

GENETIC DIVERGENCE AND HYBRID SPECIATION

Mark A. Chapman^{1,2} and John M. Burke^{1,3}

¹University of Georgia, Department of Plant Biology, Miller Plant Sciences Building, Athens, Georgia 30602

²E-mail: mchapman@plantbio.uga.edu

³E-mail: jmburke@uga.edu

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Although the evolutionary importance of natural hybridization has been debated for decades, it has become increasingly clear that hybridization plays a fundamental role in the evolution of many plant and animal taxa, sometimes resulting in the formation of entirely new species. Although some hybrid species retain the base chromosome number of their parents, others combine the full chromosomal complements of their progenitors. Hybrid speciation can thus produce two fundamentally different types of evolutionary lineages, yet relatively little is known about the factors influencing ploidy level in hybrid neospecies. We estimated genetic divergence between species pairs that have given rise to homoploid and polyploid hybrid species and found that divergence is significantly greater for the parents of polyploids, even after controlling for potentially confounding factors. Our data thus provide the first direct evidence in support of the notion that the extent of genomic divergence between hybridizing species influences the likelihood of diploid versus polyploid hybrid speciation.

KEY WORDS: Allopolyploid, genetic divergence, homoploid, hybridization, polyploidy, speciation.

Natural hybridization has played a role in the evolution of a wide variety of both plant and animal taxa (e.g., Arnold 1997; Rieseberg 1997; Otto and Whitton 2000; Le Comber and Smith 2004). Such interspecific hybridization may have important evolutionary consequences depending on the frequency of intermating and the fitness of the resulting hybrid progeny (Barton and Hewitt 1985; Burke and Arnold 2001). For example, if hybrids are viable and fertile, and if there are repeated opportunities for hybridization, extensive gene flow may result in the extinction of one of the hybridizing taxa via genetic assimilation (e.g., Haddon 1984; Ayres et al. 2004; Konishi and Takata 2004; Rosenfield et al. 2004; Genovart et al. 2005), or even the merging of the two taxa into a single evolutionary lineage (e.g., Echelle and Connor 1989; Hegde et al. 2006; Taylor et al. 2006). In contrast, persistent gene flow accompanied by reduced hybrid fitness can result in a stable hybrid zone, allowing for genetic exchange in certain genomic regions (including the possible introgression of beneficial alleles), but preventing the merging of the taxa (Barton and Hewitt 1985).

Alternatively, if the hybrids are fertile and viable, and at least partially reproductively isolated from their parents, the end result may be the production of a hybrid neospecies.

Although there are a great number of cases in which two species have come together to form a small number of hybrids, or even a hybrid zone consisting of thousands of hybrid individuals, the number of well-documented cases of hybrid speciation is much smaller, especially for animals (Coyne and Orr 2004). Perhaps the biggest reason for the paucity of hybrid species is the difficulty associated with producing a reproductively isolated hybrid lineage that can escape close competition with its parental taxa. One possible path to reproductive isolation in hybrids is the segregation and recombination of chromosomal rearrangements or genic incompatibilities that distinguish the parental taxa (i.e., recombinational speciation resulting in a homoploid hybrid species; Grant 1981; Templeton 1981; Ungerer et al. 1998; Rieseberg 2001). Alternatively, chromosome doubling in the hybrid offspring (i.e., allopolyploidy; Ramsey and Schemske 1998) can result in the

immediate isolation of hybrids from their parents. In either case, backcrossing results in offspring with reduced fitness, whereas the hybrids are fully viable and fertile when crossed inter se. The invasion and utilization of a novel habitat also appear to be a critical component of hybrid speciation due to both the additional, environmentally mediated reproductive isolation resulting from niche separation and a reduction in the degree of direct competition between hybrids and their parents (Buerkle et al. 2000, 2003). The importance of niche divergence is corroborated by increased ecological tolerances in a number of putative homoploid hybrid species (Gross and Rieseberg 2005).

Although both of these modes of hybrid speciation are known to have occurred in nature, allopolyploidy appears to be more common than homoploid hybrid speciation, and considerably more is known about its overall evolutionary significance (Otto and Whitton 2000). According to Grant (1981), the three primary factors required for the formation of allopolyploids are (1) the existence of diploid species carrying distinct genomes, (2) natural hybridization between these species, and (3) a mechanism to increase the opportunity for the production of polyploid offspring by hybrid individuals (e.g., a long-lived perennial growth habit or an autogamous breeding system). If one or more of these conditions is lacking, Grant (1981) argued that polyploidy will be rare or absent. However, even when these conditions are satisfied, the frequency of polyploid taxa is quite variable across taxonomic groups, suggesting that other factors influence the likelihood of allopolyploid establishment.

