

1 OPINION

2

3 **Does hybridization between divergent progenitors drive whole-**
4 **genome duplication?**

5

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19 Key words: polyploidy, whole-genome duplication, hybrid speciation, evolutionary
20 paradigm

21 Running title: Parental divergence and polyploidy

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23 Word count: abstract, 99; main text, 2711.

24

25 **Abstract**

26

27 Hybridization and whole-genome duplication are both potential mechanisms of rapid
28 speciation which sometimes act in concert. Recent surveys, showing that homoploid
29 hybrid species tend to be derived from parents that are less evolutionarily divergent than
30 parents of polyploid hybrid species (allopolyploids), have been interpreted as supporting
31 a hypothesis that high divergence between hybridizing species drives whole-genome
32 duplication. Here we argue that such conclusions stem from problems in sampling
33 (especially the omission of autopolyploids) and null model selection, and underestimate
34 the importance of selection. The data simply demonstrate that hybridization between
35 divergent parents has a higher probability of successfully producing a species if followed
36 by polyploidization.

37

38 **Introduction**

39

40 Hybrid speciation occurs when two existing species cross and produce phenotypically
41 distinct offspring (or later-generation progeny) which then become reproductively
42 isolated from their parents. This mode of speciation was once regarded as unimportant
43 (Darwin 1859; Mayr 1963) but is now seen as a significant evolutionary process in both
44 plants (Rieseberg & Willis 2007; Soltis & Soltis 2009) and animals (Jesús Mavárez &
45 Linares 2008; Mallet 2007). This new view of hybrid speciation has developed through
46 both the discovery of numerous hybrid species, and an increased understanding of
47 processes which allow the obstacles to hybrid speciation to be overcome (Mallet 2007;
48 Rieseberg 1997).

49

50 Several obstacles stand in the way of successful hybrid speciation (reviewed in Grant
51 1981; Mallet 2007; Rieseberg 1997; Soltis & Soltis 2009). Geographic separation or
52 reproductive differences may prevent crossing between two potential parent species. If
53 hybridization does occur, the progeny may abort or have low fitness due either to genetic
54 incompatibilities between the parental genomes, or the generation of maladaptive
55 phenotypes. Even if the hybrid has high fitness, it may never achieve reproductive
56 isolation and will backcross with its parents, leading to introgression. Successful hybrid
57 speciation is therefore a comparatively rare event that occurs under a limited range of
58 conditions.

59

60 One process involved in the success of hybrid species is transgressive segregation: the
61 expression of trait values that exceed the range between the parental means (McDade
62 1990; Rieseberg *et al.* 2003). Transgressive segregation raises the likelihood of speciation
63 as the hybrids have new phenotypes not found in the parental species and may therefore
64 occupy an underused fitness peak on the local adaptive landscape (Mallet 2007). Under a
65 scenario involving transgressive segregation, ecological differentiation will occur rapidly
66 following hybridization, conferring a degree of reproductive isolation which may then be
67 reinforced with pre-zygotic isolation.

68

69 Another process frequently allowing success of hybrids is whole-genome duplication,
70 which is commonly found in plants and more rarely in animals (Mable 2004; Otto &
71 Whitton 2000; Rieseberg & Willis 2007). After genome doubling, backcrosses with
72 parental diploid species will generate progeny with an odd number of genome sets and
73 typically low fitness, providing post-zygotic reproductive isolation (Ramsey & Schemske
74 1998). Parental genomic incompatibilities may be overcome because genome doubling
75 causes every chromosome to have an identical homologue – thus pairing between
76 divergent parental chromosomes at meiosis is not necessary for successful gamete
77 formation. Whole-genome duplication can occasionally confer phenotypic changes such
78 as increased size or a change in sexual system, which may also contribute to speciation
79 (Otto & Whitton 2000).

