Insights from genomes into the evolutionary importance and prevalence of hybridization in nature

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Abstract: Hybridization is an important evolutionary phenomenon that has fascinated biologists for centuries. Indeed, Darwin considered hybridization in the *Origin of Species*, concluding that hybrid sterility did not contradict his theory of natural selection and the gradual evolution of species. Prior to the advent of whole genome sequencing, it was clear that hybridization had played a role in the evolutionary history of many extant taxa, particularly plants, and hybridization was used regularly in crop breeding and development. The extent that hybridization has contributed to the evolution of Earth's biodiversity has, however, been the topic of much debate. Analyses of whole genomes are providing further insight into this significant evolutionary problem. Recent studies have documented ancient hybridization in a diverse array of taxa including mammals, birds, fish, fungi, and insects. Convincing evidence for adaptive introgression is being documented in an increasing number of systems, though demonstrating the adaptive function of introgressed genomic regions remains difficult. And finally, several new homoploid hybrid speciation events have been reported. Here we review the current state of the field, specifically evaluating the additional insights we have gained from having access to whole genome data, and the challenges that remain with respect to understanding the evolutionary relevance and frequency of ancient hybridization, adaptive introgression, and hybrid speciation in nature.

Introduction: Hybridization between species, subspecies, or lineages within species, has long been viewed as a powerful tool for understanding evolutionary biology (Lotsy 1916; Anderson 1949; Harrison 1990; Stebbins 1959). The study of hybridization has had two primary foci over the past century, one concerned with understanding species barriers, and the other with the role of hybridization in generating novel gene combinations on which selection can act. The utility of using hybrid zones to understand the process of speciation (*i.e.,* to identify regions of the genome likely involved in the maintenance of reproductive isolation) is clear (Harrison and Larson 2014; Gompert *et al.* 2017), and has been the focus of hundreds of studies (*e.g.,* Turner and Harr 2014; Taylor *et al.* 2014b; Scordato *et al.* 2017; Rafati *et al.* 2018; Sung *et al.* 2018). The crossing experiments that occur in natural hybrid zones would often be impossible to replicate in the lab, and the importance of studying hybridization in nature for understanding evolution is hard to overstate.

      The importance of hybridization in processes of adaptation and speciation has, however, been rigorously debated in the literature with a more recent consensus forming that hybridization can, and often does, play a creative role in evolution

(Seehausen 2004; Abbott *et al.* 2016; Roux *et al.* 2016). Importantly, the role that hybridization plays in the evolutionary history of different taxa is variable (*i.e.*, periods of introgression, hybrid speciation, adaptive introgression), and the emphasis on certain outcomes of hybridization (*e.g.*, hybrid speciation) appears to be skewed in the literature (see Schumer *et al.* 2014). Historically, botanists and zoologists approached this problem with different perspectives. The importance of hybridization in the evolution and diversification of plants is well-documented, and a creative role for hybridization in the evolution of plants has long been accepted (Rieseberg 1997; Baack and Rieseberg 2007; Arnold 2015). Zoologists, on the other hand, have generally viewed hybridization as a useful tool for studying species barriers and reproductive isolation, but not as a source of genetic novelty on which selection can act (Barton 2013). There has been a significant shift since the mid 1900's in this view (Barton 2001; Stebbins 1959), and many now view hybridization as a potentially creative force in evolution and adaptation for both plants and animals (Mallet 2007; Abbott *et al.* 2013).

It is becoming increasingly evident that hybridization, whether intermittent or ongoing, has played a major role in the evolutionary history of many taxa, including hominids (Vernot and Akey 2014; Sankararaman *et al.* 2014; Huerta-Sánchez *et al.* 2014; Dutheil *et al.* 2015; Dannemann *et al.* 2016; Simonti *et al.* 2016; Hackinger *et al.* 2016; Vernot *et al.* 2016; Foote 2018). For a long time, however, it has been difficult to fully understand the extent to which hybridization between closely related taxa has influenced their evolutionary history. This is, in part, because we lacked the tools to dissect genomes at high resolution. Over the past decade this landscape has rapidly changed and we can now sequence whole genomes from most non-model taxa at relatively low cost. Studies that leverage genome-spanning data in their analyses are uncovering signatures of hybridization in taxa that were never really expected to have a history of hybridization (*e.g.*, Palkopoulou *et al.* 2018) and confirming previous hypothesis about the role of hybridization in diversification (*e.g.*, Seehausen 2004; Meier *et al.* 2017)

In the era of high-throughput sequencing we are better equipped than ever before to determine the extent to which hybridization has played a role in the evolution of life on Earth (Payseur and Rieseberg 2016). We have the tools, and are developing the methods, to understand (1) the frequency of hybridization within evolutionary lineages (*e.g.*, Marcet-Houben and Gabaldón 2015; Toews *et al.* 2016; Schumer *et al.* 2017), particularly for taxa for which this was previously impossible (*e.g.,* Lunt *et al.* 2014), (2) the context for hybridization between taxa (*e.g.,* geographic variation, Mandeville *et al.* 2017, temporal variation,Taylor *et al.* 2014a; Colella *et al.* 2018), and (3) what happens after hybridization occurs (*i.e.,* how much of the genome remains after the initial hybridization event?; Do reproductive barriers evolve between hybrid individuals and their parental taxa?) (*e.g.,* Schumer *et al.* 2017; Runemark *et al.* 2018b; a).