Polyploid species are often thought to arise via a "triploid bridge," wherein the fusion of reduced (n) and unreduced ($2n$) gamete produces a triploid ($3n$) offspring. Such triploids can, on occasion, produce viable n , $2n$, or $3n$ gametes, and can thus give rise to tetraploids via backcrossing with diploids, crossing with other triploids, or selfing (Ramsey and Schemske 1998). In plants, allotriploids are often observed in the progeny of controlled crosses between interspecific F_1 hybrids or in the backcross progeny of F_1 hybrids and their parents (Ramsey and Schemske 1998). There is also evidence that the formation of unreduced ($2n$) gametes in hybrids may be related to the genetic distance between the parents of the hybrid. For example, $2n$ gametes are more common in intersectional *Lilium* F_1 hybrids than in intrasectional hybrids (van Tuyl et al. 1989), with haploid (n) gametes sometimes being entirely absent in hybrids from wider crosses (J. van Tuyl, pers. comm., 2006).

In addition to affecting the rate of production of unreduced versus reduced gametes in the F_1 generation, the level of genetic divergence between two hybridizing individuals also affects the fertility and fitness of homoploid F_1 hybrids. In the first study to look at this phenomenon, Coyne and Orr (1989, 1997) showed that reproductive isolation (i.e., hybrid inviability and sterility) between *Drosophila* species increased in parallel with genetic di-

vergence. This same pattern has since been found in sea stars (Foltz 1997), frogs (Sasa et al. 1998), lepidopterans (Presgraves 2002), birds (Price and Bouvier 2002; Tubaro and Lijtmaer 2002), and three species of angiosperms (Moyle et al. 2004). Although the rate and pattern of the evolution of pre- and postzygotic isolation varies amongst species (Edmands 2002; Mendelson et al. 2004), in all cases reproductive isolation is correlated with ecological isolation (Funk et al. 2006). Moreover, a number of authors have argued that the rate of formation of fertile/viable hybrids between distantly related species should be lower than that between more closely related species (e.g., Mallet 2005; Schranz et al. 2005).

Despite the negative correlation between genetic divergence and the fitness of homoploid hybrids, allopolyploids between even the most widely divergent taxa can in some cases show very little reduction in fitness. In fact, it has even been suggested that the fertility of polyploid hybrids might increase with increasing parental genetic divergence (e.g., Stebbins 1950; Bogart 1972; Mable and Bogart 1995; reviewed in Mable 2004). This is perhaps due to preferential pairing of homologous chromosomes, and thus fewer meiotic abnormalities, in the polyploid progeny of wider crosses.

The observations and ideas outlined above suggest that genetic divergence may play an important role in determining the outcome of hybrid speciation (i.e., the production of homoploid vs. allopolyploid neospecies). More specifically, (1) hybrids between increasingly divergent parental taxa are more likely to produce unreduced gametes, and (2) polyploid offspring derived from such divergent crosses may be more fit than diploid offspring. To investigate this possibility, we analyzed the relationship between the genetic divergence of species pairs that are known to have formed hybrid species and the ploidy level of the resulting hybrid species. Because of the rarity of well-documented cases of hybrid speciation in animals (Coyne and Orr 2004), we restricted our analysis to plants.

Materials and Methods

SELECTION OF TAXA AND COLLECTION OF DNA SEQUENCES

We compiled a representative list of hybrid species and their parental taxa using published reviews on homoploid/polyploid hybrid speciation (Rieseberg 1997; Ramsey and Schemske 1998; Otto and Whitton 2000; Gross and Rieseberg 2005) and by searching the Web of Science (<http://portal.isiknowledge.com/portal.cgi>) for articles using the keywords "hybrid species," "homoploid," and "polyploid." When selecting taxa for inclusion in our study, we considered only hybrid taxa that have evolved naturally. For example, the diploid hybrid species *Senecio squaridus* was excluded from consideration because it did not evolve in situ; instead, hybrids were transplanted to a different country

