HOW COMMON IS HOMOPLOID HYBRID SPECIATION?

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Hybridization has long been considered a process that prevents divergence between species. In contrast to this historical view, an increasing number of empirical studies claim to show evidence for hybrid speciation without a ploidy change. However, the importance of hybridization as a route to speciation is poorly understood, and many claims have been made with insufficient evidence that hybridization played a role in the speciation process. We propose criteria to determine the strength of evidence for homoploid hybrid speciation. Based on an evaluation of the literature using this framework, we conclude that although hybridization appears to be common, evidence for an important role of hybridization in homoploid speciation is more circumscribed.

KEY WORDS: Hybrid speciation, hybrid swarm, hybridization, reproductive isolation.

Homoploid hybrid speciation, or speciation via hybridization without a change in chromosome number, has historically been considered vanishingly rare. This is because hybrids are often thought to be ecologically intermediate (Coyne and Orr 2004) and frequently have only weak premating isolation from parental species due to overlapping signals and preferences (Christophe and Baudoin 1998; Velthuis et al. 2005; Ganem et al. 2008). However, hybridization could trigger reproductive isolation between hybrid and parental lineages in cases in which hybrids have recombinant phenotypes (Fig. 1) that allow them to colonize niches unavailable to either parental species (Gross and Rieseberg 2005) and/or reject parental species as potential mates (Mavarez et al. 2006; Melo et al. 2009). Chromosomal rearrangements resulting from hybridization could also contribute to reproductive isolation (Grant 1971; Rieseberg et al. 1995; Rieseberg 1997). Each of these situations could ultimately lead to hybrid speciation.

Although homoploid hybrid speciation was historically considered rare, perspectives on its importance as an evolutionary process have changed rapidly over the past decade. A large number of recent empirical studies have reinforced the notion that homoploid hybrid speciation may be common (discussed in Abbott et al. 2013). Reviews on the topic reflect a shifting view on the importance of homoploid hybrid speciation among evolutionary biologists. For example, in their 2008 review Mavarez and Linares state ". . . several new putative examples in butterflies, ants, flies and fishes . . . [suggest that homoploid hybrid speciation] is far more common than previously thought." In a seminal review on the topic, Mallet (2005) concludes "Enough suspected homoploid hybrid species exist to indicate that it may be as common in animals as in plants . . . it would hardly be surprising if hybridization had triggered the origin of *Homo sapiens*, the most invasive mammal on the planet." Other discussions of homoploid hybrid speciation have echoed this view (Olson and Stenlid 2002; Schardl and Craven 2003; Nolte and Tautz 2010; Seehausen 2013). Yet, as we argue here, many purported examples fail to provide concrete evidence of homoploid hybrid speciation.

What evidence is required to demonstrate that hybrid speciation has occurred? Past reviews have used a variety of definitions for hybrid speciation. These definitions range from requiring that hybrid species form stable genetically distinct populations (Mallet 2007) to requiring that traits derived from hybridization were likely important in the speciation process (Mavarez and
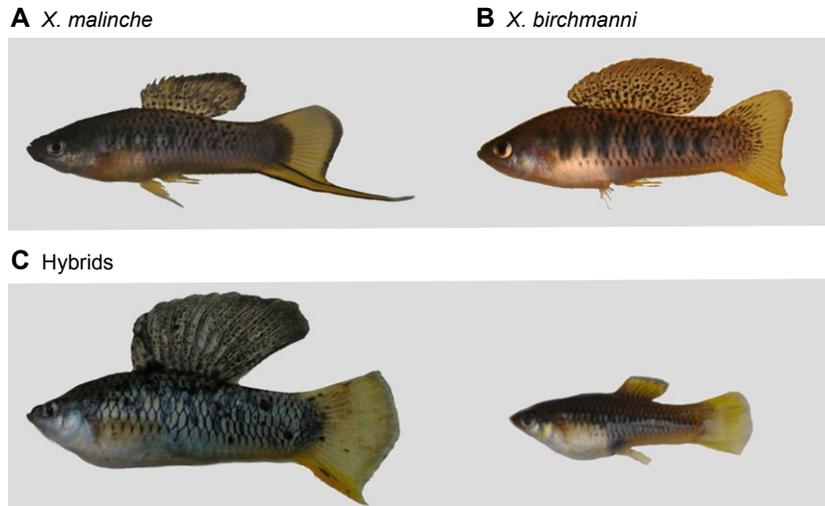
A *X. malinche*　　　　　　　　**B** *X. birchmanni*
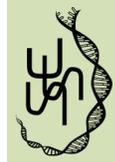
C Hybrids

Figure 1. Hybridization between sister species *Xiphophorus malinche* (A) and *X. birchmanni* (B) produces novel phenotypes (C). Novel combinations of sexually selected traits and preferences in hybrids could lead to reproductive isolation and ultimately hybrid speciation. However, in this case hybrid females do not exhibit strong mate preferences and parental females actually discriminate less against hybrid males than heterospecifics.

