of, and the volumes attained by radiations.

Phylogenetic predictions of hybridization preceding adaptive radiations

Emergence of adaptive radiations from hybrid populations predicts a phylogenetic signature that is recoverable with the use of molecular and statistical genetic methods. If a radiation was directly preceded by hybridization between two ancestral species, different regions of the genome of member species of the radiation will carry conflicting information about its immediate ancestors. Cytonuclear discordance is one possible form of such genealogical conflict. Parental alleles of nonrecombining cytoplasmic genes (CYTOTYPES) remain distinct in a hybrid clade. Given sufficient time, one will become fixed and one lost. By contrast, recombination will cause parental alleles of nuclear genes to converge in the hybrid clade. Hence, given sufficient time for recombination, nuclear genes of hybrid taxa will become intermediate to the parental alleles. If time was insufficient for the convergence of

nuclear genes, hybrid taxa will still be intermediate at the genome level, and different nuclear genes will suggest different ancestral relationships. Genealogical discordance will be difficult to detect if one parental cytotype became fixed and if the same parent species also contributed most of the nuclear genome of the hybrid taxon. In such cases, many nuclear genes would have to be investigated to confirm or reject hybrid origin. Technically, this is now quite possible (Box 4).

Special cases that would violate the above prediction but not the hypothesis of hybrid origin are: (i) repeated colonization of an underutilized environment from the same source population if colonization events are separated sufficiently long in time for the first population of colonists to have diverged in quantitative traits by the time the second wave of immigrants arrives ([56] Figure 1b); or (ii) when founder populations arising from a single colonization event, diverge in spatial isolation in different sections of the same adaptive zone and later become sympatric and hybridize in the process of filling up the adaptive zone (Figure 1c). Although two hybridizing taxa merge into a hybrid swarm, in both cases only one ancestral taxon remains outside the radiation. The phylogeny of the radiation would suggest paraphyly or monophyly, respectively. However, in contrast to truly monophyletic origins, both special cases predict that some genetic divergence among members of the radiation predates the main burst of the radiation.

Phylogenetic predictions of functional diversification during a syngameon phase

Present-time syngameon-like conditions have been demonstrated in several young adaptive radiations [21], including Darwin's finches [29,57], African cichlid fish [58] and *Heliconius* butterflies [32,55]. Phylogenetic analysis might also enable detection of past syngameon conditions because, in terms of the coalescence of selectively neutral genes, species in a syngameon form essentially a single gene pool. For neutral genes (and genes under the same directional

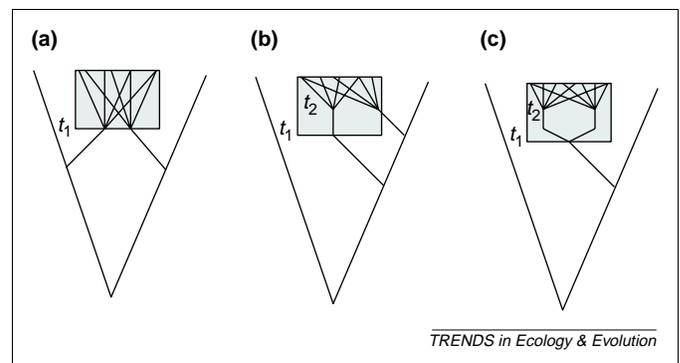


Figure 1. Genealogies expected under different scenarios of adaptive radiation from a hybrid population. **(a)** at t_1 , two species colonize the new environment (blue box), fuse into a hybrid swarm, and radiate, generating diphyletic genealogies of the radiation. **(b)** sequential double colonization of the new environment by the same species (at t_1 and t_2), fusion into a hybrid swarm followed by radiation, generating paraphyletic genealogies; **(c)** two founder populations arising from a single colonization event at t_1 begin to diverge in spatial isolation in different sections of the new environment and later (t_2) become sympatric, fuse into a hybrid swarm, followed by radiation, generating monophyletic genealogies. In contrast to true monophyly, all three cases predict that some genetic divergence among members of the radiation predates the main burst of the radiation.