where they became established (James and Abbott 2005). To have a common metric with which to compare divergence amongst species pairs, we then searched Genbank (<http://www.ncbi.nlm.nih.gov/genbank/>) for DNA sequences from the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA (rRNA) genes. To supplement the Genbank data, we obtained tissue samples from the parents of two additional allopolyploids and sequenced the ITS region ourselves. PCR primer sequences were taken from Baldwin (1993), and sequencing was performed on an MJ Research BaseStation automated DNA sequencer (MJ Research, South San Francisco, CA) using DYEnamic ET sequencing chemistry (Amersham Biosciences, Piscataway, NJ) following the manufacturer's protocols. Details regarding species analyzed, Genbank accession numbers for all sequences analyzed, and supporting references can be found in Table 1.

DNA SEQUENCE DIVERGENCE AND STATISTICAL ANALYSES

Sequences were aligned using ClustalW (Thompson et al. 1994), gaps were scored using GAPCODER (Young and Healy 2003), and Kimura's (1980) two-parameter (K2P) genetic distance was calculated using PAUP* version 4.0b10 (Swofford 2001). K2P was chosen as a suitable measure of genetic distance because (1) the base composition was virtually identical between sequences within each dataset (homogeneity of base frequencies test in PAUP*) and (2) it does not constrain the results by implying transition and transversion rates were equal. The sequences of the individual rRNA genes were not available for all species, so our analysis focused on the ITS regions only. When multiple sequences were available for a given taxon, we estimated the K2P distance for all possible interspecific pairs of sequences and averaged our results. We omitted from our analysis rare cases in which a pair of species has given rise to both diploid and polyploid hybrid species. Finally, in cases in which a pair of species has given rise to more than one diploid or more than one polyploid hybrid species, we entered the divergence data into our analysis just once. We arcsine-square-root transformed all K2P distances prior to analysis to achieve a normal distribution, and performed our statistical analyses using JMP version 5 (SAS Institute, Inc., Cary, NC). For two species pairs in particular, we had to exercise caution when utilizing sequences from Genbank, as follows: First, the paternal progenitor of *Gossypium bickii* has not been confirmed; it is either *G. australe* or *G. nelsonii* (Wendel et al. 1991). These two species are very closely related and approximately equally diverged from *G. sturtianum* (the maternal parent of *G. bickii*) and hence the average distance between *G. australe*/*G. nelsonii* and *G. sturtianum* was calculated. Second, sequence AB032051, which is supposedly derived from *Eupatorium sessilifolium* (Ito et al. 2000), most likely represents an accession of *E. semiserratum* (Siripun and

Schilling 2006). Thus, this sequence was excluded from the analysis of *Eupatorium* species.

Results and Discussion

The average genetic distance between the parents of the different "types" of hybrid species (i.e., homoploid vs. polyploid) were significantly different ($t = 3.87$, $df = 1$, $P = 0.0004$; Fig. 1), with the parents of allopolyploids being more than twice as divergent as the parents of homoploid hybrid species. A nonparametric (Wilcoxon rank sum) test on the untransformed data confirmed this result ($\chi^2 = 11.50$; $df = 1$; $P \leq 0.0007$). Although the species pairs included in this survey represented a wide range of phylogenetically diverse angiosperms, it is still possible that this pattern that we observed arose as a by-product of taxonomic sampling. We thus performed a two-way analysis of variance (ANOVA) with the type of hybrid species and taxonomic order of each species pair as the main effects (the data were not sufficiently balanced to test for lower-level taxonomic effects). Here again, the type of hybrid species had a significant effect ($F = 16.36$; $df = 1, 26$; $P \leq 0.0004$), whereas the effects of order were nonsignificant ($F = 0.86$; $df = 10, 26$; $P = 0.58$).