Linares 2008). In agreement with a number of previous reviews (Gross and Rieseberg 2005; Hegarty and Hiscock 2005; Arnold 2006; Mallet 2007; Jiggins et al. 2008; Mavarez and Linares 2008; Abbott et al. 2010; Abbott et al. 2013), we define hybrid speciation as a speciation event in which hybridization is crucial in the establishment of reproductive isolation. Although we agree with previous reviews on the definition, we focus this piece on establishing standards for the genetic and phenotypic evidence required to demonstrate that homoploid hybrid speciation has occurred. To demonstrate that hybrid speciation has occurred given this definition, three criteria must be satisfied: (1) reproductive isolation of hybrid lineages from the parental species, (2) evidence of hybridization in the genome, and (3) evidence that this reproductive isolation is a consequence of hybridization. By contrast, a large number of empirical studies have simply used genetic evidence of hybridization (Criterion 2) as support for hybrid speciation (see below).

In our discussion, we evaluate the strength of evidence for homoploid hybrid speciation in studies published in the last decade against these three criteria. We argue that much of the evidence presented in proposed cases of homoploid hybrid speciation does not provide strong support for the hypothesis of hybrid speciation. In addition, we outline the evidence required to support hybrid speciation and suggest promising directions for future studies.

EVIDENCE USED TO SUPPORT HYBRID SPECIATION

Most studies that have made claims about hybrid speciation rely primarily on genetic data. Although genetic evidence of hybridization is an important part of demonstrating hybrid speciation,

hybridization is common in the absence of hybrid speciation (Mallet 2007). Studies often do not explicitly address whether reproductive isolation between parental and hybrid species was likely driven by hybridization. Here we discuss specific studies that have proposed homoploid hybrid speciation, and evaluate the current strength of evidence for these cases.

Genetic evidence for hybridization

Prior to the advent of large scale sequencing, genetic analyses of hybridization centered on phylogenetic discordance of organellar and nuclear markers (Arnold et al. 1988; Asmussen et al. 1989; Bullini 1994; Dowling and Secor 1997). Accordingly, mitochondrial DNA from one species in combination with nuclear markers closely related to another species has been used to support hybrid speciation in a number of cases (Meyer et al. 2006; Larsen et al. 2010; Kang et al. 2013; Amaral et al. 2014). However, species boundaries are remarkably permeable to mitochondrial sequences (Ferris et al. 1983; Shaw 2002; Bachtrog et al. 2006), and even in the absence of gene flow they may be significantly influenced by incomplete lineage sorting (ILS, reviewed in Ballard and Rand 2005).

The majority of proposed cases of hybrid speciation are based on analysis of nuclear genetic markers that demonstrate hybrid ancestry in the genome. Early studies relied on a small number of nuclear markers to infer that hybrid speciation has occurred (Nolte et al. 2005; Schwarz et al. 2005; Mavarez et al. 2006; Brelsford et al. 2011; Hermansen et al. 2011). Using a small number of genomic regions to infer hybridization can be problematic because of confounding processes such as ILS. More recent
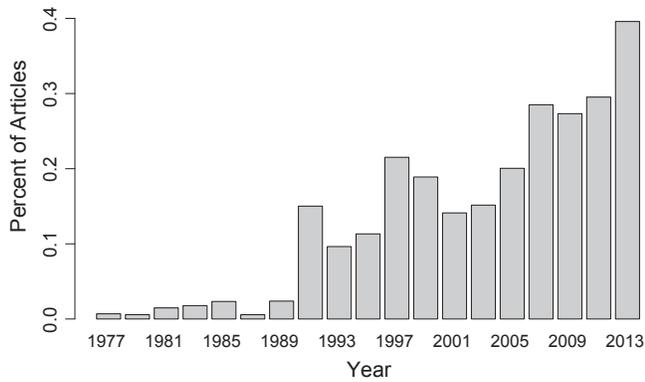
Figure 2. A rapid increase in recent years in the percent of articles including the terms "hybrid speciation" or "hybrid origin" (but excluding articles with the terms polyploidy, autopolyploid, and allopolyploid). The number articles was determined by a Web of Science search; the total number of articles was defined as all the articles published per year in the evolutionary biology subject category.

studies have used dense genome-wide markers or even whole-genome sequences to characterize hybridization throughout the genome, allowing researchers to distinguish between ILS and hybridization using gene tree methods, model-based approaches, or the length of ancestry tracts associated with discordant gene trees (Kunte et al. 2011; Heliconius Genome 2012; Stukenbrock et al. 2012; Cui et al. 2013; Nice et al. 2013; Schumer et al. 2013).