Box 4. Methods for detecting hybrid origin in the absence of obvious cytonuclear discordance

Historical population genetics

Divergence population genetics (DPG; [71] and references therein) is a coalescent-based approach that compares patterns of sequence divergence between species across multiple recombining genes against predictions made by isolation models of speciation, models of speciation with gene flow and isolation models with lineage sorting. Isolation models predict that, with the number of shared polymorphisms decreasing and that of fixed differences increasing over time, net divergence for each gene is proportional to the time since speciation. If gene flow has occurred at some but not all loci, as expected in models of speciation under disruptive selection, large variance across loci is expected both in the numbers of shared polymorphisms and sites fixed for alternative alleles [71]. An analogous situation arises if a hybrid species (or one of several species in a clade of hybrid origin) that obtained some genes from each parent is compared with its parent species.

The Wang, Wakeley and Hey test [71] can be used to compare the sum of the across loci variance in the numbers of polymorphic sites shared between the putative hybrid and either of its putative parents plus the variance in fixed differences, against a distribution of expected values obtained from coalescent simulations of isolation with lineage sorting. A related approach measures linkage disequilibria (LD test) among polymorphisms within and between species [71]. Both tests depend on the accuracy of parameter estimations from the genetic data, and their power is strongly affected by the amount of recombination. A simpler approach might be to compare levels of coalescence observed in nuclear sequences to levels predicted from the observed coalescence levels in mitochondrial DNA, assuming polymorphisms in the latter are selectively neutral ([32]; Fig. 2, main text).

Least squares distance methods to reconstruct reticulate evolution

Phylogenetic networks or reticulograms can be constructed from gene sequence or allele frequency data using optimality criteria based on a least squares function that aims at minimizing the squared deviations between pairwise genetic distances and pairwise distances in a network. Methods have been developed to test whether a reticulogram explains the data significantly better than a tree [72].

Tree-based methods

Tree-based methods for identifying hybrid taxa through their homoplasy effects on a phylogeny estimate have had mixed success when morphological characters were used for which hybrids are intermediate between their parents [73]. Character intermediacy makes it difficult to distinguish between hybrids and intermediate species. However, character state variation at individual nucleotide sites in the DNA sequence has no intermediate condition. In phylogeny estimates, therefore, hybrids behave like one parent in some, and like the other parent in other characters. This between-character inconsistency as opposed to intermediacy of hybrids can be used to identify their hybrid status.

Genomic methods of assaying polymorphisms at many unlinked nuclear loci [e.g. amplified fragment length polymorphisms (AFLPs)] can provide large datasets for such analyses. Hybrid taxa will be intermediate overall to the parent species but identical to one or the other parent at each individual AFLP locus [74]. This phylogenomic inconsistency of a hybrid taxon introduces homoplasies in parts of a phylogenetic tree constructed from AFLP genotypes. The detection of this homoplasy effect can be used to test a hypothesis of hybrid origin, and the partitioning of the homoplasies over nodes in the tree can be used to infer the parental taxa. Inclusion of a hybrid taxon, whose ancestors are distant edges on the tree, is predicted to increase conflict, and therefore weaken support for nodes, more than will inclusion of a nonhybrid taxon. By reconstructing a tree with and without the putative hybrid taxon, and recording the difference that its inclusion makes to the support for each node, the homoplasy effect of a putative hybrid taxon can be investigated for each node and compared to a distribution of non-hybrid effects generated by adding and removing in turn each other taxon in the tree. Homoplasies introduced by hybridization between edges on a tree are partitioned exclusively over nodes that separate the sister taxa of those that generated the hybrid. Theoretically, the test can discriminate between emergence of an adaptive radiation from a hybrid population and hybridization between terminal taxa in the radiation. The former, but not the latter, predicts that the inclusion of any species of the radiation will have similar homoplasy effects, and implicate the same parental taxa as progenitors of the radiation.

selection in all taxa of the radiation), but not for genes under divergent selection, this predicts genealogies in which haplotypes of the adaptive radiation are separated from each other by branches of short or zero length, but by a much longer branch from haplotypes that are ancestral to the entire radiation and geographically isolated from it. For genes under divergent selection, branches separating haplotypes within the radiation would not differ in length from those that separate them from ancestral haplotypes (Figure 2). Although short branches within the radiation would also be consistent with recent radiation, the contrast between neutral and selected genes is not. Moreover, to explain neutral genealogies with short branches within, but a long branch under the radiation, other than by invoking a syngameon, requires the assumption that either radiation did not begin until well after colonization of the adaptive zone, or that the nearest relatives of the radiation outside the adaptive zone went extinct.