Overall, it appears that outcomes of hybridization are more variable than was expected (*e.g.,* Schumer *et al.* 2017; Mandeville *et al.* 2017). Prior to high-throughput tools, geographically expansive, or temporally repeated, high-resolution investigations of hybridization in nature were difficult. Recent studies are making the important point that hybridization is geographically variable, even between the same two taxa (Harrison and Larson 2016). Combined with longitudinal studies that follow hybridization within

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93 populations over multiple generations (*e.g.,* Schumer *et al.* 2017) we also see variation
94 in the extent to which reproductive isolation exists within hybrid populations between the
95 same two parental taxa. The geographic context of hybridization, along with the initial
96 outcome of hybridization (*i.e.,* extent of hybridization and time period of hybridization) is
97 important and it would appear that generalizations from investigations of single hybrid
98 zone transects in one time period should be avoided. Interestingly, a large number of
99 the studies reporting ancient hybridization are focused on mammals (Fig 1), a significant
100 advance since Stebbins (1959) pointed out that few studies on hybridization in
101 mammals existed. Finally, many discoveries are being made in clades that have been
102 difficult to study (*e.g.,* fungi) because we typically have used morphology to identify
103 hybrids. Overall, during a short period of time, and thanks in large part to the rapid
104 development of sequencing technology, we have gained many interesting and exciting
105 insights into the role of hybridization in adaptation and speciation.
106   Progress in understanding the frequency of hybridization, geographic and
107 temporal variation in hybridization, and the consequences of hybridization is allowing us
108 to begin to address other, more complex questions about the ultimate consequences of
109 hybridization for species formation. What follows is a discussion of advances being
110 made in understanding three aspects of hybridization—ancient hybridization, adaptive
111 introgression, and hybrid speciation—into which the advent of whole genome
112 sequencing has been shedding significant insight. When hybridization is not ongoing, it
113 can be difficult to detect using traditional methods. For many taxa, however, it appears
114 that ancient hybridization occurred at some point in their evolutionary history (Fig 1). At
115 the same time, linking introgression of gene regions and adaptive function is
116 exceedingly difficult, yet recent studies have convincingly demonstrated that specific
117 gene regions serve adaptive roles in the receiving lineages (Fig 2). And finally, the hotly
118 contested frequency and importance of homoploid hybrid speciation is being addressed
119 in a diverse array of taxa. Several new studies shed additional light on this
120 phenomenon, but few fulfil the criteria for homoploid hybrid speciation laid out in
121 Schumer et al. (2014) (Fig 3).
122
123 ***Ancient hybridization: more common than we thought***
124
125 A growing number of studies are documenting ancient hybridization between lineages
126 we may not have expected to hybridize, or more complex patterns of hybridization than
127 would have been predicted (Fig 1). We consider hybridization ancient if there is no
128 contemporary evidence of hybridization between taxa, either because contemporary
129 lineages are allopatric or one (or more) of the hybridizing lineages have gone extinct
130 (*e.g.,* Sankararaman *et al.* 2014; Palkopoulou *et al.* 2018). Two aspects of advances in
131 sequencing technology are allowing rigorous study of ancient hybridization. First, we
132 can now sequence whole genomes at low cost, which provides the resolution necessary
133 to detect small genomic blocks of hybrid origin (*e.g.,*Sankararaman *et al.* 2014; Toews
134 *et al.* 2016). With traditional panels of a handful of markers this was not possible.
135 Second, because a number of new sequencing technologies rely on fragmentation
136 during the library preparation stage, degraded DNA from museum specimens and the
137 subfossil record can be incorporated into genomic studies (Jones and Good 2015;