Genomic mosaicism, or substantial contribution of genetic material from two parental lineages, is often considered a key signature of hybrid speciation (discussed in Jiggins et al. 2008). Although genomic mosaicism provides strong evidence for past hybridization, additional evidence is needed to demonstrate that hybridization was likely important in the speciation process (see below). For example, a number of wolf species have clear genome-wide admixture with other species, but this likely occurred through secondary hybridization with coyotes (vonHoldt et al. 2011). However, genomic mosaicism is consistent with

scenarios in which hybrid swarms are established and later undergo allopatric speciation, which some authors have considered a form of hybrid speciation (James and Abbott 2005; Mavarez and Linares 2008; Hermansen et al. 2011). A concern with these cases is that it is unclear that hybridization per se was a key element in the subsequent development of barriers to gene flow in these allopatric populations. For example, in swordtail fish, isolated ∼100-year-old hybrid populations are complete mosaics of the two parental species and certain genomic regions are beginning to fix for alternate parental species (M. Schumer et al., unpubl. data). Despite this genomic mosaicism in swordtails, hybridization-derived traits do not promote reproductive isolation and in fact weaken sexual isolation between parentals and hybrids (Fisher et al. 2009)—highlighting the limitations of the mosaic genome criteria.

Another major limitation with using genomic mosaicism as the central evidence for hybrid speciation is that, when admixture proportions differ from 50–50, it is unclear what level of contribution from the minor parent should be considered sufficient to warrant evidence for hybrid speciation. Given that a range of variation in parental contribution is consistent with hybrid speciation, there is no reason to rule out the possibility that only the genomic regions associated with the establishment of reproductive isolation will show evidence of being derived from hybridization.

Importance of hybridization in speciation

The strongest cases for hybrid speciation combine data on genome-wide patterns of hybridization with evidence that hybridization has resulted in unique trait combinations or genetic incompatibilities that promote reproductive isolation. Only a handful of studies have taken the next step to show that these phenotypes are derived from hybridization and are important in maintaining reproductive isolation.

The first compelling example comes from plants in the sunflower genus *Helianthus*. Not only has genomic mosaicism been shown in the three homoploid hybrid species derived from hybridization between *H. annuus* and *H. petiolaris* (Rieseberg
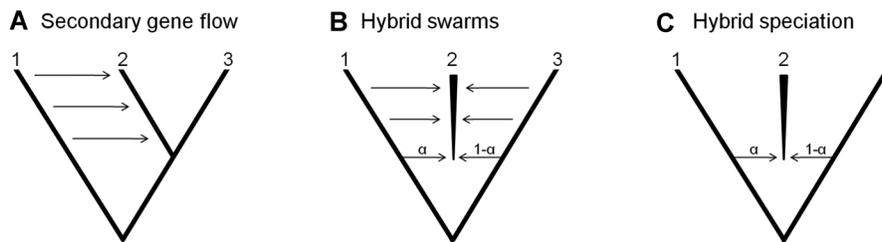

Figure 3. Different evolutionary scenarios can produce genome-wide signatures of hybridization. (A) Secondary gene flow, (B) hybrid swarms that lack reproductive isolation from parental species, and (C) hybridization resulting in reproductive isolation between hybrids and parental lineages (α = genomic contribution from parent 1). The similarity of genetic patterns derived from these different processes underscores the importance of other evidence for hybrid speciation. Model selection approaches may be useful in distinguishing between the processes underlying genome-wide signatures of hybridization.

et al. 1995), a series of later studies demonstrated that hybridization was crucial in the development of reproductive isolation between hybrid and parental species (Gross and Rieseberg 2005). In addition to genomic reorganization that promotes isolation (Rieseberg et al. 1995), quantitative trait locus (QTL) mapping using artificial hybrids between *H. annuus* and *H. petiolaris* demonstrated that the extreme phenotypes of *Helianthus* hybrid species (which allow for survival in their present environments) were derived from recombination between the parental genomes (Rieseberg et al. 2003).