80

81 **The role of genetic divergence**

82

83 The extent to which genetic divergence between parental species affects hybrid
84 speciation processes is currently under debate. The probability of ecological
85 differentiation following hybridization would seem to increase with the level of
86 divergence between the hybridizing parental species, as increased divergence would
87 likely expand the range of possible intermediate phenotypes. Recent evidence also
88 suggests that transgressive segregation in hybrids increases with genetic distance between
89 parental species (Stelkens & Seehausen 2009). Wide hybrids may therefore have more
90 evolutionary potential than hybrids between closely related species.

91

92 However, genetic differentiation between parental species is likely to be positively
93 correlated with some of the obstacles to hybridization. Under a general model of
94 allopatric speciation, geographic separation is likely to be more pronounced between
95 distantly related species, reducing the incidence of hybridization between distant
96 relatives. In addition, genetic incompatibilities due to, for example, chromosomal
97 rearrangements, Dobzhansky-Muller incompatibilities, or differential loss of duplicated
98 genes, are also likely to increase with genetic divergence, leading to hybrid inviability
99 (Lynch 1991; Orr 1995). Thus, wide hybrids (i.e., hybrids between divergent parents) are
100 less likely to form than those between close relatives, and may have lower fitness.

101

102 As some of the negative effects of genetic divergence between parental species can be
103 overcome by whole-genome duplication, we would expect formation of hybrid polyploid
104 species to be successful over a greater range of parental divergences than homoploid
105 hybrid species. At high parental divergences, allopolyploids may benefit from effects of

106 transgressive phenotypes, while homoploid hybrids between highly divergent parents,
107 which are likely to be sterile, may not similarly benefit. Thus, we might expect the
108 average divergence between parents of allopolyploids to be higher than that of homoploid
109 hybrid species, simply because polyploidy enables wide hybrids to be viable.

110

111 Several researchers have made the intriguing suggestion that rather than being a random
112 mutation that fortuitously confers viability to a wide hybrid, successful polyploidy is
113 determined by wide hybridization: genomic differentiation between parental species in
114 fact drives successful whole-genome duplication in their hybrids. Winge (1917) first
115 suggested that “occasional hybridization might be the cause” (p. 13) of polyploidy. He
116 developed “a scheme showing the different degrees of physiogenetic likeness between
117 gametes – and thus also between their chromosomes – endeavouring at the same time to
118 ascertain what results we can expect in each case from the fusion of gametes” (p. 196). In
119 this scheme a hybrid in which the chromosomes from parental species were unable to pair
120 would have to undergo chromosome doubling in the zygote to have “any possibility at all
121 of propagating” (p. 199). Subsequent workers proposed that this doubling would occur
122 through unreduced gamete formation. Darlington (1937) suggested that gametic doubling
123 occurs in hybrids because meiosis has proved unworkable. Similarly, Grant (1981)
124 considered that reduced chromosome pairing in hybrids between parents whose
125 chromosomes had different structures would “set the stage” for unreduced gamete
126 formation. Ramsey and Schemske (1998) found unreduced gametes to occur at a
127 frequency of 28% in hybrids but only 0.6% in non-hybrids. This could suggest that
128 unreduced gamete formation is actually triggered in hybrids.

129

130 Darlington (1937) also suggested another level at which this drive could occur. He
131 proposed an inverse relationship between the fertility of a diploid hybrid and that of a
132 tetraploid to which it gives rise. He reasoned that at low parental divergences, homoploid
133 hybrids will be fertile because chromosomes will be able to pair at meiosis, but
134 allopolyploids will be of low fertility because pairing will occur between both duplicated
135 chromosomes and homeologous chromosomes from each parent, causing uneven
136 segregation. In contrast, at high parental divergences, homoploid hybrids will be sterile
137 due to failure of chromosome pairing, but allopolyploids will be fertile due to consistent
138 bivalent formation at meiosis. This has sometimes been called “Darlington’s rule” (not to
139 be confused with Darlington’s rule in biogeography which states that with every ten-fold
140 increase in area, the number of species doubles). A literature survey by Clausen, Keck
141 and Hiesey (1945) seemed to support this rule by showing that the success and constancy
142 of allopolyploids is “linked with the degree of relationship found between their parents”
143 (p. 2). They argued that the “parent species...should be closely enough related to produce
144 a vigorous F_1 hybrid, but remotely enough so that the balance between their combined
145 genomes can be perpetuated” (p. 68-69). Stebbins (1950) agreed with this conclusion.
146 However, a recent survey of neopolyploids (Ramsey & Schemske 2002) did not show
147 significantly lower fertility in autopolyploids than allopolyploids, and we now know that
148 many allopolyploids do not show consistent bivalent formation and that non-homologous
149 transposition can occur between parental genomes (Leitch & Leitch 2008). These
150 findings suggest that although Darlington’s rule may describe the average fertility of