138 Holmes *et al.* 2016). Indeed, findings from paleogenomics feature heavily in recent
139 advances in our understanding of ancient hybridization.
140   Paleogenomic studies are playing a major role in furthering our understanding of
141 the frequency and taxonomic distribution of ancient hybridization. Most recently, ancient
142 admixture has been documented between brown and polar bears, and appears to have
143 been widespread during the last glacial maximum when polar bear distributions were
144 more expansive than present day (Cahill *et al.* 2018). These findings have important
145 implications for understanding outcomes of hybridization as polar bear distributions
146 contract and hybridization with brown bears once again becomes common. Ancient
147 hybridization has also been rigorously documented between humans and Neanderthals
148 (Sankararaman *et al.* 2014), where the potential adaptive introgression of skin and hair
149 colour has been inferred. Interestingly, patterns of Neanderthal ancestry in human
150 genomes predominantly reflect selection against Neanderthal derived regions,
151 especially on the sex chromosomes. Finally, complex hybridization dynamics in
152 elephantids (including extinct straight-tusked elephants and mammoths) have recently
153 been reported (Palkopoulou *et al.* 2018). It appears that interspecies hybridization was a
154 common occurrence between straight-tusked elephants and extant forest elephants
155 when their ranges broadly overlapped, and the authors present evidence of widespread
156 hybridization between mammoths along a band of range overlap.
157   Understanding the long-term outcomes of ancient hybridization is difficult. On the
158 timescale of multiple generations, Schumer et al. (2017) examined multiple hybrid
159 populations of swordtail fishes and found that the maintenance of assortative mating
160 and the genomic distribution of hybrid ancestry varied significantly among hybrid
161 lineages, even though they all arose from the same two hybridizing parents. Overall, we
162 still have much to learn about how genomes evolve following hybridization events
163 (Payseur and Rieseberg 2016), particularly the role of selection in purging introgressed
164 alleles (Schumer *et al.* 2016; 2018b). This should be a major focus as studies document
165 additional cases of ancient hybridization. In some taxa, (*e.g.,*Sankararaman *et al.* 2014)
166 it appears that selection following hybridization resulted in regions of the genome devoid
167 of genetic material from one of the hybridizing taxa. In the Human-Neanderthal
168 example, there is potential evidence that genes underlying skin and hair color conferred
169 adaptive advantages following introgression. However, throughout the genome there is
170 a dearth of Neanderthal ancestry, especially on the sex chromosomes, which may play
171 significant roles in the maintenance of species boundaries (Dutheil et al. 2015). In other
172 taxa, it has been difficult to distinguish ancient hybridization from other processes like
173 whole genome duplications until recently. For example, results from whole genome
174 studies in baker's yeast have clarified the evolutionary history of this economically
175 important fungus. It appears that hybridization did play a role in the evolution of baker's
176 yeast, and that this hybridization event was followed by a whole genome duplication
177 (Marcet-Houben and Gabaldón 2015).
178   Certain long-term outcomes of ancient hybridization are of particular interest. The
179 role that hybridization may play in adaptive radiations was theorized in Seehausen
180 (2004) and recent results using genome-spanning data in African cichlids have provided
181 support for the idea that hybrid swarms can provide the genetic novelty for adaptive
182 radiations (Meier *et al.* 2017; see also Richards *et al.* 2017). Importantly, Seehausen
183 (2004) hypothesized that a number of other adaptive radiations may have been the

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184   product of (or have been facilitated by) rampant hybridization (*e.g.,* Darwin's finches),
185   within which genome-spanning data have documented widespread hybridization
186   (Lamichhaney *et al.* 2015). Given how significantly the resolution of genomic data in
187   many systems has changed since 2004 it will be exciting to see if this hypothesis holds
188   up for other classic adaptive radiations like Lake Baikal sculpin, Hawaiian
189   honeycreepers, silverswords, and laupaula crickets (Seehausen 2004). Indeed, a recent
190   review of the genomics of adaptive radiations reported evidence for hybridization in all
191   systems for which multiple sequenced genomes exist (Berner and Salzburger 2015).
192   More recently, vonHoldt *et al.* (2016) used whole genome data to document widespread
193   ancient hybridization and introgression in North American canids, suggesting that
194   eastern and red wolf genomes contain significant contributions from grey wolves and
195   coyotes to their ancestry, and may be of hybrid origin (see below). Finally, Svardal *et al.*
196   (2017), have recently provided evidence of ancient admixture in vervet monkeys. It is
197   notable that many of the most recent examples of ancient hybridization come from
198   mammals, a group that was historically not expected to hybridize as regularly as several
199   other vertebrate taxa (*e.g.,* de Manuel et al. 2016).
200   Given the number of new systems within which ancient hybridization has been
201   documented, it seems likely that, as additional studies are conducted in a greater
202   number of taxa, evidence of ancient hybridization will increase. An important
203   consideration with respect to ancient hybridization is contemporary conservation. How
204   should detection of ancient hybridization influence conservation? (discussed in vonHoldt
205   *et al.* 2017). Current policies have provisions for hybridization, but many are focused at
206   the level of species. The results reported by vonHoldt *et al.* (2016) and Toews et al
207   (2016) are both interesting cases where hybridization appears to have played a major
208   role in the evolutionary history of taxa currently listed, or being considered for listing,
209   under the Endangered Species Act. As our understanding of the frequency of ancient
210   hybridization increases it should inform policy (*e.g.* vonHoldt *et al.* 2017).
211
212   ***Adaptive introgression: linking genomic regions to phenotypes with known***
213   ***function***
214
215   It is likely that hybridization often leads to the introgression of adaptive gene regions
216   (reviewed in Hedrick 2013; Arnold and Kunte 2017; Suarez-Gonzalez *et al.* 2018);
217   however, it is necessary to demonstrate an adaptive function for the introgressed
218   genomic regions prior to claiming the discovery of adaptive introgression (Suarez-
219   Gonzalez *et al.* 2018). In their recent review of adaptive introgression, Arnold and Kunte
220   (2017) state that there is overwhelming evidence for the occurrence of adaptive
221   introgression, but acknowledge the need for, and frequent absence of, fitness
222   estimates. Importantly, for a number of examples of adaptive introgression they
223   highlight, no direct link has been made between genotype and phenotype, and no
224   adaptive function of phenotype has been rigorously demonstrated (see Tables S1a and
225   S1b in Arnold and Kunte 2017). Demonstrating the adaptive function of a specific gene
226   region is a difficult task in many systems and will remain a challenge. This is especially
227   true for organisms that cannot be easily incorporated into experiments (*e.g.,* free living
228   wolves, tropical birds, see Table S1b in Arnold and Kunte 2017). In the absence of
229   experiments that convincingly demonstrate an adaptive function of a specific genomic