The best-supported case of hybrid speciation in animals comes from butterflies. In the passion-vine butterfly *Heliconius heurippa*, the genomic regions underlying wing patterning are derived from hybridization (Salazar et al. 2010). Artificial hybrids between *H. heurippa*'s putative parental species recreate this wing pattern (Mavarez et al. 2006). Laboratory backcross hybrids resembling *H. heurippa* have increased preferences for their own wing pattern (Melo et al. 2009), suggesting that reduced gene flow could have been an immediate result of hybridization. In mate preference tests including parental species, preference for wing pattern is also likely to significantly reduce gene flow, though there is weaker assortative mating between *H. heurippa* and the putative parental species *H. cydno* (Mavarez et al. 2006).

Many studies that propose hybrid speciation on the basis of genetic signatures of hybridization (see above) also examine morphology or behavior for evidence of intermediate or recombinant phenotypes (Gompert et al. 2006; Meyer et al. 2006; Hermansen et al. 2011; Nice et al. 2013), or use current geographical distributions to support predictions that a population was formed via hybridization (Brelsford et al. 2011; Hermansen et al. 2011). Although such data are consistent with a hybrid speciation scenario, they do not demonstrate that these traits arose from hybridization without additional evidence. The persistence of genetic variation present in the ancestral population can result in novel phenotypes without those phenotypes being caused by hybridization (e.g., as a result of collapse of local adaptation or a balanced polymorphism). In addition, populations can exhibit a mixture of traits due to gene flow, without those traits being reproductive isolating factors. The two cases discussed above provide an excellent model for approaches that can provide evidence that hybridization played a role in the establishment of reproductive isolation. Even in the absence of hybridization-derived phenotypes, inversions, duplications, and other genetic changes that lack obvious phenotypic effects can also contribute to reproductive isolation (e.g., Greig et al. 2002) and deserve more experimental attention (discussed below).

REQUIRED EVIDENCE FOR HYBRID SPECIATION

Despite a surge in the number of studies that have proposed hybrid speciation (Fig. 2), only a handful have presented strong evidence
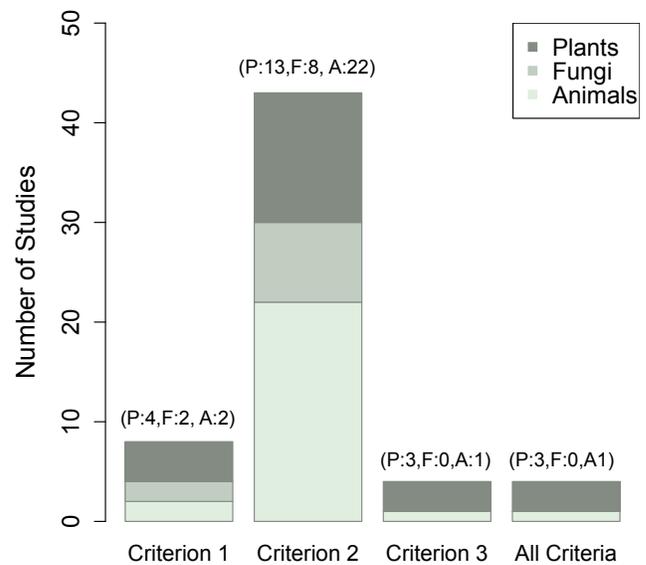

Figure 4. The number of proposed cases of homoploid hybrid speciation categorized by the level of strength of evidence. Criterion 1, strong reproductive isolation between parental and hybrid lineages; Criterion 2, genetic evidence of hybridization; Criterion 3, evidence of hybridization derived reproductive isolation. *P*, number of studies in plant species; *F*, number in fungal species; *A*, number in animal species. See Table S1 for a list of case studies.

for a role of hybridization in speciation (Fig. 4). We suggest that there are three components of required support for hybrid speciation: (1) showing reproductive isolation from parental species, (2) documenting past hybridization, and (3) demonstrating that isolating mechanisms were derived from hybridization.