Demonstrating that hybridization generated functional diversity

This requires a demonstration that different genes contributing to the same quantitative trait, or genes that determine different traits in the same species, have been acquired from two different parental species. One way to

test it is to cross individuals of a species (A) that combines traits from two putative parent species (B, C) to individuals of species B and C. The separate crosses are then investigated to see whether genetic factors for each trait inherited from A and from B or C segregate as complementary alleles of the same gene, as expected if A obtained traits from B and C, or independently as alleles of different genes. If the putative parent species are unknown, it could be tested whether the same alleles determine the same derived trait in two species of a radiation that are not sister taxa. Mapping of the genes to the same or different chromosomal regions with molecular markers in the unrelated species is an alternative approach. The likelihood that sharing of complementary alleles for functional traits is due to an ancestral polymorphism is inversely proportional to the number of clades that separate the test species in the phylogeny without sharing the trait. Robust phylogenies are, therefore, required for robust test design.

Testing whether hybridization elevates the likelihood of adaptive radiation

Demonstrating that hybridization has preceded an adaptive radiation and has generated diversity is insufficient evidence for hybridization facilitating that radiation.

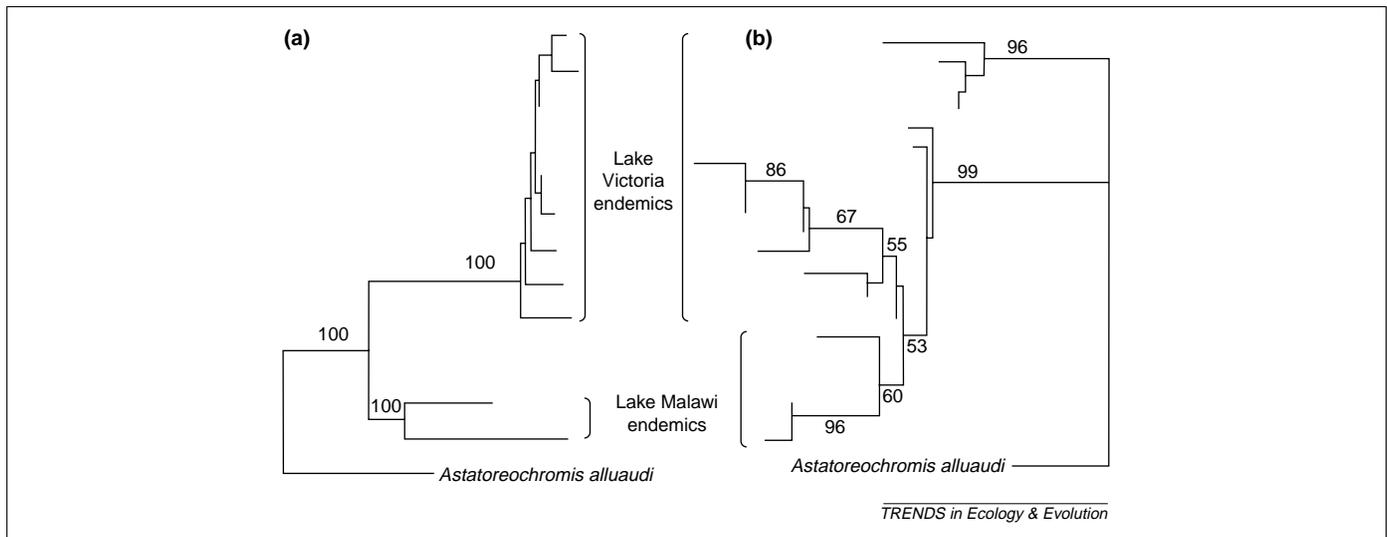


Figure 2. Phylogenetic prediction of the syngameon hypothesis. Contrasting genealogies for two genes in the adaptive radiation of Lake Victoria cichlid fish: (a) mitochondrial control region (sequences obtained from GenBank), (b) exons 2–5 of the long wave sensitive opsin gene [79]. The opsin gene is likely to be under divergent selection whereas the mitochondrial gene is probably not. Divergence in a syngameon predicts neutral gene genealogies in which all haplotypes within the radiation are separated from each other by branches of short or zero length, but by a much longer branch from haplotypes that are ancestral to the radiation and geographically isolated from it, as is the case in the cichlid mtDNA. For genes under divergent selection instead, it predicts that branches separating haplotypes within the radiation do not differ in length from those that separate them from ancestral and geographically isolated haplotypes, as is the case in the cichlid opsin gene genealogy. (a) based on [52,77,78]; (b) reproduced, with permission, from [79].