These results suggest that increased evolutionary divergence increases the likelihood that hybrid species will be formed at the polyploid versus homoploid level. The observed pattern could, however, be produced if the allopolyploids included in our sample were all much older than the homoploid hybrid species. Under such circumstances, differences in genetic divergence could simply be attributed to a difference in the amount of time that has passed since the hybrid speciation event, with the parents of polyploids having more opportunity for divergence after the fact. Unfortunately, data concerning the age of hybrid species are sparse, with only two of our examples having published estimates based on molecular data: the three homoploid hybrid sunflower species are thought to have arisen between 75,000 and 20,800 years ago (Schwarzbach and Rieseberg 2002; Welch and Rieseberg 2002; Gross et al. 2003), whereas allopolyploid cotton is thought to have arisen about 1.5 million years ago (Senchina et al. 2003). However, the authors of 26 of the 38 studies included in our survey stated whether the hybrid species in question were thought to be of "ancient" or "recent" origin. Although this is largely subjective, our dataset comprised four ancient diploids, two recent diploids, 10 ancient polyploids, and 10 recent polyploids (Table 1). It therefore seems unlikely that the pattern documented reflects a difference in the ages of the two types of hybrid species. It is also worth noting that if the parents of the hybrid species are polyploid themselves, then relaxed purifying selection may give rise to a greater estimate of parental divergence (e.g., Aagaard et al. 2006). However, the vast majority of parent species are, if anything, considered to be paleopolyploids, and for the three parent species pairs in which

Table 1. Summary of taxa included in our analysis. K2P genetic distance (\pm SE), phylogenetic order, life-history strategy, and age (R, recent; A, ancient; or -, unknown) are also provided for each. References refer to the papers documenting the hybrid origin of the species in question and (in bold) the Genbank numbers of the ITS sequences used.

Category	Hybrid species	Parents ^a	Genetic distance	Order ^b	Life history	Age	Reference ^c
Diploid	<i>Argyranthemum sundingii/lemsii</i>	<i>A. broussonetii</i> <i>A. frutescens</i>	0.00000	ASTE	Perennial	A	1
Diploid	<i>Arisaema ehimense</i>	<i>A. tosaense</i> <i>A. serratum</i>	0.00195	MONO	Perennial	—	2
Diploid	<i>Encelia virginensis</i>	<i>E. actoni</i> <i>E. frutescens</i>	0.01364	ASTE	Perennial	—	3
Diploid	<i>Gossypium bickii</i>	<i>G. sturtianum</i> <i>G. australe/G. nelsonii</i>	0.03488 \pm 0.00048	MALV	Perennial	A	4
Diploid	<i>Helianthus anomalus/deserticola/paradoxus</i>	<i>H. annuus</i> <i>H. petiolaris</i>	0.00925 \pm 0.00044	ASTE	Annual	A	5, 6
Diploid	<i>Hippophae goniocharpa</i>	<i>H. rhamnoides</i> ssp. <i>sinensis</i> <i>H. neurocarpa</i>	0.09190	ROSA	Perennial	—	7
Diploid	<i>Hyobanche glabrata</i>	<i>H. sanguinea</i> <i>H. rubra</i>	0.03669 \pm 0.00003	LAMI	Perennial	—	8
Diploid	<i>Paeonia emodi</i>	<i>P. veitchii</i> <i>P. lactiflora</i>	0.00823	SAXI	Perennial	—	9
Diploid	<i>Phlomis</i> \times <i>margaritae</i>	<i>P. composita</i> <i>P. purpurea</i>	0.03799 \pm 0.00103	LAMI	Perennial	R	10
Diploid	<i>Scaevola kilaueae</i>	<i>S. coriacea</i> <i>S. chamissoniana</i>	0.02027 \pm 0.00028	ASTE	Perennial	R	11
Diploid	<i>Scaevola procera</i>	<i>S. gaudichaudii</i> <i>S. mollis</i>	0.01808 \pm 0.00129	ASTE	Perennial	A	11
Diploid	<i>Eupatorium godfreyanum</i>	<i>E. rotundifolium</i> <i>E. sessilifolium</i>	0.04212 \pm 0.00036	ASTE	Perennial	—	12
Polyploid	<i>Achillea alpina/wilsonia</i>	<i>A. acuminata</i> <i>A. asiatica</i>	0.04527	ASTE	Perennial	—	13
Polyploid	<i>Achillea virescens</i>	<i>A. nobilis</i> agg. <i>A. millefolium</i> agg.	0.01377 \pm 0.00628	ASTE	Perennial	A	14
Polyploid	<i>Arabidopsis suecica</i>	<i>A. thaliana</i> <i>A. arenosa</i>	0.08292 \pm 0.00866	BRAS	Annual	R	15
Polyploid	<i>Arachis hypogaea</i>	<i>A. duranensis</i> <i>A. ipaensis</i>	0.03943 \pm 0.00401	FABA	Annual	R	16
Polyploid	<i>Artemisia douglasiana</i> (6 \times)	<i>A. suksdorfii</i> (2 \times) <i>A. ludoviciana</i> (4 \times)	0.03264 \pm 0.02003	ASTE	Perennial	—	17
Polyploid	<i>Symphyotrichum</i> (<i>Aster</i>) <i>Ascendens</i>	<i>S. falcatum/A. falcatus</i> <i>S. spathulatum/</i> <i>A. occidentalis</i>	0.13184 \pm 0.00050	ASTE	Perennial	—	18
Polyploid	<i>Brassica napus</i>	<i>B. rapa</i> <i>B. rapa</i>	0.08173 \pm 0.00302	BRAS	Annual	R	19
Polyploid	<i>Cardamine schulzii</i> (4 \times)	<i>C. rivularis</i> <i>C. amara</i>	0.05451 \pm 0.00082	BRAS	Perennial	R	20
Polyploid	<i>Cardamine silana</i>	<i>C. apennina</i> <i>C. acris</i>	0.01565 \pm 0.00042	BRAS	Perennial	A	21
Polyploid	<i>Clarkia delicata</i>	<i>C. epilobioides</i> <i>C. unguiculata</i>	0.04004 \pm 0.00163	MYRT	Annual	R	22
Polyploid	<i>Clarkia similis</i>	<i>C. epilobioides</i> <i>C. modesta</i>	0.03818 \pm 0.00210	MYRT	Annual	R	22