Criterion 1, reproductive isolation

Studies proposing hybrid speciation should demonstrate a strong reproductive isolating mechanism between the putative parental and hybrid species. This may include temporal, behavioral, or geographic isolation, or reduced fitness in hybrids between the parental and hybrid species. Reproductive isolation between species is often presumed, and is the biggest Achilles' heel of studies on hybrid speciation. For example, even in *H. heurippa*, which is the strongest example of hybrid speciation in animals, reproductive isolation from *H. cydno* is relatively weak based on mate preference trials (Mavarez et al. 2006). Some studies have analyzed genetic data to demonstrate that predicted hybrid species do not show genotypes consistent with early generation hybrids (Schwarz et al. 2005; Gompert et al. 2006; Kunte et al. 2011), or tested whether markers show strong linkage disequilibrium (LD) or deviate from Hardy–Weinberg equilibrium (HWE, Schwarz et al. 2005; Gompert et al. 2006). However, signatures such as LD or deviations from HWE decay quickly following hybridization and do not distinguish hybrid species from a hybrid swarm that is not reproductively isolated from parental species. Another

approach has been the use of multivariate genetic analyses to illustrate genetic differentiation between hybrid and parental species (Sherman and Burke 2009; Brelsford et al. 2011; Kunte et al. 2011). One limitation of this approach is that even populations with high levels of gene flow can exhibit genetic differentiation, especially given spatial separation (Rosenberg et al. 2002; Novembre et al. 2008; Vonholdt et al. 2010). Experiments determining the strength of behavioral or ecological isolation, reduced fitness as a result of gene flow (e.g., Sherman and Burke 2009), or quantifying the extent of present-day gene flow can provide more conclusive evidence for reproductive isolation.

Criterion 2, evidence of hybridization

Genetic evidence of hybridization is a prerequisite to proposing that hybridization played a role in speciation. Whole-genome sequencing will give the most detailed information about genome-wide ancestry in putative hybrid species, but at a minimum, enough data should be collected to exclude ILS as a null hypothesis (e.g., Kunte et al. 2011; Nice et al. 2013; Schumer et al. 2013). For example, model selection approaches applied to genomic data can help distinguish between ILS and hybridization (Nice et al. 2013), and may even be able to distinguish between a single pulse of hybridization and ongoing gene flow (Fig. 3). An advantage of performing whole-genome sequencing is that the regions responsible for reproductive isolation are more likely to be surveyed, while subgenomic methods may fail to sample these regions.

Setting aside the concern of distinguishing hybridization from processes such as ILS, evidence of hybridization in the genome, even genomic mosaicism (Fig. 3C), does not provide conclusive evidence for the hypothesis of hybrid speciation. Due to advances in sequencing technology, genome-wide sequence information has recently become available for a large number of species. This has led to the realization that many species have large proportions of their genomes derived from hybridization. For example, Cui et al. (2013) estimate that in swordtail fish >10% (and up to 40%) of the genomes of most species are of hybrid origin. Genome-wide signals of hybridization such as these could be caused by hybrid speciation (Fig. 3C), but they are also consistent with other forms of gene flow (Fig. 3A, B).

Criterion 3, hybridization-derived isolation

The most compelling cases for hybrid speciation combine genetic evidence of hybridization with evidence that hybridization led to the emergence of reproductive isolation, either through changes in genomic architecture such as chromosomal rearrangements or trait combinations derived from hybridization that ecologically or reproductively differentiate hybrids and parental species. This evidence may be derived from QTL mapping of the traits of interest followed by determination of ancestry in these regions in the putative hybrid species (e.g., Rieseberg et al. 2003; Heliconius Genome 2012). In cases in which there is high confidence in the putative parental species, investigating genomic rearrangements and recombinant phenotypes in artificial hybrids can be highly informative (Rieseberg et al. 1995; Mavarez et al. 2006).

The key shortcoming in most studies proposing hybrid speciation is the lack of evidence that hybridization played a role in the speciation process. In most proposed cases of homoploid hybrid speciation, authors have suggested that certain trait combinations were derived from hybridization and contributed to the emergence of reproductive isolation. However, as discussed above, there are a number of explanations for mosaic phenotypes (persistence of ancestral variation or recent gene flow without the development of reproductive isolation). Some of the isolating mechanisms proposed in putative hybrid species, including novel mate preferences (Meyer et al. 2006), host preference (Gompert et al. 2006) or habitat shifts (Newcombe et al. 2000; Nolte et al. 2005; Gonthier et al. 2007), and temperature tolerance (Kunte et al. 2011), could be powerful isolating mechanisms but require further investigation to determine whether they are hybridization-derived traits and whether they significantly contribute to reproductive isolation. Increased pathogenicity or access to new hosts is a frequently proposed mechanisms for hybrid speciation in fungal hybrid species (Olson and Stenlid 2002; Schardl and Craven 2003) but is also largely untested.