A test of the latter hypothesis is to investigate one of its biogeographical predictions, that is, that the geographical distribution of adaptive radiations should be significantly associated with contact zones between closely related species or divergent populations of allopatric origin. Examples could be contact zones between (sub)species from different climate (e.g. glacial) refugia, lakes that captured headwaters of more than one river system, or islands that were colonized from more than one direction. The test would involve mapping the distribution of close relatives of radiations, and would ask whether the localities where radiations occurred are situated significantly more often inside overlap zones than would be expected by chance.

Genealogical discordance and ancestral hybridization in adaptive radiations

During the past decade, monophyly was inferred from gene sequence data for many geologically young adaptive radiations, suggesting rapid diversification from single ancestral species [39–45]. Most of the genealogies were of cytoplasmic (mitochondrial or chloroplast) genes. Such genealogies cannot distinguish between origin from a single species (true monophyly) and origin from a hybrid swarm in which one parental haplotype happened to become fixed. The latter was implicitly assumed to be highly unlikely, but increasing evidence suggests that this assumption is problematic.

Recent work on molecular phylogenetics of hybridizing species reveals that the cytotype of just one species often becomes fixed in both hybridizing species or the resulting hybrid swarm, as first shown in the *Gila* species complex of cyprinid fish [20] and North American charr [59]. Subsequent studies of several small fish species radiations found that sympatric species pairs appeared monophyletic in their mitochondrial but poly- or paraphyletic in their

Table 1. Cytonuclear discordance affecting basal branches in phylogenies of adaptive radiations

Radiation	Cytonuclear discordance	Refs	
		Cytoplasmic DNA	Nuclear DNA
Darwin's finches	Yes ^a	[44]	[75]
Hawaiian honeycreepers	? ^b	[63]	[63]
Hawaiian crickets	Yes ^c	[43]	[31]
Hawaiian silverswords	Yes ^c	[40]	[64]
Hawaiian drosophilids	? ^d	[30]	[30]
Lake Victoria cichlids	Yes ^e	[77]	[78,79]
Lake Malawi cichlids	Yes ^f	[80]	[78]
Lake Tanganyika cichlids	Yes ^g	[82]	[83]
Lake Baikal sculpins	Yes ^h	[50]	[84]
Columbine flowers	No	[41]	[41]

^aSee Box 5.

^bAlthough there is no cytonuclear discordance apparent between the basal branches in the entire radiation, such discordance is apparent between the basal branches of clade B, which represents the main radiation of 12 species.

^cSee main text.

^dPhylogeny does not enable us to judge whether the reported cytonuclear discordance is due to hybridization at the base or between the tips.

^eAn AFLP phylogeny [78] resolved *Thoracochromis* species from Lake Edward as very close relatives of the radiation, whereas they are distantly related in a mtDNA genealogy [77]. Whereas all Lake Victoria region endemics are monophyletic and separated by a long branch from their relatives in a mtDNA genealogy, they are not monophyletic and tremendously variable in an opsin gene tree (Fig. 2 [79]).

^fA mtDNA genealogy [80] resolves *Rhamphochromis* and *Diplotaxodon* as sister taxa to all other endemic Lake Malawi haplochromines including *Copadichromis virginalis*, separated from them by a long branch. An AFLP phylogeny [78] that used the same individuals found *C. virginalis* basal to *Ramphochromis*, *Diplotaxodon* and the main radiation.

^gVariation at 21 allozyme loci [83] suggests the panafrikan river-dwelling cichlid genus *Oreochromis* is the closest relative of the modern radiation in Lake Tanganyika whereas a mitochondrial genealogy [82] suggests that ancient endemic lineages of Lake Tanganyika are. Also, allozymes resolved the Eretmodini lineage as a relatively young group, and sister taxon of the rapidly radiated Tropheini group [83], whereas mitochondrial genes suggest they are the deepest branch in the modern radiation [82].

^hA rod opsin genealogy [84] resolves the genus *Cottocomephorus* as sister taxon to all other endemic Lake Baikal cottid genera together, and *Cottus kessleri* and *Paracottus kneri* as sister taxa within the main radiation. In contrast, *C. kessleri* is resolved as member of a clade with *Cottocomephorus* in a mtDNA genealogy [50].

nuclear genes, suggesting hybridization of colonizing species, followed by fixation of one parental mtDNA haplotype (e.g. [56,60]). The latter can happen by chance, but selection on cytonuclear genotypes has been invoked in some cases [61,62]. In two-species radiations [55,60], such a pattern might be equally consistent with reinforcement of reproductive isolation between incipient species that

were already partially isolated when they became sympatric and with sympatric speciation from a hybrid swarm.