230 region, methods that detect signatures of positive selection can be used (*e.g.,* Suarez-
231 Gonzalez *et al.* 2016); however, inferring adaptive introgression from genomic
232 signatures alone should be done with caution. Although linking introgression of specific
233 genomic regions to phenotypes with demonstrated adaptive functions is rare, and
234 difficult, several recent examples that use whole genome data exist in the literature
235 (Table 1; Fig 2).
236   Not surprisingly, it is easiest to link introgression to adaptive functions when the
237 introgressed region confers drug or disease resistance (*e.g.,* Song *et al.* 2011; Liu *et al.*
238 2015), or the gene region is responsible for a well-studied trait (*e.g.,* wing colour pattern,
239 The *Heliconius* Consortium *et al.* 2012; Wallbank *et al.* 2016). This most often occurs for
240 phenotypic traits controlled by single genes of large effect, or when multiple genes for a
241 given phenotypic trait are on the same chromosome (*e.g.,* Suarez-Gonzalez *et al.*
242 2016), or linked in an inversion (*e.g.,* Tuttle *et al.* 2016; reviewed in Wellenreuther and
243 Bernatchez 2018). Perhaps the biggest advance in our understanding of adaptive
244 introgression is that we can now link specific genomic regions to their phenotypic trait,
245 which was difficult (except in ~~non-~~model organisms) until very recently. We can now
246 pinpoint gene regions that underlie phenotypes of interest, with known adaptive
247 functions, often by taking advantage of natural admixture, and then examine the
248 behaviour of these genomic regions when hybridization occurs (*e.g.,* Jones *et al.* 2018).
249   A number of recent studies that report adaptive introgression following
250 hybridization have used both whole genome and experimental data (Table 1; Fig 2). Liu
251 et al. (2015), following up on Song et al (2011), confirmed recent adaptive introgression
252 of a warfarin resistance allele from *Mus spretus* to *M. mus domesticus*, also reporting
253 two previously unknown ancient hybridization events between these lineages. Suarez-
254 Gonzalez et al. (2016), reported adaptive introgression from *Populus balsamifera* to *P.*
255 *trichocarpa*. Importantly, they used whole chromosome sequencing to investigate
256 specific gene regions for which they also evaluated function in a common garden, and
257 linked these functions back to patterns of introgression in the wild. Similar inferences
258 have been made for the common bean by Rendon-Anaya et al. (2017); however, the
259 adaptive function of introgressed regions was inferred using genomic analyses rather
260 than common gardens. Jones *et al.* (2018) used whole exome and whole genome
261 sequencing to uncover the genetic basis of seasonal coat colour change in snowshoe
262 hares, and went on to demonstrate that the allele that confers brown coat colour likely
263 introgressed into the snowshoe hare genome from the closely related black-tailed
264 jackrabbit (Fig 2). In this case, the adaptive function of the introgressed allele is clear:
265 hares that do not match their background experience significantly higher rates of
266 mortality (Zimova *et al.* 2016). These new studies are taking advantage of whole
267 genome data paired with fitness estimates and functional understanding of traits to
268 rigorously document adaptive introgression in nature. Adaptive introgression is likely
269 more common than was once assumed; however, we caution against making
270 inferences of adaptive introgression in the absence of appropriate data, such as fitness
271 estimates and a demonstrated adaptive function for a specific gene region. Although
272 these examples focus on a single gene of large effect, or taxa where experimental
273 studies in common gardens are possible, we are excited by our increasing ability to link
274 genotype to phenotype in many different systems.
275