A gray area in Criterion 3 includes cases in which hybridization is associated with partial reproductive isolation, which may or may not be followed by more complete isolation. This situation has been observed in ecologically differentiated young hybrid lineages (Nolte et al. 2005; Schwarz et al. 2005, 2007). For example, in hybrid tephritid fruit flies there is some assortative mating driven by host plant preference; researchers have proposed that this is the first step in hybrid speciation (Schwarz et al. 2007). However, many populations exhibit assortative mating without incipient speciation; for example, human populations exhibit positive assortative mate preferences by height (Silventoinen et al. 2003). Determining the degree of reproductive isolation that constitutes "incipient speciation" is subjective and tied to controversies in evolutionary biology over the species concept (Coyne and Orr 2004).

ROADMAP FOR FUTURE STUDIES ON HOMOPLOID HYBRID SPECIATION

Understanding the prevalence of homoploid hybrid speciation requires consideration not only of the signal of hybridization in the genome but also the mechanisms through which hybridization can generate reproductive isolation; so far only a few studies have investigated these mechanisms and this promises to be an exciting direction for future research.

Understanding the mechanisms of hybrid speciation

We currently know very little about the range of genetic mechanisms that could be involved in homoploid hybrid speciation, because most work to date has focused on identifying whether extant species were generated by hybridization. Simulation studies and experimental approaches in model organisms can begin to characterize these mechanisms and may reveal likely pathways to hybrid speciation. Experimental work in yeast has shown that hybrid speciation can occur through changes in genomic architecture such as duplications that isolate hybrids lineages from both parental species (Greig et al. 2002). Because selection is a key component of theoretical models of hybrid speciation (Buerkle et al. 2000), similar experiments selecting for hybrid traits may demonstrate how rapidly reproductive isolation can evolve in these scenarios. However, laboratory-based experiments will not expose hybrid genomes to the same combinations of selective pressures present in natural environments, and are limited to organisms with rapid generation times.

Experimental approaches to identify existing hybrid species

Demonstrating that hybridization has played a role in speciation is a difficult but tractable question. If there is high confidence in the parental species of a species of hybrid origin, a number of experimental approaches can be used to investigate the role of hybridization in the speciation event. Crosses between parental species can reveal whether traits observed in the hybrid species are produced through recombination between the parental genomes. Using QTL mapping to identify the genomic regions underlying these traits will demonstrate whether hybrid species show expected ancestry patterns at these genomic regions. The most convincing cases of homoploid hybrid speciation to date have taken advantage of these approaches (e.g., Rieseberg et al. 2003; Mavarez et al. 2006).

Another tool for studying the role of hybridization in speciation is incipient hybrid species that have not fixed for most parental alleles. Investigating signatures of selection on hybrid genomes (such as LD between unlinked sites) can help us evaluate the role that selection is playing in fixation of parental alleles relative to drift. Similarly, characterizing LD or chromosomal rearrangements in artificial hybrids can demonstrate whether the observed genetic architecture of a hybrid species is generated by selection or interactions between the parental genomes. Most research to date has focused on the role of hybridization-derived traits in facilitating reproductive isolation (e.g., wing color, host preference, habitat tolerance) but fixation of incompatibility loci from each parental species could also promote reproductive isolation from both parents.

CONCLUSIONS

We are beginning to recognize that hybridization is a ubiquitous process in evolution—before, during, and after speciation (Mallet 2005). This has led to many species having hybrid genomes (e.g., Cui et al. 2013), but leaves unresolved whether hybridization played a role in speciation. With currently used standards of evidence for hybrid speciation a large number species and hybrid populations would qualify as hybrid species. Using permissive definitions of hybrid speciation could lead to a misunderstanding of the importance of hybridization in speciation and this directly conflicts with theoretical predictions that homoploid hybrid speciation is likely to be rare (Servedio et al. 2013). We argue that though hybridization is clearly an important evolutionary process, and may frequently contribute to evolutionary success through mechanisms such as heterosis and adaptive introgression, there are few cases that show a decisive role for hybridization in homoploid speciation. Only three proposed cases of homoploid hybrid speciation in plants and one in animals currently satisfy all three criteria set forth in this article (Fig. 4). Future research guided by these criteria will allow us to more conclusively determine the importance of hybridization as a mechanism of homoploid speciation.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. List of recently proposed cases of homoploid hybrid speciation represented in Figure 4 and criteria satisfied.