Continued.

Table 1. Continued.

Category	Hybrid species	Parents ^a	Genetic distance	Order ^b	Life history	Age	Reference ^c
Polyploid	<i>Draba ladina</i>	<i>D. aizoides</i> <i>D. tomentosa</i>	0.05995±0.00079	BRAS	Perennial	R	23
Polyploid	<i>Erythronium quinaultense</i>	<i>E. montanum</i> <i>E. revolutum</i>	0.09909	MONO	Perennial	—	24
Polyploid	<i>Gossypium hirsutum</i>	<i>G. raimondii</i> <i>G. arboreum</i>	0.10094	MALV	Perennial	A	25
Polyploid	Hawaiian Silversword spp.	<i>Anisocarpus scabridus</i> <i>Carlquistia muirii</i>	0.07893	ASTE	Perennial	A	26
Polyploid	<i>Nicotiana arentsii</i>	<i>N. undulata</i> <i>N. wigandioides</i>	0.04616	SOLA	Perennial	A	27
Polyploid	<i>Nicotiana rustica</i>	<i>N. paniculata</i> <i>N. undulata</i>	0.06397	SOLA	Perennial	A	27
Polyploid	<i>Platanthera huronensis</i>	<i>P. dilatata</i> <i>P. aquilonis</i>	0.04566±0.00029	MONO	Perennial	—	28
Polyploid	<i>Primula scotica</i>	<i>P. farinosa</i> (2×) <i>P. halleri</i> (4×)	0.03170±0.00114	ERIC	Perennial	A	29
Polyploid	<i>Primula egalikensis</i>	<i>P. mistassinica</i> <i>P. nutans</i>	0.06205±0.00032	ERIC	Perennial	A	29
Polyploid	<i>Rubus maximus</i> (<i>R. maximiformus</i>)	<i>R. idaeus</i> (2×) <i>R. caesius</i> (4×)	0.04838±0.00050	ROSA	Perennial	—	30
Polyploid	<i>Saxifraga osloensis</i>	<i>S. adscendens</i> <i>S. tridactylites</i>	0.16035±0.00475	SAXI	Perennial	A	31
Polyploid	<i>Spartina anglica</i>	<i>S. maritime</i> <i>S. alterniflora</i>	0.11511±0.00002	MONO	Perennial	R	32
Polyploid	<i>Spiranthes diluvialis</i>	<i>S. magnicamporum</i> <i>S. romanzoffiana</i>	0.05637	MONO	Perennial	A	33
Polyploid	<i>Tragopogon mirus</i>	<i>T. dubius</i> <i>T. porrifolius</i>	0.02966±0.00457	ASTE	Annual	R	34
Polyploid	<i>Tragopogon miscellus</i>	<i>T. dubius</i> <i>T. pratensis</i>	0.05064±0.01125	ASTE	Annual	R	34

^aWhere the parent species differ in chromosome number the ploidy is noted.