Hybrid Speciation: still a rare phenomenon

Hybrid speciation is defined as a speciation event where hybridization has played a crucial role in the evolution of reproductive barriers between a hybrid lineage and its parent lineages. The emphasis on hybridization directly contributing to the evolution of reproductive isolation is important, but continues to generate debate in the evolutionary biology community (*e.g.* Feliner *et al.* 2017; Schumer *et al.* 2018a). It is not sufficient to infer hybrid speciation from mosaic ancestry alone. Admixture could represent what remains after hybrid ancestry has been purged from critical regions of the genome (Schumer *et al.* 2016; 2018b). The classic examples of homoploid hybrid speciation are the *Helianthus* sunflowers. Three independent lineages have evolved from hybridization between *H. annuus* and *H. petiolarsis* (Rieseberg *et al.* 1995). *Helianthus* fulfils the three criteria (see Schumer *et al.* 2014) to define a hybrid species: 1) hybrid lineages are reproductively isolated from their parent lineages through genomic rearrangements and adaptation to xeric habitats uninhabitable to the parent species (Rieseberg *et al.* 1995), 2) they have been clearly demonstrated to be a product of hybridization through lab studies that recreated the genomic composition of hybrid lineages (Rieseberg *et al.* 1996), and 3) hybridization led directly to the extreme phenotypes that allowed hybrids to persist in xeric habitats (Rieseberg *et al.* 2003).

Two recent examples of potential hybrid speciation are featured in Fig 3. These examples use whole genome data to clarify patterns of genomic divergence in potential hybrid species and, among recent claims of hybrid speciation, are supported by the best evidence of reproductive isolation stemming from hybridization. Lamichhaney *et al.* (2018) use a classic long-term field study of Darwin's Finch (*Geospiza fortis x G. conirostris*) to directly document homoploid hybrid speciation, from initial hybridization to reproductive isolation of the hybrid lineage after only three generations. Although the timescale of their study is short from an evolutionary perspective, they convincingly demonstrate the speed with which reproductive isolation can be reached between hybrids and their parental taxa following hybridization in vertebrates. In a very different organism, Leduc *et al.* (2016; 2017) use whole genome sequencing to investigate the budding yeast *Saccharomyces paradoxus* and find evidence supporting homoploid hybrid speciation. They suggest that chromosomal architecture in the hybrid lineage plays a role in the maintenance of reproductive isolation between that lineage and the parental taxa, and also report that the hybrid lineage inhabits a unique ecological niche that may further isolate it from the parental taxa.

Even in the absence of clear evidence for homoploid hybrid speciation, the role of hybridization and introgression in the evolution of many taxa is interesting in and of itself. Some intriguing examples of potential hybrid speciation that were difficult to study without whole genome data come from a variety of fungi (see Table 1 in Mixão and Gabaldón 2017; Depotter *et al.* 2016). A common theme among these studies is the suggestion that having a hybrid origin has allowed these fungi to colonize novel environments/hosts (*e.g.,* Leducq *et al.* 2016; 2017) or become more pathogenic than their parental taxa. Further, many of these species have implications for human health (*e.g,* Pryszcz *et al.* 2015; Schröder *et al.* 2016), or agriculture (*e.g.,* Menardo *et al.* 2016). The morphological similarity of many of these taxa made it previously impossible to know if hybridization played a role in their evolution. In many of these cases it is

difficult to determine if hybridization *per se* contributed to reproductive isolation between the hybrid taxon and the parental taxa. That said, Leduc et al. (2016) provide convincing evidence that the hybrid species is reproductively isolated from its parental taxa due to chromosomal incompatibilities. Similar investigations should be carried out in other fungal systems to fully evaluate the status of these taxa as homoploid hybrid species.

Additional recent examples of possible hybrid speciation come from nematodes, birds, and fish. Lunt *et al.* (2014) use whole genome data and report complex hybrid origins of a widely-distributed crop pest within the root knot nematode group (*Meloidogyne Sp.*). They confirm a previous hypothesis that the widespread tropical species, *M. incognita*, is of hybrid origin—a double-hybrid derived from one hybrid parent species (*M. floridensis*) and an as yet unknown parent species. Lunt *et al.* (2014) hypothesize that the complex hybrid origin of *M. incognita* has facilitated the increased pathogenicity of the species, which is considered one of the most important tropical crops pests globally. The extent of reproductive isolation of the hybrid lineage remains unknown in this case, but is assumed to be significant.