151 hybrids and allopolyploids, selection may subsequently play a large role in preserving
152 fertile autopolyploids, as well as allopolyploids that formed from closely related parents.

153

154 **New evidence**

155

156 Three recent studies have re-visited the hypothesis that high divergence between
157 hybridizing species drives whole-genome duplication, using molecular methods to assess
158 divergence between the progenitor species of natural polyploid species, assuming as
159 Darlington (1937) did that genetic differentiation will correlate with structural
160 differentiation of chromosomes: Chapman and Burke (2007), Buggs *et al.* (2008) and
161 Paun *et al.* (2009). Chapman and Burke (2007) provided the first study that directly
162 compares the genetic distance between the parental species of homoploid and polyploid
163 hybrid species. They calculated Kimura's two-parameter (K2P) genetic distance between
164 DNA sequences from the internal transcribed spacer (ITS) region of nuclear ribosomal
165 RNA genes of 12 species pairs that have given rise to homoploid hybrid species and 26
166 species pairs that have given rise to allopolyploid species. They compared all hybrid
167 versus allopolyploid parental pairs and found a significantly larger divergence between
168 the parents of allopolyploids. They concluded that "the extent of evolutionary divergence
169 between hybridizing taxa plays an important role in determining the outcome of hybrid
170 speciation" (p. 1778).

171

172 Buggs *et al.* (2008) tested the hypothesis that closely related parents are less likely to
173 form a successful polyploid than more divergent parents. They examined molecular

174 phylogenies of eight genera that contain polyploids, using node-based and clade-based
175 methods of calculating the phylogenetic distance between parental pairs. They compared
176 these with expected divergences based on the null hypothesis that hybridization would
177 occur successfully at random between all species of a genus. They found that the
178 phylogenetic divergence between parents of polyploids was not significantly different
179 from the divergence expected under the null hypothesis. The same analysis on homoploid
180 hybrids in the same genera found a lower divergence between the parents of homoploid
181 hybrids than the null expectation, even when unstable hybrids were included. They
182 concluded that “contrasting patterns of divergence between the parents of polyploids and
183 homoploid hybrids are...determined by the restriction of homoploid hybrid formation to
184 low parental divergence, rather than the restriction of polyploid formation to high
185 parental divergence” (p. 87).

186

187 Paun *et al.* (2009) conducted an additional analysis that combined and improved some of
188 the approaches of Chapman and Burke (2007) and Buggs *et al.* (2008). For 16 homoploid
189 hybrids and 32 allopolyploids, they calculated uncorrected p-distances and K2P distances
190 between parental pairs using nuclear and/or chloroplast sequences. They converted each
191 of these distances to a genetic divergence index (GDI) by dividing parental divergence by
192 the average genetic distance between all pairs in each genus based on the same molecular
193 markers. The GDI gave very similar results for both distance measures, and parents of
194 polyploids were found to be significantly more divergent than parents of hybrids (Figure
195 1). Fitting a heuristic model to their data, Paun *et al.* (2009) suggested that at a GDI of
196 around 0.75, there is an equal probability of a hybrid being homoploid or allopolyploid,

197 but above this point, allopolyploidy is more likely, and below this, homoploidy is more
198 likely. They concluded that “parental divergence drives ploidy”.