^bASTE = asterales, BRAS = brassicales, ERIC = ericales, FABA = fabales, LAMI = lamiales, MALV = malvales, MONO = monocots, MYRT = myrtales, ROSA = rosales, SAXI = saxifragales, SOLA = solanales.

^cReferences and Genbank IDs: ¹Brochmann et al., *Plant Syst. Evol.* 220:77 L77792, L77739/94; ²Maki and Murata, *Heredity* 86:87 EF017383–4; ³Allan et al., *Plant Syst. Evol.* 205:205 EF017385–6, AF496995; ⁴Wendel et al., *Evolution* 45:694 AF057749–53/59/60/61–63, U12720, U56786/89; ⁵Welch and Rieseberg, *Evolution* 5:2126; ⁶Rieseberg, *Am. J. Bot.* 78:1218 AF047916/17/18/24/27; ⁷Sun et al., *Belg. J. Bot.* 136:91 AF440241/53; ⁸Wolfe and Randle, *Syst. Bot.* 26:120 AF120218–23; ⁹Sang et al., *Proc. Natl. Acad. Sci. U.S.A.* 92:6813 U27682/95; ¹⁰Aparicio et al., *Ann. Bot.* 85:7 AY792819, AY839235/6; ¹¹Howarth and Baum, *Evolution* 59:948 AY102735/39/47/60, AY894502–6, AY894510–12/15/16; ¹²Siripun and Schilling, *Am. J. Bot.* 93:319 DQ236179–84, DQ236192–7, AB032048, AF177813/53; ¹³Guo et al., *Mol. Ecol.* 15:133 AY603208/09/43; ¹⁴Guo et al., *New Phytol.* 166:273 AF046939, AF155265/302, AY603185–7, AY603212; ¹⁵O’Kane et al., *Syst. Bot.* 21:559 X52320; AJ232900, U43229–33; U52181–4/7/8; AY662287; X98628; ¹⁶Kochert et al., *Am. J. Bot.* 83:1282 AY862310/13; AY615240/57, AY862311; ¹⁷Clausen et al., *Carnegie Inst. Wash. Publication* 564:1 AF514347–50; AF061387/0471; ¹⁸Allen, *Am. J. Bot.* 72:268 EF017387–96; ¹⁹Song and Osborn, *Genome* 35:992 AY833603; DQ003650–6; AY722423; AF039994/40038; AF128095–101; ²⁰Franzke and Mummenhoff, *Theor. Appl. Genet.* 98:831 AF077981/2; AF265166–168/181/185–187/201, AY260579–584/618/619, AY662295; AJ232908; ²¹Perny et al., *Bot. J. Linn. Soc.* 148:101 AY245977/5978/5984/5989/5993/6001–3/6007/6008/6014/6019/6023/6031–6033; ²²Ford and Gottlieb, *Evolution* 53:1060 EF017397–404; ²³Widmer and Baltisberger, *Am. J. Bot.* 86:1282 AF120721/25–6; AF146511–12; AY134193; ²⁴Allen, *Syst. Bot.* 26:263 AF485296/98; ²⁵Wendel and Cronn, *Adv. Agronom.* 78:139 U12712/18; ²⁶Barrier et al., *Mol. Biol. Evol.* 16:1105 M93798/99; ²⁷Chase et al., *Ann. Bot.* 92:107 AJ492413/34/35; ²⁸Wallace, *Int. J. Plant Sci.* 164:907 EF025518–36; ²⁹Guggisberg et al., in prep DQ993712–22, 41–9, 63–71; ³⁰Stebbins, *Variat. Evol. Plants* (Columbia Univ. Press, New York) AF055755–7/76, AF362705; ³¹Nilsson and Jorde, *Nord. J. Bot.* 18:425 EF028686–8; ³²Raybould et al., *Biol. J. Linn. Soc. Lond.* 43:111 AF272775–6, AJ489793–8; ³³Arft and Ranker, *Am. J. Bot.* 85:110 AF301440–1; ³⁴Ownbey, *Am. J. Bot.* 37:487 AY525376–7, AY645813/33500/33503, AY508167/69, AJ633494/6, L35855.