Two other bird species, the Italian sparrow (*Passer italiae*) and the golden-crowned manakin (*Lepidothrix vilasboasi*), are proposed to be of hybrid origin and have been examined using high-resolution genomic datasets (Hermansen *et al.* 2011; Trier *et al.* 2014; Barrera-Guzmán *et al.* 2017; Runemark *et al.* 2018b; a; Elgvin *et al.* 2017). Elgvin *et al.* (2017) and Runemark *et al.* (2018a,b), build on the work of Trier *et al.* (2014) and Hermansen *et al.* (2014), to more thoroughly explore genomic variation in the Italian sparrow and its purported parental taxa, house (*P. domesticus*) and Spanish (*P. hispaniolensis*) sparrows. Eglvin *et al.* (2017) document mosaic parental inheritance and genomic divergence within the hybrid lineage that is novel compared to parental divergence. Runemark *et al.* (2018a,b) go on to report variation and constraint in the genomic composition of Italian sparrow populations on four islands in the Mediterranean. Unlike Lamichhaney *et al.* (2018), these studies have inferred reproductive isolation from patterns in genetic and genomic data and, although hybridization has played a role in their evolutionary history, it remains unclear if they fulfil the criteria for homoploid hybrid speciation (*e.g.,* Rosenthal *et al.* 2018). Additional field-based data on reproductive isolation will help fill this knowledge gap. Finally, the previously mentioned ancient hybridization detected between Nile and Congolese cichlid lineages appears to have facilitated the adaptive radiation of Haplochromine cichlids in Lake Victoria; however, whether hybridization itself played a role in reproductive isolation remains unknown. The ease with which many of these species can be housed in aquaria will facilitate future investigations.

Clearly, there are an increasing number of taxa for which hybridization has likely played an important role in their evolution. Some appear to be of hybrid origin, but whether they are homoploid hybrid species, or have experienced ongoing or irregular gene flow with close relatives throughout their evolutionary history remains unclear in most cases. Generally, however, there is insufficient data to demonstrate homoploid hybrid speciation. Prematurely calling them such leads to a misunderstanding of the frequency and importance of homoploid hybrid speciation versus the contributions that ancient and contemporary hybridization and adaptive introgression (previously discussed) can make to genomic variation within species (Schumer *et al.* 2014; 2018a;

367 b). It will be important to incorporate statistical tests that account for the timing of
368 hybridization (*e.g.* Hibbins and Hahn 2018) in future studies of hybrid speciation.
369
370 **Conclusions and looking forward**
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372 Admittedly, the three broad discussion points in this review are less distinct than they
373 have been presented. Ancient introgression may often have been adaptive, but our
374 ability to directly link introgression to adaptation in most systems is limited. Before
375 labelling something as adaptive, which often happens in the absence of sufficient data,
376 it is critically important to demonstrate adaptive function. At the same time, it is
377 important to recognize that even if hybridization leads to introgression of adaptive
378 alleles, it can also lead to strong negative selection against foreign alleles (*e.g.,*
379 Sankararaman et al. 2014). Moving forwards, haplotype-based investigations of
380 hybridization should be adopted whenever possible to provide greater insight into the
381 breakdown of linkage blocks and the generality of genomic architecture following
382 contemporary and ancient hybridization (Hvala *et al.* 2018). It is an exciting time to be
383 studying hybridization, and a time during which we should utilize model-based
384 estimates, incorporate natural selection and geographic variation, should explicitly
385 measure reproductive isolation in the field, and work on hybrid zones where
386 backcrossing and introgression occur (Harrison and Larson 2014; Payseur and
387 Rieseberg 2016). The increased resolution with which we can survey the genome for
388 signs of hybridization is providing insight not possible even 10 years ago. As we
389 accumulate more studies of a diverse array of taxa we will be able to generate a robust
390 understanding of the role of ancient hybridization across taxa, be better able to link
391 introgression to adaptive functions, and rigorously test the requirements of homoploid
392 hybrid speciation. These advances will continue to illuminate the many, and varied,
393 ways in which hybridization plays a creative force in the generation of new species.
394
395 **FIGURES:**
396
397 **Fig 1. Increasing evidence for widespread ancient hybridization**. With increasing
398 frequency, genome-spanning datasets are revealing that hybridization has played a role
399 in the evolution of a diverse, and globally distributed, array of taxa. Though it is often
400 hard to infer that ancient hybridization has played an adaptive role in the evolution of
401 extant taxa, it certainly appears to be a common phenomenon. There are many taxa,
402 including humans, for which there were no obvious reasons to suspect that hybridization
403 played a role in their evolutionary history. Clarifying the importance of hybridization in
404 the evolutionary history of these groups is an important next step in their study. (A)
405 Hybridization between coyotes and grey wolves in north America (vonHoldt et al. 2016);
406 (B) Polar bear and brown bear hybridization during the last glacial maximum (Cahill et
407 al. 2018); (C) Human and Neanderthal hybridization following expansion of humans out
408 of Africa (Sankararaman et al. 2014); (D) Baker's yeast hybridization prior to whole
409 genome duplication (Marcet-Houben and Gabaldón 2015); (E) Common bean
410 hybridization with wild ancestors in South America (Rendon-Anaya et al. 2017); (F)
411 Chimpanzee Bonobo hybridization (de Manuel et al. 2016); (G) Rampant ancient
412 hybridization between extant and extinct elephantids (Palkopoulou *et al.* 2018); (H)

Ancient hybridization between Congolese and Nile cichlid lineages appears to have led to the evolution of the Lake Victoria Super Flock of haplochromine cichlids (Meier et al. 2017).