199

200 Although Paun *et al.* (2009) calculated the average divergence between all species pairs
201 in each genus, they did not use this as a null hypothesis for the expected divergence
202 between parents of allopolyploids as in Buggs *et al.* (2008). If we carry out a two-tailed
203 paired *t*-test on the genetic distances between parental pairs and the average genetic
204 distance between all species pairs in their respective genera, using the data from Table S1
205 of Paun *et al.* (2009), we find a significant difference between these values for homoploid
206 hybrids ($t = 3.427$, d.f. = 15, $P < 0.01$), but no significant difference for allopolyploids (t
207 = 1.533, d.f. = 31, $P > 0.1$). A Wilcoxon matched-pairs signed-ranks test gives similar P
208 values. Significantly, this result agrees with that of Buggs *et al.* (2008): homoploid hybrid
209 formation occurs at low parental divergence, but polyploid formation fits a model of
210 random hybridization.

211

212 **Null models and sampling issues**

213

214 The three studies summarized above therefore provide the same general pattern of results,
215 despite differences in methodology and sampling. They allow us to predict that wide
216 hybridization has a higher probability of producing a successful species if followed by
217 genome doubling. The authors of the three studies disagree over whether or not the
218 results constitute good evidence for high parental divergence driving ploidy. This
219 difference is partly due to the use of different null hypotheses. By basing their

220 conclusions on comparisons between homoploid hybrids and allopolyploids, Chapman
221 and Burke (2007) and Paun *et al.* (2009) seem to view the parental divergence of
222 homoploid hybrids as a null hypothesis of the distribution expected of allopolyploids
223 without the action of drive. This is problematic for two reasons. First, homoploid hybrids
224 are likely to be restricted in the parental divergences under which they can form. Second,
225 polyploid and hybrid species may not be comparable: while polyploid species typically
226 have post-zygotic reproductive isolation from their parents, homoploid hybrids are likely
227 to be more interfertile with their parents than the parents are with each other. Most
228 homoploid hybrid species, such as *Helianthus anomalus*, *H. deserticola*, and *H.*
229 *paradoxus* (Rieseberg 2003), are introgressed ecological forms that survive in habitats
230 unused by the parental species and are not clearly able to coexist with their parents due to
231 a lack of post-zygotic isolation (Rieseberg 1997).

232

233 Buggs *et al.* (2008) view random hybridization as a better null model than homoploid
234 hybrid species formation as the former assumes that parental divergence has no *a priori*
235 influence on the probability of allopolyploidization. This random hybrid formation model
236 is likely to be overly simplistic, as acknowledged by Buggs *et al.* (2008), but fits the data
237 well. Sang *et al.* (2004) suggested a model for the origin of tetraploids as a function of
238 genomic divergence between diploid progenitors, but as they suggest, this needs to be
239 made more mathematically rigorous, with better natural population estimates of the rates
240 of hybridization, unreduced gamete formation, and establishment of the resulting
241 lineages. It seems, for example, that wide hybridization may increase rates of unreduced
242 gamete formation (see above) but, as Ramsey and Schemske (1998) argue, this rise in

243 mutation rate is likely to be at least cancelled out by low hybridization rates and may
244 therefore not increase the frequency of allopolyploids at high parental divergences.

245

246 Our ability to draw firm conclusions is also restricted by sampling limitations. Chapman
247 and Burke (2007), Buggs *et al.* (2008) and Paun *et al.* (2009) agree that improved
248 sampling is needed of homoploid hybrids, which are very difficult to detect and may in
249 fact be quite rare in nature. We suggest that the lack of allopolyploids between closely
250 related species (Figure 1) may also be due to sampling bias as these may also be hard to
251 detect (Rieseberg & Willis 2007); for example, a recently discovered allotetraploid
252 formed by interspecific hybridization between *Mimulus nasutus* and *M. guttatus* was
253 identified as the former species until it gave anomalous results in a crossing experiment
254 (Sweigart *et al.* 2008). There is an urgent need for thorough molecular analysis of many
255 additional hybridizing plant groups, particularly those genera containing hybrids as well
256 as polyploids, such as the genera *Crepis*, *Clarkia*, *Betula*, and *Gilia*.