To distinguish between reinforcement and radiation from a hybrid swarm, nuclear phylogenies for adaptive radiations of more than two species are required (Table 1). Some research groups have explicitly tested the congruence between monophyly-suggesting cytoplasmic

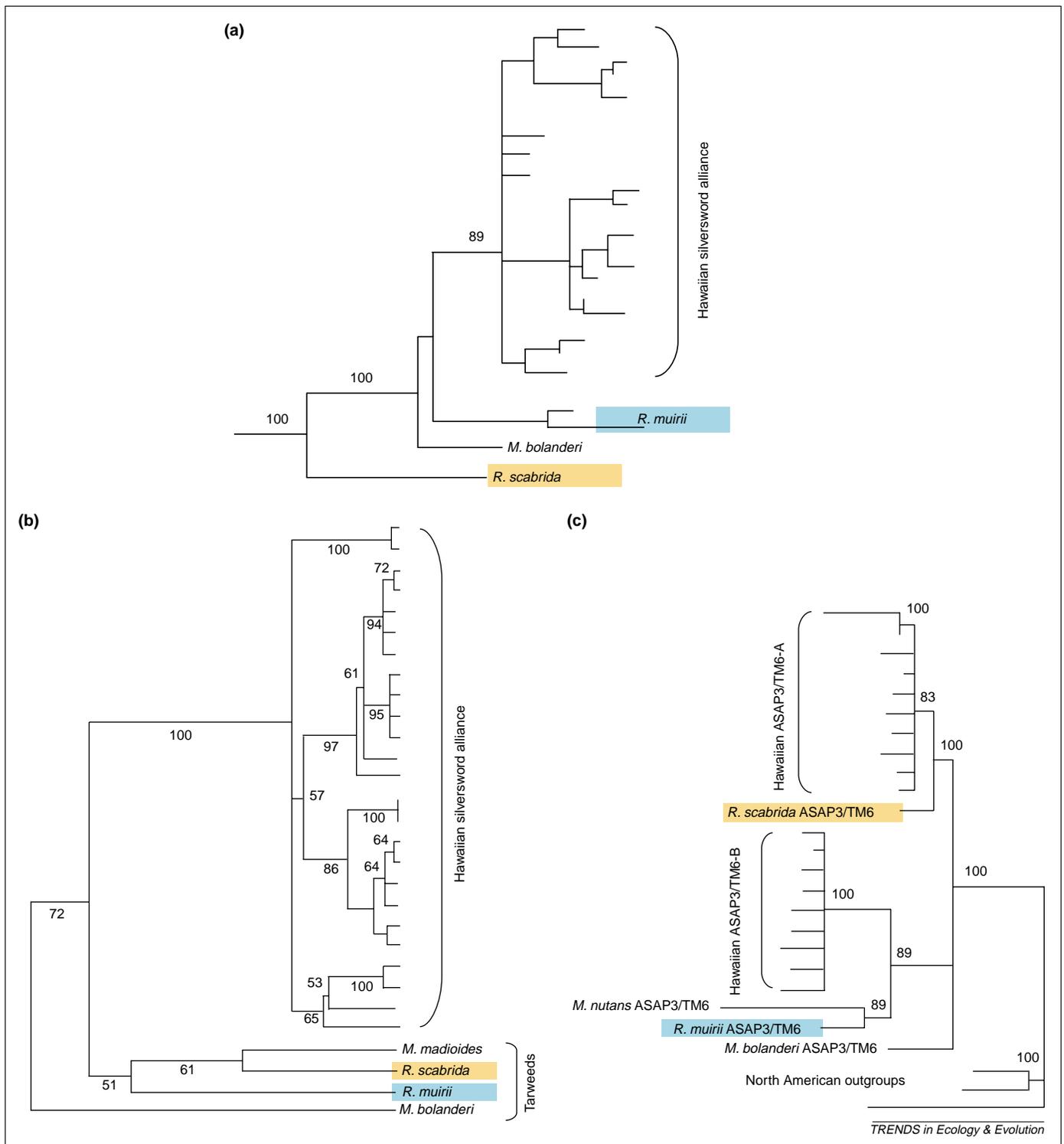


Figure 3. Discordant gene trees suggest a hybrid origin for the Hawaiian silversword radiation. **(a)** A chloroplast DNA genealogy [40], and **(b)** a nuclear gene (ITS) sequence after concerted evolution [81] suggest that the Hawaiian silversword radiation is monophyletic. **(c)** A phylogeny based on a nuclear floral homeotic gene resolves two different North American tarweed species (in shaded boxes) as the closest relatives of the two different copies of the gene found in each species of the Hawaiian radiation [64]. The Hawaiian silverswords are allopolyploid whereas their North American relatives are diploid. Reproduced with permission from [40,64,81].

genealogies and nuclear phylogenies for the same adaptive radiation ([30,41,63], [31] versus [43], [40] versus [64]). One in five studies found good cytonuclear congruence [41], two were inconclusive [30,63], and two revealed strong evidence for ancestral hybridization ([31] versus [43], [40] versus [64]): The Hawaiian silversword alliance, one of the most spectacular radiations of flowering plants, appears to be derived from a hybrid population between two or more species of North American tarweed [64]. Strikingly, all species in the radiation share the same chloroplast haplotype of just one parent species (Figure 3a), a nuclear ITS-sequence that is different from both parental sequences (Figure 3b), and carry duplicate copies (all silverswords are allopolyploid) of nuclear floral homeotic genes, of which one is derived from one parent species, and the other one from the other parent species (Figure 3c).

Similarly, radiations of *Laupala* crickets on different islands in the Hawaiian archipelago are reciprocally monophyletic in their mtDNA [43], but a nuclear gene phylogeny is in strong conflict with this [31], consistent with hybridization between unrelated species and fixation of one mitochondrial haplotype preceding radiations within and between islands. For instance, the largely Hawaiian *Laupala pacifica* group of 13 species might have radiated after hybridization between a species from Oahu (*L. kokeensi*) and one from Maui (*L. eukolea*).