Fig 2. Adaptive introgression is difficult to demonstrate. Convincingly demonstrating that introgression is adaptive is difficult, especially if introgression is ancient. Many of the most convincing examples of adaptive introgression come from contemporary introgression events (*e.g.,* A), or from traits with very clear adaptive function. For single loci of large effect (*e.g.,* C) this is easier than for multigene traits (*e.g.,* B). (A) Liu *et al.* 2015 build on the work of Song *et al.* 2011, which reported introgression of genes involved in warfarin resistance from *M. spretus* to *M. m. domesticus*. Using 22 whole genomes, Liu *et al.* 2015 find evidence of three hybridization events between *M. spretus* and *M. m. domesticus*, one ancient and two more recent. Importantly, they recovered the same introgressed region on chromosome 7 that contains *Vkorc1*, which is important for warfarin resistance, and find functional enrichment of olfactory receptors in introgressed regions. (B) Suarez-Gonzalez *et al.* 2016 expand on previous work examining *Populus trichocarpa* landscape genomics using whole chromosome sequencing to investigate introgression of candidate genes involved in local adaptation from *P. balsamifera* into *P. trichocarpa* within its northern range. Importantly, they report signals of selection on genes in introgressed regions of the genome. Functional trait and gene expression analyses in a common garden setting reveal correlations of these genomic regions with traits that are adaptive at the northern range limit of *P. trichocarpa* where the growing season is shorter (*e.g.,* higher photosynthetic rates and faster growth). (C) Jones *et al.* 2018 use a combination of whole genome and whole exome sequencing to demonstrate that cis-regulatory variation controls seasonal expression of the *Agouti* gene, which underlies seasonal coat color change in snowshoe hares (*Lepus americanus*). Further, their analyses indicate that the allele for brown coat color introgressed from the black-tailed jackrabbit (*L. californicus*) and swept to high frequency in snowshoe hare populations in habitats with mild winters. Background matching is critical for snowshoe hare survival. As such, introgression of this allele into snowshoe hares in areas with mild winters facilitates their survival. (D) The *Heliconius* Genome Consortium 2012 used whole genome resequencing to document introgression between species, particularly of two genomic regions that control mimicry patterns between three species of *Heliconius* that are co-mimics. *Heliconius* butterflies are unpalatable to vertebrate predators and are considered a classic example of Müllerian mimicry: their warning color patterns enable multiple species to share the cost of predator education. Wing patterns are also important in mate selection.

Fig 3. Hybrid speciation. The prevalence of homoploid hybrid speciation in nature is debated, but thus far appears to be rare. An important component of documenting this form of speciation is providing evidence that hybridization itself led to reproductive isolation between the hybrid species and its parental taxa (see Schumer et al. 2014). Although there are a growing number of reports of hybrid speciation in the literature, few systems have convincingly demonstrated the criteria outlined in Schumer et al. (2014). The two systems included here are recent examples of reported homoploid hybrid

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speciation that use whole genomes to clarify patterns of divergence and selection in
hybrid lineages. Differentiating between homoploid hybrid speciation and introgression
from ancient hybridization will be an important avenue of future research.
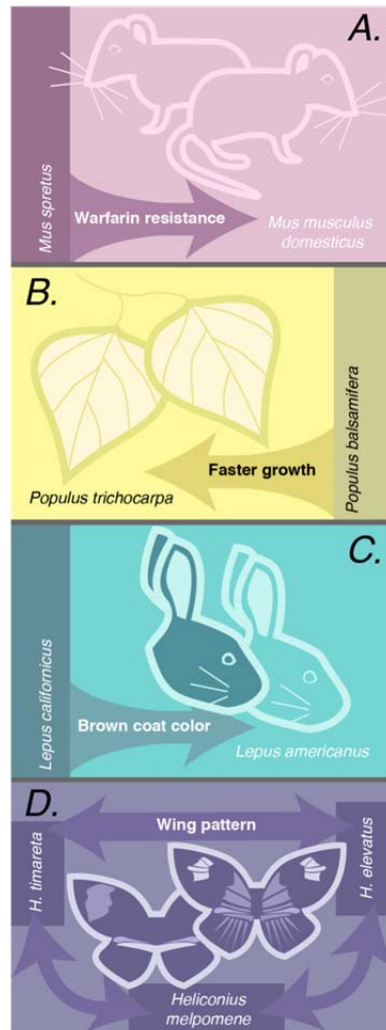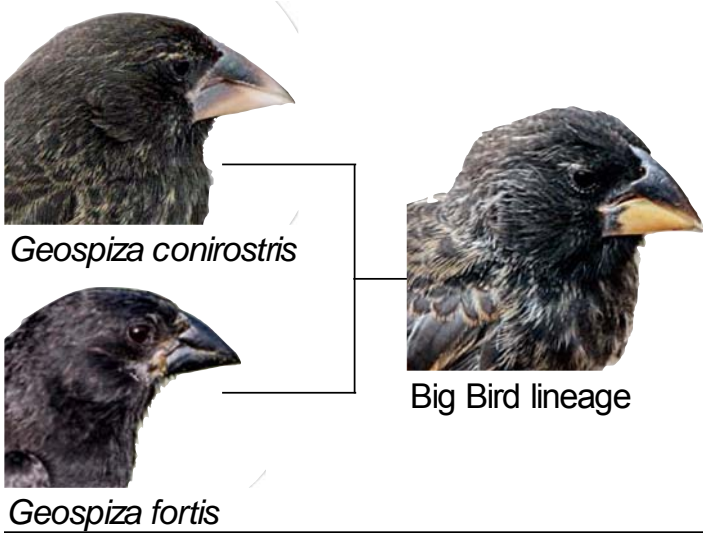