257

258 The authors of the three studies disagree about the relevance of autopolyploids (defined
259 here as polyploids formed within a species) to the issue of parental divergence and
260 whole-genome duplication. We have long recognized that there is a continuum from true
261 autopolyploid to allopolyploid, with “hybrid autopolyploid” and “segmental
262 allopolyploid” as intermediate points in this continuum (Stebbins 1950). In a restriction
263 that appears to stem from the use of homoploid hybrids as a null model, Paun *et al.*
264 (2009) argue that autopolyploids should not be included in the analysis as they do not
265 directly correspond to hybrid speciation processes at the diploid level. In contrast, Buggs

266 *et al.* (2008) argue that the hypothesis that parental divergence drives polyploidy cannot
267 be tested fully without including divergences at or close to zero (e.g., autopolyploids).

268

269 These issues regarding the comparability of autopolyploids, allopolyploids and
270 homoploid hybrids are part of the broader problem of defining species. Classing two
271 groups as “species” seems to imply that they are comparable evolutionary units, but due
272 to the use of different species concepts and types of information, the classification of
273 certain groups as species is not standardized and somewhat arbitrary. Autopolyploids
274 seem to occur very frequently in nature (e.g. Ramsey & Schemske 1998; Soltis & Soltis
275 1993) but are rarely classified as separate species (Soltis *et al.* 2007), despite sometimes
276 strong reproductive isolation from their parents. Reliance upon named species as a unit of
277 comparison in surveys therefore introduces a strong bias against successful
278 autopolyploidization events, whose frequent occurrence in nature certainly contradicts the
279 idea that whole-genome duplication is less likely at low parental divergence. Likewise,
280 with homoploid hybrids, the division between a recurrent unstable hybrid and a hybrid
281 species is not straightforward.

282

283 If in our sampling of polyploids we were able to count all polyploidization events that
284 have led to an established population (whether classified by taxonomists as a species or
285 not), it is possible that the number of events would be highest for closely related parents
286 and actually decline with parental divergence. This distribution would directly contradict
287 the idea that parental divergence drives polyploidy, and would also contrast with the

288 random pattern of allopolyploid species formation in relation to parental divergence
289 noted by Buggs *et al.* (2008) in their survey and shown here in that of Paun *et al.* (2009).
290 Paun *et al.* (2009) make the interesting suggestion that inclusion of autopolyploids would
291 cause a bimodal distribution of polyploid frequency in relation to parental divergence,
292 indicating the presence of different phenomena. Whilst such a distribution could be an
293 artifact of the lack of detection of polyploids of intermediate parental divergence (i.e.
294 allopolyploids with closely related parents; see above), it might also be explained by
295 selection. For an unoccupied fitness peak that is close to an occupied peak to be filled by
296 a new variant, that variant must be reproductively isolated. Polyploidy typically confers
297 reproductive isolation from its parents at both low and high parental divergences; in
298 contrast, a homoploid hybrid is likely to occur and be reproductively isolated from its
299 parents only at intermediate parental divergences (i.e. low enough for the hybrids to be
300 viable but high enough for the hybrid to be isolated from both parents). Because
301 homoploid hybrids often come into existence before an allopolyploid forms (following
302 the Class 2 mode of allopolyploidization; Harlan & De Wet 1975), there will not be
303 strong selection for polyploidy at intermediate divergences, unless the new polyploid
304 would occupy a different adaptive peak from that of the extant homoploid hybrid.

305

306 **Conclusion**

307

308 In our view, the idea that parental divergence drives polyploidy is based on two factors.
309 The first is an over-emphasis on potential bias in mutational mechanisms (e.g., unreduced
310 gamete formation) without sufficient consideration of subsequent selection on the newly

311 formed hybrid or polyploid. The second is misinterpretation of survey data due to
312 problems in sampling (particularly the omission of autopolyploids) and null model
313 selection. The three recent studies reviewed here of the relationship between parental
314 divergence and hybrid speciation (Buggs *et al.* 2008; Chapman & Burke 2007; Paun *et al.*
315 2009) do not provide convincing evidence that polyploid species are less likely to form
316 successfully at lower parental divergences and therefore do not demonstrate that parental
317 divergence drives ecologically successful whole-genome duplication. Instead, they
318 simply allow us to predict that wide hybridization has a higher probability of producing a
319 successful species if followed by polyploidization.