I found published nuclear phylogenies for several other adaptive radiations with monophyletic cytoplasmic genealogies. Cytonuclear discordance consistent with hybridization preceding the burst of radiation is apparent in all of them: Darwin's finches, three African cichlid fish radiations and Lake Baikal sculpins (Box 5, Table 1).

Box 5. Cytonuclear discordance between basal branches of the Darwin's finch radiation

A phylogeny of the radiation of Darwin's finches based on allele variation at 16 microsatellite loci (Figure 1b) resolves three allopatric species with ancestral morphology, the two warbler finches (*Certidea olivacea* and *C. fusca*) and the Cocos finch, as basal and the ground and tree finches as two derived clades [75]. By contrast, a mitochondrial genealogy of the same taxa (Figure 1a: branches with discordant positions are connected by a dotted line) resolves the Cocos finch as a member of the rapidly radiated clade of tree and ground finches, separated from the warbler finches by a long branch [44]. This discordance could be explained if, well after the population on Cocos Island had become isolated from its relatives on the Galapagos and after the Galapagos population had split into warbler finches and vegetarian finch, some Cocos finches arrived on the Galapagos and hybridized with

a resident population related to the vegetarian finch. The mitochondrial haplotype of the Cocos finch became fixed in the hybrid population, which then quickly radiated into ten species of tree and ground finches (black arrows indicate begin of rapid radiation in mtDNA tree). The alternative explanations, that the Cocos finch is a recent product of the rapid radiation into tree and ground finches, or that its mtDNA similarity to them is due to ongoing hybridization, are difficult to reconcile with the nuclear phylogeny and with geography respectively. The ancestral hybridization scenario is biologically plausible: absence of premating isolation at the base of the Darwin's finch phylogeny has recently been shown between *C. olivacea* and *C. fusca* that have been isolated for between 1.5 and 2 million years [76]. Modified, with permission, from [44,75].