Figure 1.

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A. Darwin's finches

Geospiza conirostris

Big Bird lineage

Geospiza fortis

B. *Saccharomyces paradoxus*

SpC

SpC*

SpB

Figure 3.

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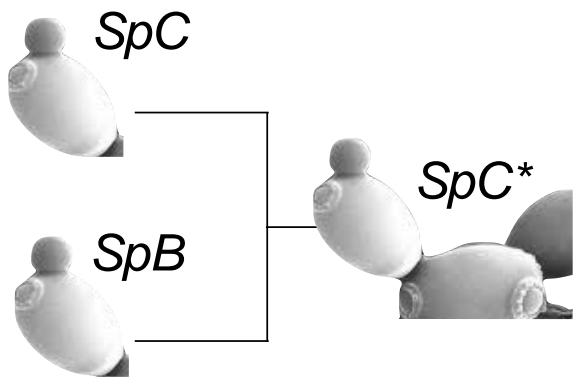
Table 1. A selection of recent studies that use whole genome data to study ancient hybridization, adaptive introgression, and hybrid speciation. A number of studies could have been placed into multiple sections.

	Taxon	Data	Authors
Ancient Hybridization			
Vervet monkey	*Chlorocebus Sp.*	n=166	Svardval et al. 2017
Bakers yeast	*Saccharomyces cerevisiae*	n=26,	Marcet-Houben et al. 2015
Eastern and red wolves	*Canis lyacon, C. rufus*	n=28	Von Holdt et al. 2016
Winged warblers	*Vermivora Sp.*	n=24	Toews, Taylor et al. 2016
Polar bear and brown bear	*Ursus maritimus* and *U. arctos*	n=13	Cahil et al. 2018
Human and Neanderthal	*Homo sapiens, H. neanderthalensis*	n=1004	Sankararaman et al. 2014
Elephants	*Loxodonta cyclotis, L. africana, Elephas maximus, Mammuthus orimigenius, M. columbi, Palaeoloxodon antiquus*	n=22	Palkopoulou et al. 2018
Bonobos and chimpanzee	*Pan paniscus, P. troglodytes*	n=75	De Manuel et al. 2016
Lake Victoria cichlids	*Pundamilia pundamilia* and *P. nyererei*	n=8	Meier et al. 2017
Adaptive Introgression			
Algerian mouse and house mouse	*Mus spretus, M. mus domesticus*	n=22	Liu et al. 2014 (Song et al. 2011)
Balsam poplar and Black cottonwood	*Populus trichocarpa and P. balsamifera*	n=118	Suarez-Gonzalez et al. 2016
Common bean	*Phaseolus vulgaris* L.	n=29	Rendon-Anaya et al. 2017
Snowshoe hare and black-tailed jackrabbit	*Lepus americanus, L. californicus*	n=82	Jones at al. 2018
Mosquitoes	*Anopheles gambiae* complex	n=11	Fontaine et al. 2015
Butterflies	*Heliconius sp.*	n=142	Wallbank et al. 2016
Sand rock-cress	*Aradibopsis arenosa*	n=24	Arnold et al. 2016
Human, Neanderthal, Denisovan	*Homo sapiens, H. neanderthalensis, H.*	n= 1004	Dannemann et al. 2016

Hybrid Speciation			
Root knot nematodes	*Meloidogyne Sp*	n=3	Lunt et al. 2014
Powdery mildew	*Blumeria graminis f. sp. triticale*	n=46	Menadro et al. 2016
Yeast	*Candida metapsilosis*	n=11	Pryszcz et al. 2015
Yeast	*Saccharomyces paradoxus*	n=161	Leduc et al. 2016, 2017
Italian sparrow	*Passer italiae*	n=73	Elgvin et al. 2017; Runemark et al. 2018a, b
Big Bird Darwin's finch	*Geospiza fortis x G. consirostris*	n=227	Lamichhaney et al. 2017
Yeast	*Candida orthopsilosis*	n=27	Schroder et al., 2016
invasive sculpin	*Cottus Sp.*	Whole genomes n=30	Dennenmoser et al. 2017

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