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391 **Figure Legend**

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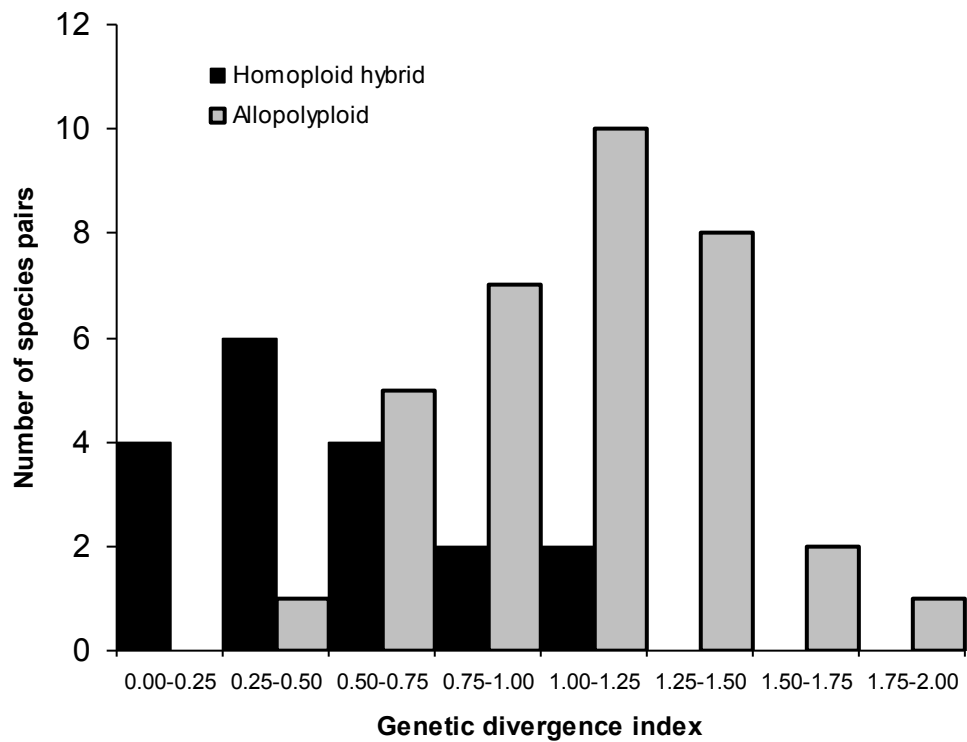
394 **Figure 1.** Genetic divergence between parents of allopolyploid species versus
395 those of homoploid hybrid species, re-plotted from Paun *et al.* (2009). The
396 genetic divergence index on the horizontal axis is the uncorrected p-distance
397 between the two species, divided by the mean p-distance of all species pairs in
398 their genus. Two species pairs that give rise to both a homoploid hybrid species
399 and an allopolyploid species were included in both counts. A two-tailed paired *t*-
400 test on the genetic distances between parental pairs and the average genetic
401 distance between all species pairs in their respective genera, shows a significant
402 difference between these values for homoploid hybrids ($t = 3.427$, d.f. = 15, $P <$
403 0.01), but no significant difference for allopolyploids ($t = 1.533$, d.f. = 31, $P > 0.1$).

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405

406 **Figure 1**

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412 **Acknowledgements**

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414 The authors thank two anonymous reviewers for helpful comments on an earlier version
415 of this paper. This work was supported in part by NSF grant DEB-0614421 and
416 the University of Florida.

417

418 **Author descriptions**

419

420 **Richard Buggs** uses molecular genetic and bioinformatics approaches to study duplicate
421 gene evolution in recent *Tragopogon* allopolyploids.

422 **Pamela Soltis**' research interests include: plant phylogenetics, polyploidy, gene family
423 evolution, phylogeography and conservation genetics.

424 **Doug Soltis** is interested in angiosperm phylogeny, genome doubling, floral
425 developmental genetics, phylogeography and molecular cytogenetics.

426