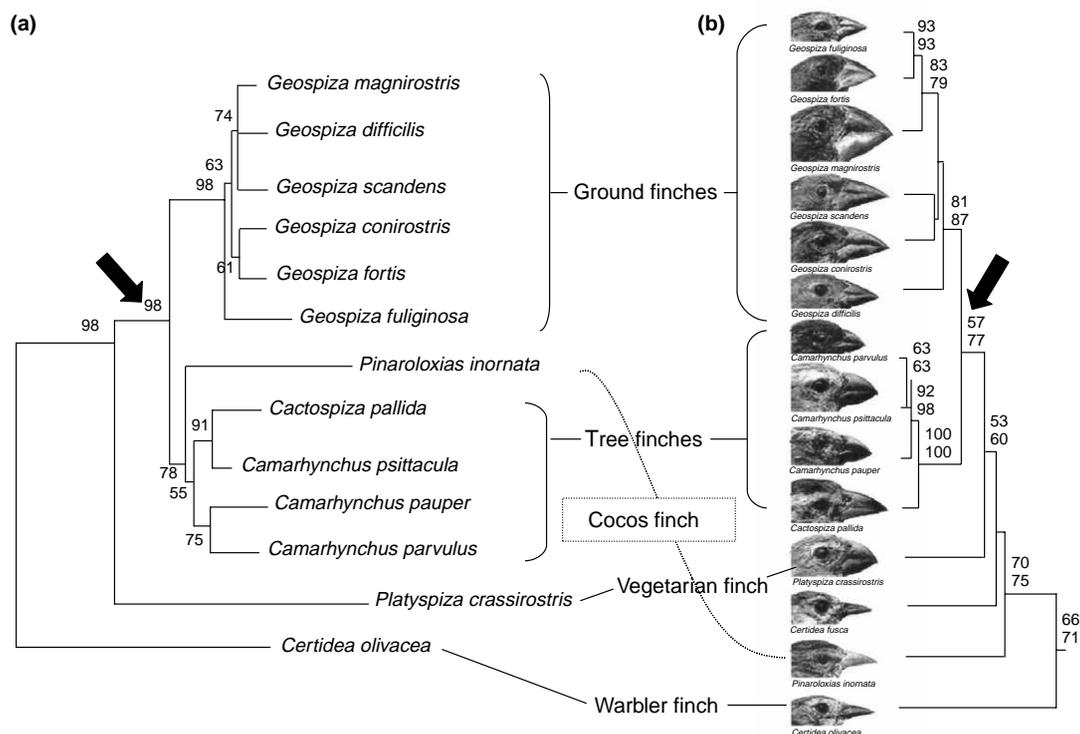


Figure 1.

Erroneous phylogenetic inferences might explain some of these cases, but the conflicting nodes in several of these incongruent trees are statistically well supported. Assuming the data quality is good, the incongruencies are difficult to explain other than by invoking interspecific hybridization, because they involve branches so distant in the trees that ancestral polymorphisms appear unlikely. Ancestral hybridization is biologically plausible in all of these cases (Table 1, Box 5). More detailed investigations into the origins of these radiations are therefore desirable.

Hybrid origin is also a distinct possibility for several allopolyploid adaptive radiations of fish for which nuclear gene phylogenies are not yet available, such as the allotetraploid suckers (Catostomidae) of North America, the allohexaploid, morphologically highly diverse African yellowfish (*Barbus*, Cyprinidae), and radiations of polyploid neotropical catfish (Callichthyidae).

Perspectives

Although Stebbins [3], Lewontin and Birch [4] and Templeton [26] developed similar concepts, a fully spelled-out hybrid swarm theory of adaptive radiation has, hitherto, been lacking. It does provide a potential explanation for elevated genetic variability and increased rates of phenotypic evolution reported from several adaptive radiations [10,21,57,65], and for the possibly elevated frequency of sympatric speciation during adaptive radiations. Moreover, the theory could provide an explanation for why older radiations (or radiations in more stable landscapes) are often less species rich than are younger radiations. As species numbers rise and underutilized resources become scarce, selection becomes stabilizing and species begin to accumulate genomic incompatibilities. Extinctions can then no longer be compensated for easily by rapid speciation. As a result, radiations would thin out as they grow old.

There is a rapidly increasing body of evidence for interspecific hybridization preceding and during adaptive radiations of plants and animals. To understand the significance of these observations and to scrutinize the hybrid swarm theory, several tests are required, including: (i) statistical tests to discriminate between real and spurious discordance resulting from poor data; (ii) historical population genetic studies to discriminate between hybridization and ancestral polymorphism; (iii) experimental studies to investigate effects of hybridization on functional diversity; and (iv) quantitative tests of biogeographical predictions. Ultimately, the utility of the hybrid swarm theory must be measured in terms of the explanatory power that it adds over and above the existing body of theory to explain the phylogenetic and geographical distribution of adaptive radiations.

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