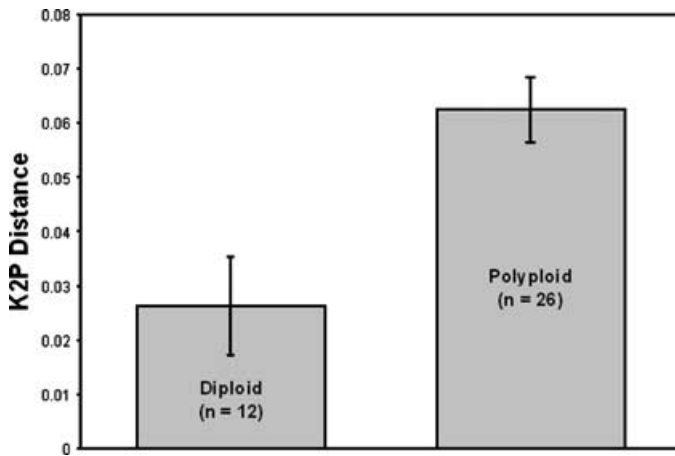


Figure 1. Genetic divergence between the parents of polyloid hybrid species versus those of diploid hybrid species. Values are based on ITS sequence divergence, and reflect Kimura's (1980) two-parameter distance \pm SE.

ploidy differs (see Table 1), the estimation of divergence is not higher than the average for the parents of polyloids.

A related possibility is that our results simply reflect differences in life history between the two types of hybrid species. Because perennial species experience fewer generations per unit time than annuals, a tendency toward perenniality in the parents of homoploid hybrid species, and annuality in the parents of allopolyploids, could produce the pattern that we observed. This would be true even if the species in question are of approximately the same age. To test for this possibility, we performed a two-way ANOVA with the type of hybrid species and annual versus perennial life history as the main effects. Once again, our results remained unchanged, with hybrid species type being highly significant ($F = 15.82$; $df = 1, 35$; $P \leq 0.0003$) and life history being nonsignificant ($F = 0.90$; $df = 1, 35$; $P = 0.35$). Thus, the elevated levels of divergence between species pairs that have given rise to allopolyploids do not appear to be an artifact of life-history differences. As noted above, it has been argued that perenniality increases the likelihood that a polyloid lineage will become established by increasing the odds that an individual will find a mate with the same cytotype (Stebbins 1950). Interestingly our data show that the majority of both polyloid and diploid hybrid species are perennial, possibly indicating that perenniality also increases the likelihood of homoploid hybrid speciation.

Our findings do not, of course, necessarily indicate that high levels of genetic divergence are required for polyloid formation. Indeed, the formation of autopolyploids clearly cannot be explained by our findings. That being said, our results do fit well with predictions and empirical evidence concerning the origin of hybrid species. For example, Sang et al. (2004) predicted that allopolyploidy would be most likely at a level of parental genetic divergence in which (1) the probability of an F_1 producing

unreduced gametes is high, (2) cross-compatibility between the parents is nonzero, and (3) hybrids are genetically distinct from the parents.

It is generally accepted that, in the absence of a "triploid bridge," allopolyploidy is relatively unlikely to occur (e.g., Harlan and De Wet 1975; Ramsey and Schemske 1998; David et al. 2004; Husband 2004). Moreover, it seems most plausible that such triploid individuals will arise via meiotic nonreduction. At lower levels of divergence, however, meiotic nonreduction is thought to be relatively rare, thereby reducing the likelihood of allopolyploid formation and leaving homoploid hybrid speciation as the most likely path to the formation of a hybrid neospecies. In contrast, higher levels of divergence are thought to increase the rate of meiotic nonreduction (e.g., van Tuyl et al. 1989), thereby increasing the likelihood that the necessary triploid bridge will be formed.

As noted above, diploid hybrids produced between divergent parents may also be less fit than allopolyploids from the same cross (Stebbins 1950; Mable 2004). Hence, there may be a maximum level of parental genomic divergence at which hybrids can survive without an increase in ploidy. Stebbins (1950, p. 327) was one of the first authors to recognize this pattern, stating: "The hybrids which are capable to give rise to allopolyploids are usually completely unable to give diploid progeny, so that interchange of genes between their parental species is impossible . . . they may belong to the same section of a genus. . . , but more often they belong to different sections, subgenera or even genera." Moreover, it has been argued that the fertility of polyloid hybrids might actually increase with increasing parental genetic divergence (e.g., Stebbins 1950; Bogart 1972; Mable and Bogart 1995; Mable 2004). Our results are fully consistent with these ideas, and suggest that the extent of evolutionary divergence between hybridizing taxa plays an important role in determining the outcome of hybrid speciation.

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